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Effect of electroacupuncture at Zusanli (ST36) and Sanyinjiao (SP6) acupoints on adrenocortical function in etomidate anesthesia patients

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: We aimed to investigate the effect of electroacupuncture at Zusanli (ST36) and Sanyinjiao (SP6) on adrenocortical function in patients with etomidate anesthesia.

Material/Methods: We randomly divided 80 patients who underwent elective surgery into 4 groups: group etomidate (ETO), group etomidate + electroacupuncture (ETO+EA), group etomidate + sham acupuncture (ETO+SEA), and group propofol (PRO). The patients in group ETO, ETO+EA, and ETO+SEA were induced with etomidate and sufentanil and maintained with intravenous infusion of etomidate and remifentanil. Group PRO was induced with propofol and sufentanil and maintained with propofol and remifentanil. Group ETO+EA received electro-acupuncture stimulation at Zusanli and Sanyinjiao throughout the operation, while group ETO+SEA received electro-acupuncture stimulation at non-acupoints. We recorded the values of MAP, HR, BIS, CVP, cortisol, ACTH, epinephrine, norepinephrine, and arterial blood gas during the perioperative period.

Results: Cortisol concentrations were significantly higher at all times except T0 in group ETO+EA compared with group ETO. The ACTH concentrations were lower in group ETO+EA than that in group ETO at point T3.

Conclusions: Electroacupuncture at ST 36 and SP 6 can mitigate the adrenal cortical inhibition induced by etomidate and can reduce the secretion of catecholamines during surgery.

MeSH Keywords: Electroacupuncture • Cortisol • ACTH • Catecholamines • Etomidate

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Background

Etomidate has become the most widely used induction agent because of its easy dosing profile, hemodynamic tolerance, limited suppression of ventilation, lack of histamine release, and protection from myocardial and cerebral ischemia [1–8]. However, adrenal cortical inhibition by etomidate has received a great deal of attention and significantly limits its use as both an anesthetic and sedative. And it indicates that etomidate inhibits adrenal steroid synthesis primarily by blocking the activity of CYP11B1, also known as 11β-hydroxylase or P450c11, according to some clinical results and *in vitro* studies [9,10].

Acupuncture, a therapeutic modality with few or no adverse effects, has been used in China and other Asian countries for thousands of years to treat a variety of conditions such as inflammatory disease and chronic pain [11]. Previous studies reported that acupuncture increases adrenocorticotropic hormone (ACTH) [12,13] and glucocorticoid levels [14]. A recent clinical trial reported that electro-acupuncture (EA) significantly alleviated the symptoms of patients with knee osteoarthritis [15]. Since adrenal glands secrete glucocorticoids such as cortisol in humans, these studies suggest that EA may activate the adrenals to increase glucocorticoid secretion, leading to suppression of inflammatory responses.

However, it has not been confirmed systematically that electro-acupuncture treatment can maintain the glucocorticoid secretion during general anesthesia maintained by etomidate. Therefore, this study was designed to clarify the effect of electro-acupuncture treatment on glucocorticoid secretion during general anesthesia induced and maintained by etomidate.

Material and Methods

The clinical trial registration number of this project was ChiCTR-TRC-11001727. After obtaining Ethics Committee approval and written informed consent, 80 patients with American Society of Anesthesiologists physical status (ASA) I or II were recruited and underwent elective surgery lasting more than 3 h but less than 4 h. Exclusion criteria included: previous history of acupuncture or emulsion allergies, ASA physical status >II, patients below 18 years or above 75 years, having a body mass index greater than 30, and those patients who had received long-term hormone therapy, had a psychiatric or liver and kidney dysfunction history, or who had received opioids in the preceding month. To avoid possible diurnal variations in cortisol secretion, each study procedure started at 8 AM.

Experimental procedure

The patients were randomized by the use of a table of random numbers into 1 of 4 groups: a group receiving etomidate anesthesia (group ETO), a group receiving propofol anesthesia (group PRO), a group receiving etomidate anesthesia plus electroacupuncture at the acupoints (group ETO+EA), and a group receiving etomidate anesthesia plus electroacupuncture at the non- acupoints (group ETO+SEA).

Premedication in all 4 groups consisted of pethidine 50 mg, promethazine 25 mg, and scopolamine 0.3 mg intramuscularly 45 min before transport to the operating room. In the operating room, standard monitoring (electrocardiogram, BIS, invasive blood pressure, SpO2 using a Datex S/5 Anesthesia Monitor [GE Healthcare, Helsinki, Finland]) was applied, and an intravenous infusion was begun in a peripheral vein and 200 ml colloid solution was infused prior to induction to compensate for overnight fluid loss. Thereafter, the patients were preoxygenated with 6 L/min O2 for 5 min by using a tight-fitting face mask that also served to sample end-tidal carbon dioxide.

After administration of 0.05 mg/kg midazolam, 0.4 μg/kg sufentanil, and 0.5 mg/kg atracurium, anesthesia was induced with either 0.3 mg/kg etomidate in group ETO, ETO+EA, and ETO+SEA or 2 mg/kg propofol in group PRO. Intubation was performed when muscles had relaxed, then the ventilation was adjusted to keep end-tidal carbon dioxide at 35–40 mmHg. Anesthesia was maintained with etomidate at a constant rate of 0.6 mg/kg/h in group ETO, ETO+EA, and ETO+SEA or 6 mg/kg/h propofol in group PRO. In addition, remifentanil was infused at a constant rate of 0.35 μg/kg/min in all 4 groups. To maintain muscle relaxation, 0.25 mg/kg atracurium was injected repeatedly whenever deemed necessary by the anesthesiologist.

Electroacupuncture

The acupuncture points selected were adopted from the Chinese acupuncture literature and the recommendations of an experienced acupuncturist, who also administered the acupuncture and sham acupuncture procedure in group ETO+EA and ETO+SEA, respectively. The needles were inserted into acupoints ST36 and SP6, respectively, and the depth of needle insertion depended on the acupoints selected. Further manipulation was then delivered to achieve Deqi sensation, which is characterized as a numb, heavy, sore and/or distending sensation. Electrical stimulation was delivered via a battery-operated stimulator (model WQ1002K, AERON Optoelectronic Technology Corp., Beijing, PRC) that emitted dense-sparse waves (dense wave: 18 Hz, duration time 1.05 sec; sparse wave: 3.85 Hz, duration time 2.85 sec) [16,17]. The stimulator was then switched on, and the intensity was gradually increased from zero to reach a strong but comfortable level. The sensation of EA is numbness, distension, and tingling. The possibility of muscle contraction was explained to participants prior to the treatment.

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Table 1. Location of sham acupoints.

Sham acupoints corresponding to	Method of location of sham acupoints	References
Sanyinjiao (SP 6)	On the medial aspect of the leg. Four body units inferior to the popliteal crease	Zaslawski et al., 1997
Zusanli (ST 36)	On the posterior aspect of the leg. Six body units inferior to the popliteal crease and one body unit lateral to the midline	Zaslawski et al., 1997

SP6 – Spleen Meridian 6; ST 36 – Stomach Meridian 36.

In sham acupuncture (group ETO+SEA), the same number of acupoints as for group ETO+EA was used for each participant. For each real acupoint, there was a corresponding sham acupoint. Their method of location is described in Table 1 [18].

Outcome measures

The changes of MAP, CVP, HR, and BIS were recorded at 8 different time points: 5 min before anesthesia induction (T0), immediately after induction (T1), 5 min after the intubation (T2), 30, 60, 120, and 180 min after the surgery began (T2–6), and at the end of the surgery (T7).

Blood gas analysis was performed twice during the surgery: 30 min (T1) and 2 h (T2) after the surgery began, and the changes in pH, PaO2, PaCO2, and lactate content were recorded.

We immediately collected 3-ml venous blood samples from the 4 groups of patients at the 4 time points: before anesthesia induction (T0), 2 h after surgery began (T1), at the end of the surgery (T2), and 2 h after the surgery was completed (T3). Samples were centrifuged for 10 min (2000 r/min), and then the serum was collected and placed in the deep cryogenic refrigerator at –70°C until needed for measurement. The serum cortisol and ACTH were detected by double-antibody sandwich ELISA method (R&D Systems, Inc. USA).

Statistical analysis

Comparisons of categorical data such as sex and the type of surgery were analyzed by chi-square test. The continuous response variables like age, weight, duration of surgery, duration of anesthesia, urine volume, blood loss, and crystalloid and colloid requirements are presented as mean ±SD. One-way ANOVA was applied to compare the means among the 4 groups. Other variables such as MAP, CVP, HR, BIS, blood gas analysis, and serum levels of cortisol and ACTH, are presented as mean ±SD and analyzed using a two-way ANOVA with post hoc Bonferroni tests (two-tailed) for normally distributed data or using a Kruskal-Wallis test (two-tailed) with a post hoc Bonferroni test for non-normally distributed data. A p-value <0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the study population

The demographic data of the patients are summarized in Table 2. There were no significant differences in age, weight, duration of surgery, duration of anesthesia, urine volume, blood loss, or crystalloid and colloid requirements among the 4 groups (all $p>0.05$).

Arterial blood gas analysis

The results of arterial blood gas analyses at different time points in each group are summarized in Table 3. There were no significant differences in pH, PaO2, PaCO2, and lactate content among the groups (all $p>0.05$).

The changes of MAP, CVP, HR, and BIS in the different groups

No significant differences were observed in CVP and BIS among the 4 groups at any time (Supplemental Figure 1A, 1B). Compared with group ETO, MAP and HR were decreased significantly at T1 (immediately after induction) in group PRO (* $p<0.05$, # $p<0.001$), and there were no significant differences in MAP and HR among groups ETO, ETO+EA, and ETO+SEA (Figures 1 and 2).

Electroacupuncture could mitigate the adrenal cortical inhibition induced by etomidate

Cortisol concentrations were significantly increased at time points T1, T2, and T3 compared to baseline values in group PRO (all $p<0.001$). In contrast, it was significantly decreased at T1, T2, and T3 compared to baseline values in groups ETO and ETO+SEA (* $p<0.01$, ^ $p<0.001$), but cortisol concentrations were only decreased at T3 in group ETO+EA (^{a} $p<0.01$) (Figure 3).

Compared with group PRO, cortisol concentrations were significantly decreased at all time points except T0 in groups ETO, ETO+EA, and ETO+SEA (# $p<0.001$). When compared with group

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Table 2. Demographic variables at baseline in each group (n=20).

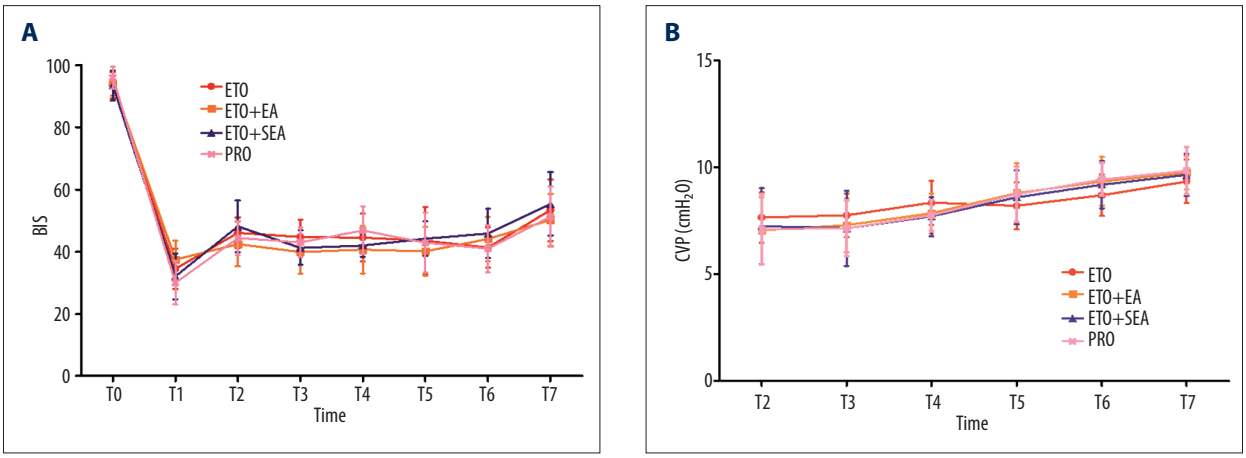
Variable	ETO	ETO+EA	ETO+SEA	PRO
Male (%)	65	60	70	55
Age (yr, mean ±SD)	60±15	56±17	56±15	59±13
Weight (kg, mean ±SD)	64±12	63±7	62±10	67±10
Duration of surgery (min, mean ±SD)	211±32	200±30	197±28	210±24
Duration of anesthesia (min, mean ±SD)	254±82	234±55	241±70	249±60
Type of surgery (number)				
General	13	15	14	14
Gynecological	4	3	5	3
Orthopedic	3	2	1	3
Blood loss (ml, mean ±SD)	162±90	170±50	180±70	170±100
Urine volume (ml, mean ±SD)	1500±428	1460±445	1425±428	1380±382
LR(ml, mean ±SD)	1380±382	1255±356	1370±387	1285±328
6% HES (ml, mean ±SD)	682±227	715±208	662±199	655±199

No significant difference was found between groups. yr – years; kg – kilogramme; min – minute; ml – milliliter; LR – Lactated Ringer's solution; HES – hydroxyethyl starch.

Table 3. Arterial blood gas analyses at different time points in each group (N=20).

Group	pH		PaO2		PaCO2		Lac	
	T1	T2	T1	T2	T1	T2	T1	T2
ETO	7.43±0.05	7.41±0.07	435.6±90.9	437.0±105.6	38.4±5.3	37.4±5.7	1.31±0.73	1.48±0.70
ETO+EA	7.41±0.04	7.40±0.06	451.8±94.4	452.3±108.9	36.1±5.3	36.9±5.9	1.35±0.77	1.28±0.57
ETO+SEA	7.39±0.08	7.41±0.05	440.1±102.9	439.9±51.2	37.1±6.8	36.3±4.9	1.26±0.72	1.24±0.67
PRO	7.41±0.08	7.41±0.04	481.4±109.1	443.8±109.0	37.8±6.0	35.0±4.2	1.52±0.75	1.48±0.64

No significant difference was found between groups. All the values were presented by mean ±SD. Lac – lactic.



Supplemental Figure 1. The changes of CVP and BIS in the different groups: No significant differences were observed in CVP and BIS among the four groups at any time.

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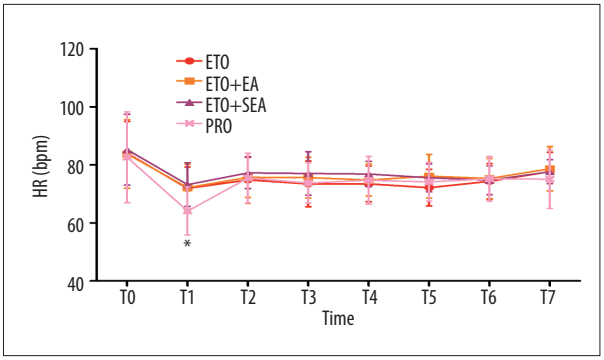

Figure 1. The change of HR in the different groups: compared with group ETO, HR was decreased significantly at T1 point in group PRO (* $p<0.05$), and there were no significant differences in MAP and HR among group ETO, ETO+EA and ETO+SEA at any time point.

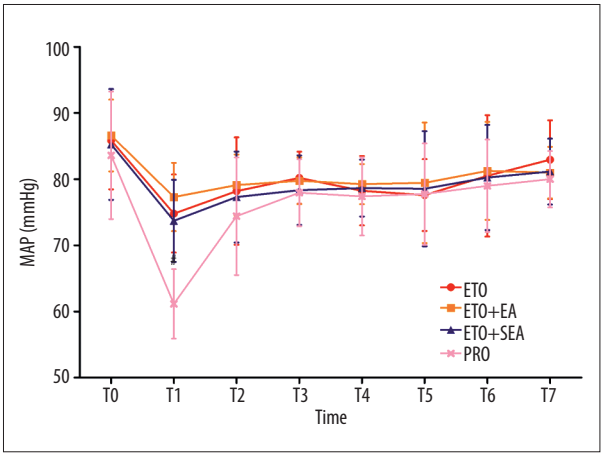

Figure 2. The change of MAP in the different groups: compared with group ETO, HR was decreased significantly at T1 point in group PRO (# $p<0.001$), and no significant differences were found in MAP among group ETO, ETO+EA and ETO+SEA at any time point.

ETO, cortisol concentrations were significantly increased at all time points except T0 in group ETO+EA (^{b} $p<0.05$, ^{c} $p<0.001$). No differences were observed between groups ETO and ETO+ SEA (Figure 3).

The ACTH levels were higher at T1, T2, and T3 than baseline values in all groups (^{a} $p<0.05$: group PRO at T1, ^{b} $p<0.01$: group ETO+EA at T1, # $p<0.001$). At time point T3, the ACTH concentrations were lower in group ETO+EA and PRO than in group ETO (* $p<0.001$), and there was no significant differences between groups ETO and ETO+SEA (Figure 4).

Discussion

The normal response to stress is characterized by stimulation of the hypothalamic-pituitary-adrenal (HPA) axis and results

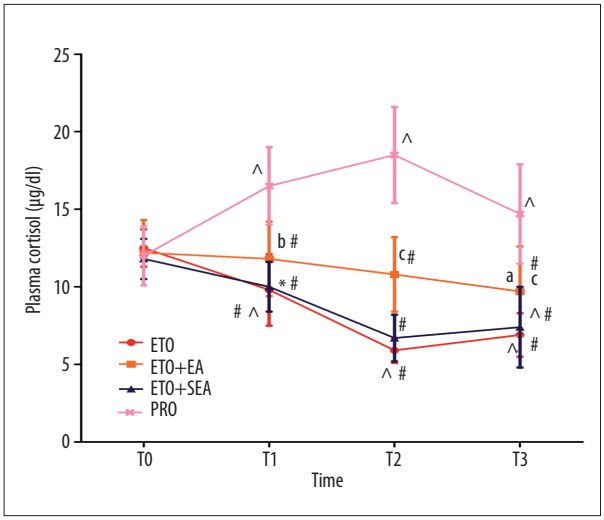

Figure 3. The comparison of cortisol levels among these four groups: cortisol concentrations were significantly increased at time points of T1, T2 and T3 compared to baseline values in group PRO (^ $p<0.001$). On the contrary, it was significantly decreased at T1, T2 and T3 compared to baseline values in group ETO and ETO+SEA (* $p<0.01$, ^ $p<0.001$), however, cortisol concentrations were only decreased at T3 in group ETO+EA (^{a} $p<0.01$). Compared with group PRO, cortisol concentrations were significantly decreased at any time except T0 in group ETO, ETO+EA and ETO+SEA (# $p<0.001$). While compared with group ETO, cortisol concentrations were significantly increased at any time except T0 in group ETO+EA (^{b} $p<0.05$, ^{c} $p<0.001$).

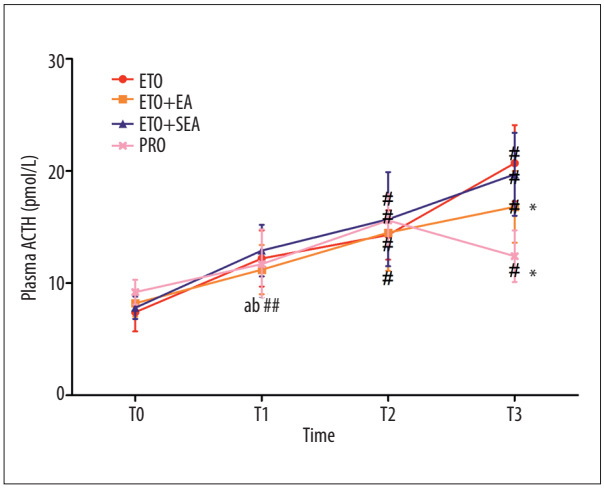

Figure 4. The comparison of ACTH levels among these four groups: The ACTH levels were higher at T1, T2 and T3 than baseline values in all groups (^{a} $p<0.05$: group PRO at T1, ^{b} $p<0.01$: group ETO+EA at T1, # $p<0.001$). At point T3, the ACTH concentrations were lower in group ETO+EA or PRO than that in group ETO (* $p<0.001$), and there was no differences significant between group ETO and ETO+ SEA.

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in increased cortisol production[19]. To observe the inhibition of etomidate on adrenal cortex during the surgery, we choose propofol as a control, which has been shown to have no effect on adrenal function in surgery patients [20,21]. In humans, the amount of cortisol present in the blood undergoes diurnal variation; the level peaks in the early morning (approximately 8 AM) and reaches its lowest level at about 4 AM, or 3–5 h after the onset of sleep [22]. So to avoid possible diurnal variations in cortisol secretion, each study procedure started at 8 AM.

We found that cortisol concentrations were significantly increased compared to baseline values at T1, T2, and T3 in group PRO. The suppression of cortisol levels we observed in patients receiving etomidate (group ETO) was in agreement with the results of previous studies [23–25].

EA can regulate the function of the HPA-axis [26], which may be the mechanism whereby electroacupuncture achieves its therapeutic effects. The previous study showed that EA of ST 36 and SP 6 can improve surgical trauma-induced HPA disorders [26], so we choose these 2 acupoints in our study. Due to the different effects of acupuncture and sham acupuncture [27], we set group ETO+SEA as a control. For each real acupoint, there was a corresponding sham acupoint, and we choose it according to findings reported in a previous study [18].

In the current study, we found that cortisol concentrations were significantly increased in group ETO+EA at all time points except T0, compared with ETO. However, no differences were observed between group ETO and ETO+SEA. This demonstrates that EA procedures can increase the cortisol concentrations, and that the sham EA causes no changes in the cortisol levels of patients, which indicates that the EA procedures used in this study could specifically mitigate the adrenal cortical inhibition induced by etomidate.

Conclusions

Our sham intervention was performed on non-acupuncture points and involved skin penetration and electrical stimulation. Therefore our differential effects of acupuncture and sham acupuncture may be due to point location. Future studies are needed to determine if the parameters of electrical stimulation are associates with the effects.

Overall, our data strongly indicate that electroacupuncture at ST 36 and SP 6 can mitigate the adrenal cortical inhibition induced by etomidate and reduce the secretion of catecholamines during the surgery. Elucidation of the fundamental mechanisms underlying these processes awaits further investigation.

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