PGC-1 upregulation via estrogen receptors: a common mechanism of salutary effects of estrogen and flutamide on heart function after trauma-hemorrhage

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DESPITE NUMEROUS ADVANCES in intensive care medicine, sepsis, organ dysfunction, and ischemia-reperfusion injury remain the major causes of death in trauma patients as well as in patients after major surgery (5, 12, 13, 16, 32, 38). Previous studies have shown prolonged depression of cardiovascular function in male rats after trauma-hemorrhagic shock, despite fluid resuscitation (2, 24). However, depletion of androgen levels by castration in male rats 2 wk before trauma-hemorrhage prevented the depression in cardiac function after trauma-hemorrhage (2, 23, 34). Alternatively, administration of the androgen receptor antagonist flutamide to intact male rats after trauma-hemorrhage prevented the depression of cardiac function (24). Moreover, administration of 17β-estradiol (E2) to male or ovariectomized female animals after trauma-hemorrhage restored depressed cardiovascular function (10, 11, 18). These studies therefore indicate that male and female sex steroids differentially influence cardiovascular function after trauma-hemorrhage. Although studies have also shown that inflammatory cytokines, inducible nitric oxide synthase, or signal transducer and activator of transcription 3 (26, 34) play a role in E2 as well as flutamide-mediated beneficial effects on organ function after trauma-hemorrhage, whether E2 and flutamide mediate their salutary effects via the same or different mechanisms remains unknown.

It is well known that mitochondria are abundant in the heart and play a critical role in the regulation of cardiac function, including ATP production. There is also evidence that trauma-hemorrhage decreases tissue ATP stores (4, 21), and in this regard, previous studies have shown that estrogens can prevent depletion of tissue ATP (30). Conversely, androgens have been shown to decrease the activities of mitochondrial complexes I, II, and IV and tissue ATP levels (25). In addition, the nuclear coactivator peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1), which is highly expressed in heart and skeletal muscle, has been characterized as a broad regulator of cellular energy metabolism and a key regulator of mitochondrial biogenesis (14, 22). PGC-1 induces mitochondrial biogenesis by activating the transcription factor nuclear respiratory factor 2 (NRF) 2, which regulates expression of mitochondrial proteins. NRF-2 in turn transactivates expression of nuclear genes required for mitochondrial function, including nuclear-encoded mitochondrial transcription factor A (Tfam), cytochrome-c oxidase subunit IV (COX IV) enzyme, and β-ATP synthase (6, 27, 28, 33). Because E2 and flutamide improved cardiac function after trauma-hemorrhage, we hypothesized that E2 and flutamide mediate their beneficial effects by upregulating PGC-1-controlled mitochondrial biogenesis and ATP production in the heart after trauma-hemorrhage. To test this hypothesis, we examined the effects of E2 and flutamide after trauma-hemorrhage on 1) cardiac function, 2) cardiac PGC-1 protein levels, 3) cardiac mitochondrial ATP levels, cytochrome-c oxidase activity, and mitochondrial DNA-encoded gene cytochrome-c oxidase subunit I (COX I) expression, 4) cardiac NRF-2 mRNA expression, and 5) cardiac Tfam, COX IV, and β-ATP synthase mRNA expression and mitochondrial protein levels.

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MATERIALS AND METHODS

**Rat trauma-hemorrhagic shock model.** Male Sprague-Dawley rats (Charles River) were fasted overnight but allowed free access to water before the experiments. Trauma-hemorrhage and resuscitation were then carried out as described previously (2, 11, 23, 24, 34). Briefly, the rats were anesthetized by isoflurane inhalation, and a 5-cm midline laparotomy was performed to induce soft tissue trauma. The abdominal wound was closed in layers, and polyethylene catheters (PE-50, Becton Dickinson, Sparks, MD) were placed in both femoral arteries and the right femoral vein. The rats were allowed to awaken and then bled rapidly within 10 min to a mean arterial pressure (MAP) of 35–40 mmHg. This hypotension was maintained until the animals could no longer maintain 35 mmHg MAP without administration of fluid in the form of Ringer lactate solution. This time was defined as maximal bleed out (MBO). After MBO, MAP was maintained at 35–40 mmHg until 40% of MBO volume was returned to the heart. Ringer lactate solution (~90 min from the onset of bleeding). The rats were then resuscitated with four times the volume of MBO with Ringer lactate over 60 min. After resuscitation, the catheters were removed and the wounds were closed. Sham-operated animals underwent surgical procedures without hemorrhage or resuscitation. The animals were killed 2 h after the end of resuscitation or sham operation. In the treatment group, E2 (50 μg/kg body wt) or flutamide (25 mg/kg body wt; Sigma, St. Louis, MO) was administered subcutaneously at the beginning of resuscitation. In another group of rats, ICI-182780 (3 mg/kg body wt ip; Tocris Cookson, Ellisville, MO) was administered with or without flutamide. Rats in the vehicle-treated group received the same volume of vehicle (corn oil or 1,2-propanediol, 1 ml/kg body wt sc; Sigma). Five to seven animals were studied in each group. This study was approved by our Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

**Determination of cardiac function.** At 2 h after trauma-hemorrhage or sham operation, the animals were anesthetized with pentobarbital sodium (30–50 mg/kg ip). Cardiac output (CO) was determined by using the indirect oxygen method (ICG) dilution technique (8). A 2.4-Fr fiber-optic catheter (Hospex Fiberoptics, Chestnut Hill, MA) was inserted to the level of the right atrium. ICG (50 μg/ml) was injected via the right jugular vein catheter. The injection of ICG was recorded by using a computer-assisted data acquisition program (Asystant+, Asyst Software, Rochester, NY). After the measurement of CO, the right carotid artery was re cannulated with PE-50 tubing, and after the measurement of blood pressure, the catheter was advanced into the left ventricle and connected to a heart performance monitoring system (DigiMed) to monitor and record positive and negative first derivatives of pressure (+dP/dtmax and −dP/dtmax). CO, stroke volume (SV), and total peripheral resistance (TPR) were calculated according to standard equations.

**Isolation of mitochondria.** After the animals were euthanized, the hearts were removed, minced, and then homogenized gently with a motor-driven grinder in 0.25 M sucrose, 0.5 mM EGTA, and 3 mM HEPES, pH 7.2, at 10 ml/g of heart. The suspension was centrifuged at 750 g for 10 min at 4°C. The supernatant was centrifuged again at 9,500 g for 10 min, and the pellet fraction-enriched mitochondria were collected and stored at −70°C for measurements of ATP levels and cytochrome-c oxidase activity.

**Cytochrome c oxidase assays.** The cytochrome-c oxidase activity of cardiac mitochondria was analyzed by an assay kit (Sigma) according to the manufacturer’s instructions. Briefly, isolated mitochondria were homogenized in assay buffer (10 mM Tris-HCl, pH 7.0, and 120 mM KC1). Approximately 50 μg of mitochondrial protein were added to 1.1 ml of reaction solution containing 50 μl of 0.22 mM fully reduced ferrocytochrome c, 10 mM Tris-HCl (pH 7.0), and 120 mM KC1. The decrease of absorbance at 550 nm was recorded over a 1-min reaction time.

**Determination of mitochondrial ATP levels.** ATP content was measured by the ATP Bioluminescence Assay kit (Roche, Mannheim, Germany) according to the manufacturer’s protocol. Briefly, the mitochondrial pellet was suspended in the lysis reagent supplied in the kit. The suspensions were pipetted and vortexed, and the protein concentrations were measured (Bio-Rad Laboratories, Hercules, CA). The samples were kept on ice until measurements were performed. For determination of ATP, 100 μl of luciferase reagent were added to the standards or 1 μg of mitochondrial proteins, and ATP was measured with a luminometer (AutoLumat LB953, Berthold, Wildbad, Germany).

**Quantitative real-time PCR.** Total RNA was prepared in TriReagent (Life Technologies, Grand Island, NY), and 2 μg of total RNA were reversed to cDNA by Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI). Real-time PCR was performed using SYBR green or TaqMan methods on a sequence detection system (PRISM 7900 HT, Applied Biosystems, Foster City, CA). The sequences of primers and probes used in this study were designed using the Prism Primer Express Program (Applied Biosystems): 5′-agttgacaagatggctgec-3′ and 5′-ctgttcctccatggcagc-3′ for NFR-2, 5′-gaaagcacaaatacaaggag-3′ and 5′-ctgctctatcagacag-3′ for Tfam, 5′-tggatgattgtagtgagta-3′ and 5′-gctgaagccgtgaaaac-3′ for COX IV, 5′-gcaacctgacagttgaat-3′, 5′-catcactgccatgaatgat-3′, and 5′-acaaaggggtcat-3′ (probe) for β-ATP synthase, and 5′-agttgcctcatgagctg-3′, 5′-ctgctctatcagttgaat-3′, and 5′-ttccactactcactgagta (probe) for COX I. The reaction mixture for TaqMan assay contained 5 μl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 0.2 μmol/l β-ATP synthase and COX I primers, 0.25 μmol/l β-ATP synthase and COX I TaqMan probes, and 40 ng of cDNA. The reaction mixture for SYBR green assay contained 10 μl of 2× SYBR green PCR Master Mix (Applied Biosystems), 0.5 μmol/l (NRF-2), 0.3 μmol/l (COX IV), or 0.4 μmol/l (Tfam) primers, and 40 ng of cDNA. All samples were tested in triplicate, and average values were used for quantification. 18S rRNA was used as an endogenous control. At each cycle, accumulation of PCR products was detected by monitoring the increase in fluorescence of the reporter dye from the TaqMan probes or double-stranded DNA-binding SYBR green. Analysis was performed by using SDS version 2.2 software (Applied Biosystems) according to the manufacturer’s instructions. The comparative cycle threshold (Ct) method (ΔΔCt) was used for quantification of gene expression.

**Western blot analysis.** For PGC-1 protein detection, heart tissues were homogenized in lysis buffer consisting of 150 mM NaCl, 1 mM EDTA, 2 mM Na3PO4, 30 mM NaF, 20 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Tissue lysates were centrifuged at 17,000 g for 10 min. For detection of Tfam, COX IV, and β-ATP synthase protein, mitochondrial pellets were suspended in 0.25 M sucrose, 0.5 mM EGTA, and 3 mM HEPES, pH 7.2. The protein concentrations were measured and separated by SDS-PAGE and transferred to nitrocellulose paper. After the membranes were immobilized with anti-PGC-1, Tfam (Santa Cruz Biotechnology, Santa Cruz, CA), COX IV (Molecular Probes, Eugene, OR), β-ATP synthase, GAPDH, and β-actin (Abcam, Cambridge, MA) antibody, horseradish peroxidase-conjugated secondary antibody was added. After the final wash, membranes were probed by using enhanced chemiluminescence (Amersham, Piscataway, NJ) and subjected to autoradiography. GAPDH and β-actin were used as loading controls.

**Statistical analysis.** Values are means ± SE. One-way ANOVA and Tukey’s test were employed for comparison among groups, and differences were considered significant at P < 0.05.
RESULTS

Effect of flutamide or E2 on cardiac function. CO, SV, \( +\frac{dP}{dt_{\text{max}}} \), and \( -\frac{dP}{dt_{\text{max}}} \) decreased (\( P < 0.05 \) for all parameters) in the vehicle-treated trauma-hemorrhage group compared with the sham-operated group (Fig. 1). In contrast, TPR increased (\( P < 0.05 \)) under these conditions (Fig. 1C). Flutamide or E2 administration after trauma-hemorrhage, however, restored these cardiac parameters to levels similar to those in the sham-operated group (Fig. 1). Our previous studies showed no significant difference in cardiac function between non-treated sham-operated animals and E2- or flutamide-treated sham-operated animals; hence, the effects of E2 and flutamide on cardiac function in sham-operated rats were not examined in this study (18, 36).

Effect of flutamide or E2 on cardiac PGC-1 protein levels. To determine the possible mechanisms of flutamide and E2 on cardiac mitochondrial function, PGC-1, a key regulator of mitochondrial biogenesis, was determined by Western blotting. Cardiac PGC-1 protein increased after trauma-hemorrhage in the vehicle-treated group compared with the sham-operated group (Fig. 2). However, administration of flutamide or E2 after trauma-hemorrhage prevented the decrease in PGC-1 protein under those conditions.
Although flutamide as well as E2 administration to sham-operated animals increased PGC-1 protein levels by 20%, the increase was not statistically significant by ANOVA.

**Effect of flutamide or E2 on cardiac mitochondrial ATP levels, cytochrome-c oxidase activity, and mitochondrial DNA-encoded COX I gene.** To evaluate the PGC-1 downstream effects, we measured mitochondrial ATP levels, cytochrome-c oxidase activity, and mitochondrial DNA-encoded COX I gene expression. Cardiac mitochondrial ATP levels (Fig. 3), cytochrome-c oxidase activity (Fig. 4), and mitochondrial DNA-encoded COX I gene expression (Fig. 5) decreased in the vehicle-treated trauma-hemorrhage group compared with the sham-operated group ($p < 0.05$). Flutamide or E2 administration after trauma-hemorrhage, however, increased mitochondrial ATP levels, cytochrome-c oxidase activity and mitochondrial DNA-encoded COX I gene expression similar to levels in the sham-operated animals.

**Effect of flutamide or E2 on cardiac NRF-2 mRNA expression.** Because NRF-2 mRNA expression can be induced by PGC-1, NRF-2 mRNA expression was determined by real-time PCR after trauma-hemorrhage. Cardiac NRF-2 mRNA expression decreased significantly in the vehicle-treated trauma-hemorrhage group ($p < 0.05$; Fig. 6) but increased with administration of flutamide or E2 and was similar to that in the sham-operated animals.

**Effect of flutamide or E2 on cardiac Tfam, COX IV, and β-ATP synthase mRNA expression and protein levels.** NRF-2 transactivates the regulators of mitochondrial function, including Tfam, COX IV, and β-ATP synthase. Accordingly, mRNA expression was determined by real-time PCR, and protein levels were measured by Western blot. Cardiac Tfam, COX IV, and β-ATP synthase mRNA expression (Fig. 7) and cardiac mitochondrial Tfam, COX IV, and β-ATP protein levels (Fig. 8) decreased significantly in the vehicle-treated trauma-hemorrhage group compared with the sham-operated group ($p < 0.05$). Cardiac Tfam, COX IV, and β-ATP synthase mRNA expression and cardiac mitochondrial Tfam, COX IV, and β-ATP synthase protein levels were increased in trauma-hemorrhage rats treated with flutamide or E2 and similar to those in the sham-operated animals.

**Effect of the estrogen receptor antagonist ICI-182780 alone or in combination with flutamide on cardiac PGC-1.** To evaluate whether flutamide mediated the upregulation of PGC-1 via the estrogen receptor (ER), rats subjected to trauma-hemorrhage were treated with the ER antagonist ICI-182780 alone or
in combination with flutamide. PGC-1 protein levels were significantly decreased in the trauma-hemorrhage group treated with vehicle or ICI-182780 alone (Fig. 9). The decrease in PGC-1 after trauma-hemorrhage was, however, not evident in rats treated with flutamide after trauma-hemorrhage. The flutamide-mediated upregulation of PGC-1 was, however, prevented in the trauma-hemorrhage rats treated with ICI-182780 in combination with flutamide (Fig. 9; \( P < 0.05 \)).

**DISCUSSION**

Previous studies have shown that CO is depressed in male rats after trauma-hemorrhage but is maintained in proestrous female rats (10, 11, 23, 24). It has also been shown that administration of E2 or flutamide improves cardiovascular function in male rats after trauma-hemorrhage (11, 18, 24). Furthermore, it has been reported that the salutary effects of E2 are mediated via the ER, because use of ICI-182780 along with E2 blocked the salutary effects of E2 (11). Although it is known that flutamide and E2 improve cardiovascular function after trauma-hemorrhage, the precise mechanism by which E2 and flutamide produce their salutary effects remains unknown.
More specifically, whether E2 and flutamide mediate their salutary effects via the same or different mechanisms is unknown. The novelty of this study is that our results clearly indicate that the salutary effects of flutamide are also mediated via the ER, because ICI-182780 blocked the effects of flutamide on PGC-1. Furthermore, we have shown an association between PGC-1 and the mitochondrial pathway in the normalization of cardiac performance with flutamide as well as E2 after trauma-hemorrhage.

The main findings of this study suggest that cardiac function, cardiac PGC-1 protein levels, mitochondrial ATP levels, cytochrome-c oxidase activity, expression of the mitochondrial DNA-encoded COX I gene, and cardiac NRF-2, Tfam, COX IV, and β-ATP synthase mRNA and protein levels in the mitochondrial fraction were markedly decreased 2 h after trauma-hemorrhage. Administration of E2 or flutamide after trauma-hemorrhage normalized cardiac function and increased PGC-1, mitochondrial ATP, cytochrome-c oxidase, mitochondrial DNA-encoded COX I gene, NRF-2, Tfam, COX IV, and β-ATP synthase levels. However, administration of the ER antagonist ICI-182780 along with flutamide after trauma-hemorrhage prevented flutamide-mediated PGC-1 upregulation. These findings therefore suggest that, similar to the effect of E2, the effect of flutamide is mediated via ER-dependent PGC-1 upregulation. Because PGC-1 plays an important role in regulation of NRF-2, Tfam, COX IV, and β-ATP synthase and production of mitochondrial ATP, cytochrome-c oxidase activity, and the mitochondrial DNA-encoded gene COX I, it is likely that upregulation of PGC-1 in trauma-hemorrhage rats treated with E2 and flutamide restores ATP production and thereby prevents alterations in cardiac function after trauma-hemorrhage. Although administration of E2 or flutamide to sham-operated animals increased PGC-1 protein levels by 20%, the increase was not statistically significant. Nonetheless, because administration of flutamide as well as E2 increased PGC-1 protein levels by 20%, it is possible that these two distinct agents upregulated PGC-1 expression through ER and that this effect is independent of trauma-hemorrhage. Moreover, the 20% increase in PGC-1 protein levels in E2- or flutamide-treated sham-operated rats suggests that E2 or flutamide upregulated PGC-1 expression through ER independent of trauma-hemorrhage. These results therefore collectively suggest that upregulation of PGC-1 is the common event through which E2 and flutamide mediate their beneficial effects on cardiac function after trauma-hemorrhage. However, although ICI-182780 blocked the flutamide-induced upregulation of PGC-1, indicating that the effects of flutamide are mediated via PGC-1, blockade of PGC-1 upregulation by agents other than ICI-182780 should provide additional proof that PGC-1 plays a pivotal role in the salutary effects of flutamide and estrogen on cardiac function after trauma-hemorrhage. However, because specific blockers of PGC-1 are not available, it is difficult to perform such a study.

Gender differences exist in the cardiovascular diseases (17). Our previous studies have also shown that male sex hormones have deleterious effects and female sex hormones produce beneficial effects on cardiovascular function after trauma-hemorrhage (3, 10, 29). In this study, E2 or flutamide treatment at the beginning of resuscitation restored all parameters of cardiac function, including CO, SV, TPR, +dP/dt\textsubscript{max}, and −dP/dt\textsubscript{max}, 2 h after trauma-hemorrhage. These findings further corroborate our previous studies and suggest that E2 or flutamide treatment prevented the depression in cardiac function 2, 20, and 24 h after trauma-hemorrhage (18, 24, 34).

Several potential mechanisms have been proposed for the salutary effects of E2 and flutamide on organ function after trauma-hemorrhage. A recent study by Yang et al. (34) suggested that the beneficial effect of E2 on cardiac function may be due in part to attenuation of IL-6 production after trauma-hemorrhage. Studies have also indicated that flutamide administration inhibits thromboxane A\textsubscript{2} production and/or enhances release of the vasodilator prostacyclin in the aortic-coronary circulation, leading to improved organ perfusion (24). Additional studies have shown that flutamide decreases cytokine release by macrophages and, thus, improves the depressed immune functions after trauma-hemorrhage (31). Although a potential role of inflammatory cytokines or other mediators in the beneficial effects of E2 and flutamide has not been ruled out, our findings suggest that E2 and flutamide mediate their effects via E2 receptors that upregulate PGC-1, which is critical for cardiac mitochondrial function.

Studies have suggested that the mitochondrion occupies a pivotal position in the physiological response to trauma and shock (9). Previous studies have shown that trauma-hemorrhage impairs mitochondrial oxidative phosphorylation and reduces energy production (15). In addition, it has been suggested that sex steroids play a role in mitochondrial function and ATP production. E2 was shown to protect against ATP depletion, mitochondrial membrane potential decline, and generation of reactive oxygen species induced by 3-nitropropionic acid (30). E2 also preserves mitochondrial structure and function in the heart during ischemia-reperfusion (37). Because ER-β is present on mitochondria, it is likely that the effect of estrogen was mediated via ER-β (35). In contrast, physiological concentrations of testosterone were able to inhibit maintenance of the proton gradient across the mitochondrial membranes, as well as ATP synthesis, and synthetic androgen exposure lowered the activities of mitochondrial complexes I, II, and IV and ATP levels (7, 25). Thus it is possible that
Estrogen and anti-androgen can protect mitochondrial function. Among the consequences of mitochondrial depression/dysfunction, loss of ATP production is clearly the most profound, especially in the heart, a tissue specialized for high-capacity ATP production. The results presented here indicate that cardiac mitochondrial ATP levels decreased significantly 2 h after trauma-hemorrhage; however, E2 or flutamide restored cardiac mitochondrial ATP levels. It is likely that the increased/restored mitochondrial ATP levels by E2 or flutamide treatment after trauma-hemorrhage help in the maintenance of myocardial energy and restoration of cardiac function.

The transcriptional coactivator PGC-1, a key regulator of cardiac mitochondrial functional capacity, participates in the transduction of physiological stimuli to energy production in the heart. Massive proliferation of mitochondria and promotion of mitochondrial respiration in cardiac myocytes in transgenic mice as a result of cardiac-specific overexpression of PGC-1 indicate that PGC-1 promotes mitochondrial biogenesis and is capable of ATP production (14). It is therefore conceivable that PGC-1 plays an important role in ATP production in the heart. In addition, PGC-1 has been shown to increase expression of the mitochondrial regulatory factors NRF-1 and NRF-2 (33), both of which are key transcriptional activators of nuclear genes encoding a range of mitochondrial enzymes. Studies have also shown that NRF-2 binds to the promoter region of nuclear genes required for mitochondrial function, including human β-ATP synthase, rat COX IV, and rat Tfam (27, 28, 33). The present study demonstrated that the protein level of PGC-1, mRNA expression of NRF-2, and mRNA expression and mitochondrial protein levels of Tfam, COX IV, and β-ATP synthase were significantly decreased after trauma-hemorrhage. However, E2 or flutamide treatment significantly attenuated the decrease in PGC-1, NRF-2, Tfam, COX IV, and β-ATP synthase mRNA expression and protein levels under those conditions. In our studies, no significant change in expression of NRF-1 after trauma-hemorrhage (data not shown) suggests that the protective effects of E2 and flutamide on cardiac function after trauma-hemorrhage are related to NRF-2 and not to NRF-1. Thus it appears that a common mechanism is responsible for producing the salutary effects of E2 and flutamide after trauma-hemorrhage, i.e., upregulation of PGC-1 and related downstream signaling molecules in the heart with trauma-hemorrhage.

It is known that estrogen mediates its action via ER-α and ER-β (20). Moreover, our previous studies showed that the salutary effects of E2 on cardiac function after trauma-hemorrhage can be abolished by administration of the specific ER antagonist ICI-182780 (11). Furthermore, our previous study also demonstrated that pretreatment with flutamide led to increased ER expression in T lymphocytes of sham-operated and trauma-hemorrhaged male rats (26). Indeed, we recently found that flutamide treatment upregulates ER-α and ER-β expression in cardiomyocytes after trauma-hemorrhage (36). Furthermore, our findings suggesting that flutamide-mediated upregulation of PGC-1 was inhibited by ICI-182780 further support the notion that the salutary effects of flutamide on cardiac function after trauma-hemorrhage are due to upregulation of ER. These findings would thus suggest that the salutary effects of flutamide and E2 are mediated through upregulation of cardiac PGC-1 via ER after trauma-hemorrhage.

Studies have shown that PGC-1 mediates nitric oxide-induced mitochondrial biogenesis (19). Moreover, our previous study also showed that administration of l-arginine after trauma-hemorrhage restored cardiac function through increased production of nitric oxide with trauma-hemorrhage (1). Whether l-arginine also restores the depressed mitochondrial function after trauma-hemorrhage remains to be determined. Nonetheless, the results collectively suggest that E2- as well as flutamide-mediated PGC-1 upregulation after trauma-hemorrhage may be through nitric oxide. This, however, remains to be determined.

In summary, our results indicate that administration of E2 or flutamide after trauma-hemorrhage restored depressed cardiac function and normalized cardiac PGC-1 levels, mitochondrial ATP, cytochrome-c oxidase activity, and the mitochondrial DNA-encoded gene COX I. Moreover, E2 or flutamide treatment also increased NRF-2, Tfam, COX IV, and β-ATP synthase expression. Furthermore, prevention of flutamide-mediated PGC-1 upregulation by treatment of trauma-hemorrhaged rats with ICI-182780 suggests that E2 and flutamide exert their beneficial effect via a common mechanism: ER-mediated upregulation of PGC-1.

GRANTS

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REFERENCES


