Original Article
Estrogen fails to facilitate resuscitation from ventricular fibrillation in male rats

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Abstract: Administration of 17β-estradiol has been shown to exert myocardial protective effects in hemorrhagic shock. We hypothesized that similar protective effects could help improve resuscitation from cardiac arrest. Three series of 18, 40, and 12 rats each, underwent ventricular fibrillation for 8 minutes followed by 8 minutes of chest compression and delivery of electrical shocks. In *series-1*, rats were randomized 1:1 to receive a bolus dose of 17β-estradiol (1 mg/kg) or 0.9% NaCl before chest compression; in *series-2*, rats were randomized 1:1:1:1 to receive a continuous infusion of 0.9% NaCl or a 17β-estradiol solution designed to attain a plasma level of 10^0, 10^2, or 10^4 nM during chest compression; and in *series-3*, rats were randomized 1:1 to receive a continuous infusion of 17β-estradiol to attain a plasma level of 10^2 nM or 0.9% NaCl during chest compression, providing inotropic support during the post-resuscitation interval using dobutamine infusion. 17β-estradiol failed to facilitate resuscitation in each of the 3 series. In *series-1* and *series-2*, resuscitability and short-term survival was reduced in 17β-estradiol groups attaining statistical significance in *series-2* when the three 17β-estradiol groups were combined (p = 0.035). In *series-3*, all rats were resuscitated and survived for 180 minutes aided by dobutamine which partially reversed post-resuscitation myocardial dysfunction but without additional benefits on myocardial function in the 17β-estradiol group. The present study failed to support a beneficial effect of 17β-estradiol for resuscitation from cardiac arrest and raised the possibility of detrimental cardiac effects compromising initial resuscitability and subsequent survival in a male rat model of ventricular fibrillation and closed chest resuscitation.

Keywords: Cardiopulmonary resuscitation, estrogen, rats, ventricular fibrillation

Introduction

Sudden out-of-hospital cardiac arrest is a problem of public health proportions with ~ 424,000 cases assessed by Emergency Medical Services annually in the United States and an average survival rate to hospital discharge of only 10.4% for those treated (~ 50%) but only 5.2% for the entire cohort [1]. Return of cardiac activity typically requires reperfusion of an ischemic myocardium by externally generated coronary blood flow through cardiopulmonary resuscitation (CPR). Yet, reperfusion concomitantly activates multiple pathogenic mechanisms, collectively known as "reperfusion injury", that further compromises myocardial function and viability in which mitochondria play a central role [2]. Current resuscitation methods do not include interventions that could ameliorate such injury. Yet, the discovery that 17β-

estradiol activates potent cell protective mechanisms that converge on mitochondria [3-8] could provide the means to ameliorate such injury and serve to establish a novel approach to cardiac resuscitation that may be both effective and feasible given the broad availability of estrogen formulations for clinical use.

17β-estradiol has been reported to be protective in rat models of hemorrhagic shock [9-13], maintaining cardiac function at baseline levels following hemorrhage. The observed effects of 17β-estradiol have been linked to activation of the PI3K/Akt pathway and to upregulation of p38 MAPK dependent eNOS [13].

In previous studies using a rat model of ventricular fibrillation (VF) and chest compression, we reported that erythropoietin facilitated resuscitation from VF by maintaining left ventricular

distensibility during VF thus enabling hemodynamically more effective chest compression and by attenuating post-resuscitation myocardial dysfunction enabling greater post-resuscitation hemodynamic stability [14]. At the molecular level, these effects of erythropoietin were associated with activation of Akt and PKCε in the heart [15] prompting us to wonder whether 17β-estradiol could also be effective for cardiac resuscitation and elicit effects similar to those of erythropoietin and those of other experimental compounds that also target mitochondria [2, 16, 17]. We tested this hypothesis in a rat model of VF and closed-chest resuscitation examining the effects of 17β-estradiol for resuscitation from cardiac arrest in various modes of administration, various doses, and using dobutamine during the post-resuscitation phase.

Materials and methods

The studies were approved by our Institutional Animal Care and Use Committee and conducted according to the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council.

Rat model of VF and resuscitation

Animal preparation: Anesthesia was induced in adult male Sprague-Dawley rats (428 to 540 grams) with sodium pentobarbital (45 mg/kg intraperitoneal) and maintained by administering 10 mg/kg intravenous every 30 minutes when needed to suppress spontaneous movements of extremities or withdrawal response to toe pinching. A lead II ECG was recorded using subcutaneous needles. The trachea was exposed through a midline incision and a 5-Fr cannula orally advanced into the trachea verifying its proper position by direct visualization through the translucent tracheal wall. The tracheal cannula was used for positive-pressure ventilation during cardiac resuscitation and the post-resuscitation interval. Fluid-filled PE50 catheters connected to pressure-transducers (Maxxim Medical) and zeroed to the midchest were advanced from the left internal carotid artery into the abdominal aorta for pressure measurement and blood sampling and from the left femoral vein into the right atrium for pressure measurement and fluid delivery. A T-type thermocouple catheter (0.64-mm diameter, IT-18, Physitemp) was advanced from the left femoral

artery into the thoracic aorta for recording thermodilution curves. The thermocouple catheter was also used to monitor core temperature, which was maintained between 36.5°C and 37.5°C with the aid of a heating lamp. Another fluid-filled PE50 catheter was advanced from the left external jugular vein into the right atrium. The catheter was connected to a stopcock fitted with another T-type thermocouple and used for injection of thermal tracer to measure cardiac output recording the injectate temperature. A 3F polyurethane catheter (C-PUM-301J, Cook, Inc.) was advanced through the right external jugular vein into the right atrium and a pre-curved guidewire fed through its lumen into the right ventricle for electrical induction of VF.

Experimental protocol: VF was induced by delivering a 60-Hz alternating current to the right ventricular endocardium (1.0-6.0 mA) for an uninterrupted interval of 3 minutes [18], and allowed to continue spontaneously for 5 additional minutes. Chest compression was then initiated using an electronically controlled and pneumatically driven (50 psi) piston device (CJ-80623; CJ Enterprises) centered on the midchest and set to deliver 200 compressions/min with a 50% duty cycle. To assess the hypothesized effects on myocardial distensibility [19], the compression depth was gradually increased adjusting the compression site over an interval of 2 minutes to attain a coronary perfusion pressure (CPP) between 22 and 24 mmHg by the end of minute 2 of chest compression, corresponding to an aortic diastolic pressure between 26 and 28 mmHg for a diastolic right atrial pressure of 4 mmHg. Subsequently, the depth of compression was increased by increments of 2 mm every minute until a maximum depth of 17 mm was reached. Positive pressure ventilation was provided using a volume controlled ventilator (Inspira, Harvard Apparatus) programmed to deliver 25 unsynchronized breaths per minute using a tidal volume of 6 ml/kg body weight and 100% oxygen. Defibrillation was attempted after 8 minutes of chest compression by delivering up to two, 5-J, biphasic waveform electrical shocks across the chest (Heartstream XL, Philips Medical Systems, MA) 5 seconds apart. If VF persisted or an organized rhythm with a mean aortic pressure ≤ 25 mmHg ensued, chest compression was resumed for 30 seconds. The defibrillation-compression cycle was repeated up to five additional times, increasing the energy of indi-

Table 1. Baseline characteristics

	Series-1 (bolus dose)		Series-2 (infusion)				Series-3 (dobutamine)	
	Saline	E_b	Saline	E_{10}^{0}	E_{10}^{2}	E_{10}^{4}	Saline	E_{10}^{2}
Weight (g)	476 ± 22	470 ± 25	470 ± 22	477 ± 30	485 ± 32	488 ± 30	483 ± 34	478 ± 25
Pentobarbital dose (mg/kg)	50.7 ± 0.3	50.9 ± 1.5	53.3 ± 2.6	54.2 ± 5.7	52.9 ± 5.2	51.4 ± 3.1	52.0 ± 3.6	52.5 ± 2.7
Preparation time (min)	107 ± 17	121 ± 31	99 ± 22	92 ± 20	96 ± 17	99 ± 24	95 ± 6	97 ± 15
Temperature (°C)	37.0 ± 0.2	37.0 ± 0.2	36.8 ± 0.2	36.9 ± 0.2	36.9 ± 0.3	36.8 ± 0.3	36.8 ± 0.4	36.9 ± 0.2
Mean aortic pressure (mmHg)	121 ± 12	123 ± 8	142 ± 12	144 ± 15	148 ± 14	136 ± 12	135 ± 11	137 ± 7
Cardiac index (ml/min·kg^{-1})	128 ± 15	129 ± 15	144 ± 15	144 ± 14	140 ± 24	147 ± 20	140 ± 22	144 ± 18
Stroke volume index (ml/kg)	0.34 ± 0.05	0.34 ± 0.04	0.39 ± 0.04	0.38 ± 0.04	0.37 ± 0.08	0.39 ± 0.07	0.38 ± 0.08	0.39 ± 0.05

Baseline measurements were taken 5 minutes before inducing ventricular fibrillation. E denotes groups treated with 17β-estradiol indicating when it was bolus dose (i.e., b) or the target plasma concentration (nM) of the infusion. Values are mean ± SD. Data from series-1 and series-3 was analyzed using t-test; data from series-2 was analyzed using one-way ANOVA showing no statistically significant differences.

Table 2. Resuscitation effort

	Series-1 (bolus dose)		Series-2 (infusion)				Series-3 (dobutamine)	
	Saline	E_b	Saline	E_{10}^0	E_{10}^2	E_{10}^4	Saline	E_{10}^2
n	9	9	10	10	10	10	6	6
Duration of VF (s)	500 ± 40	489 ± 15	439 ± 66	442 ± 68	369 ± 98	426 ± 78	463 ± 41	428 ± 110
Spontaneous Defibrillation (n)	1	0	5	3	7	4	2	2
DF Shocks (n)	2.3 ± 2.1	4.1 ± 4.5	1.6 ± 0.9	2.9 ± 4.1	1 ± 0	4.7 ± 5.7	1 ± 0	1.5 ± 1.0
Cumulative DF Shock Energy (J)	13 ± 14	25 ± 31	8 ± 5	17 ± 26	5	30 ± 39	5	8 ± 6
ROCA (n)	9	7	10	9	8	8	6	6
PR Shocks (n)	2[1]	1[1]	5 ± 2.6[3]	3[2]	3.3 ± 1.7[4]	1[1]	0[0]	0[0]
Cumulative PR Shock Energy (J)	14 ± 0	5 ± 0	25 ± 13	15 ± 0	16 ± 9	5 ± 0	0 ± 0	0 ± 0

VF, ventricular fibrillation; DF, defibrillation; ROCA, return of cardiac activity defined as an organized rhythm with an aortic pulse pressure > 5 mmHg; PR, post-resuscitation; n, number. E denotes groups treated with 17β-estradiol indicating when it was bolus dose (i.e., b) or the target plasma concentration (nM) of the infusion. Values are mean ± SD. Numbers in brackets denote number of animals receiving electrical shock during post-resuscitation. The data for spontaneous defibrillation and ROCA were analyzed using Chi-square test; the rest of the data were analyzed using one-way ANOVA.
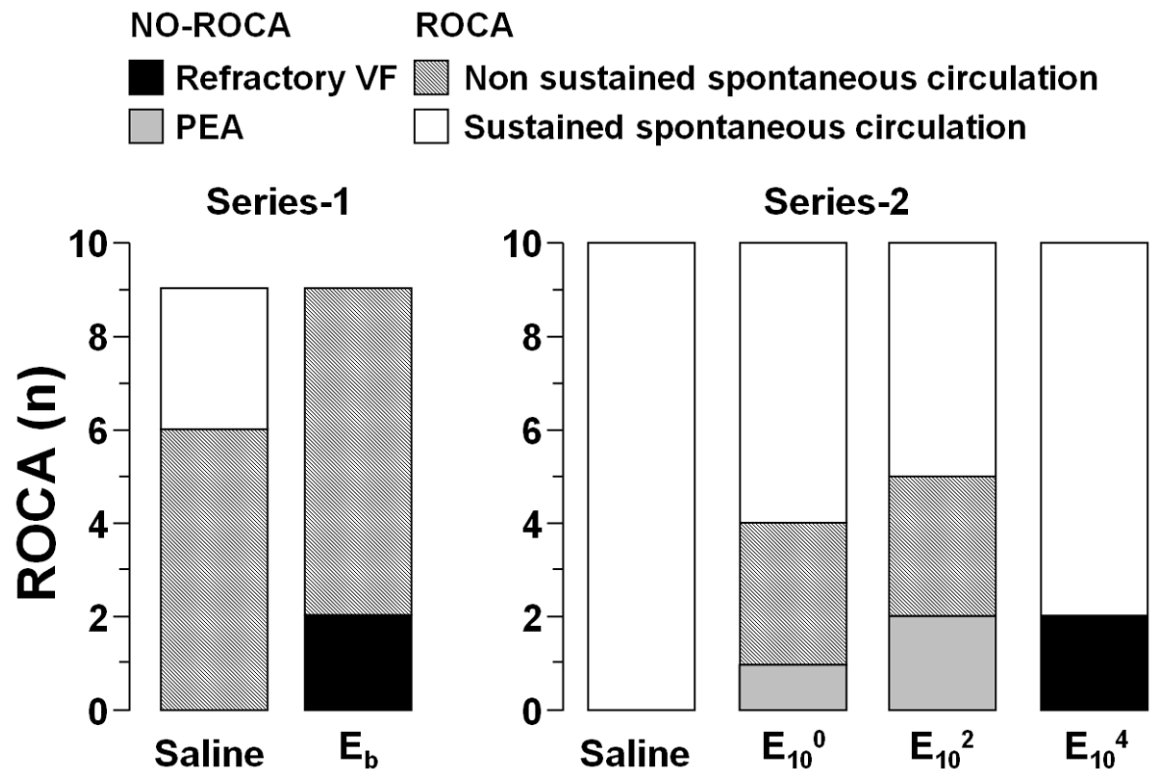

Figure 1. Resuscitability in *series-1* and *series-2*. Restoration of cardiac activity (ROCA) was defined as the return of an organized electrical activity with an aortic pulse pressure > 5 mmHg. No-ROCA included animals that had either refractory ventricular fibrillation (refractory VF) or restoration of an organized electrical activity with an aortic pulse pressure ≤ 5 mmHg corresponding to what could be clinically equivalent to pulseless electrical activity (PEA). Sustained circulation indicated rats that survived the entire 180 minute post-resuscitation interval. Differences between or among groups were analyzed using Chi-square test. No statistically significant difference was found between groups in *series-1* and among groups in *series-2*. Also no statistically significant difference was found between saline and the three 17β-estradiol groups combined in *series-2*.

vidual shocks (if VF persists) to 7-J for the subsequent five cycles. Return of cardiac activity (ROCA) was defined as return of an organized rhythm with an aortic pulse pressure (aortic systolic minus diastolic pressure) > 5 mmHg. After ROCA, the ventilator rate was increased to 60 breaths per minute using 100% oxygen for the initial 15 minutes and 50% oxy-
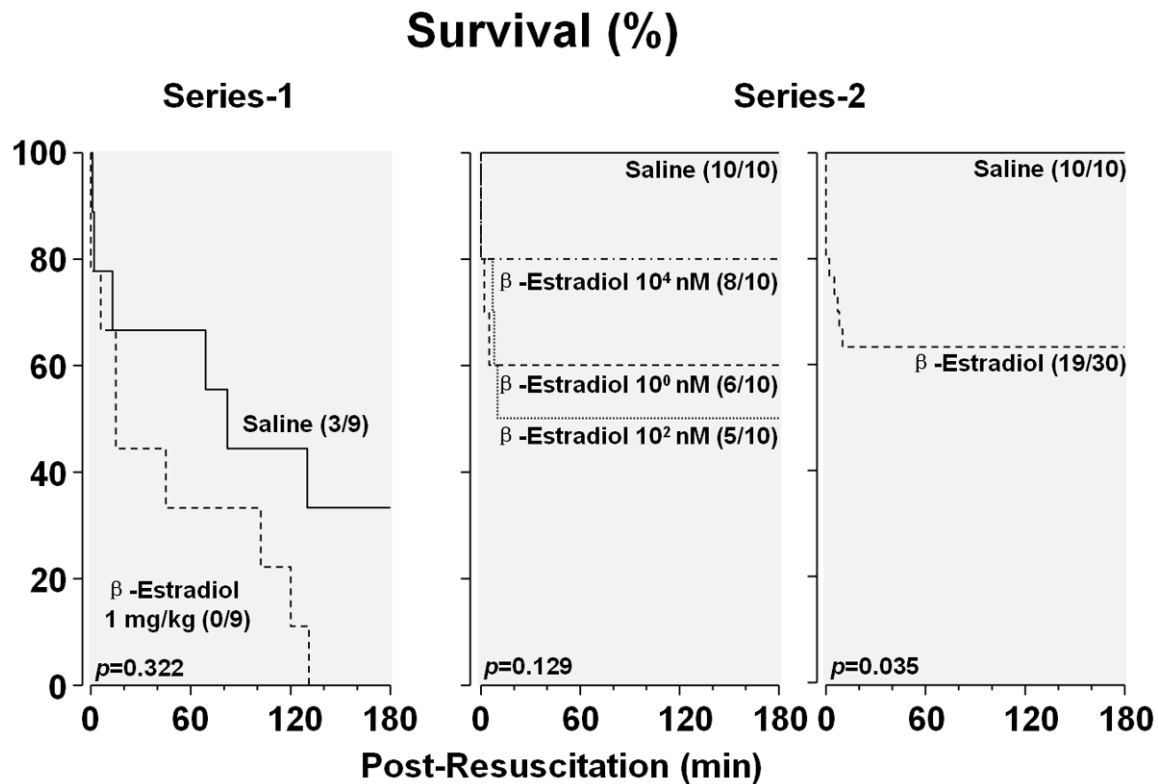
Survival (%)

Figure 2. Survival curves for *series-1* and for *series-2*, showing for the latter the comparison among four groups and the comparison between saline and the three 17β-estradiol groups combined. Differences in survival were analyzed by Kaplan-Meier using the Gehan-Breslow statistical test with the *p*-values noted in each graph.

gen for the remaining 180 minute post-resuscitation interval. Return of spontaneous circulation was defined as the return of an organized rhythm with a mean aortic pressure > 40 mmHg for > 5 minutes.

Experimental series: Three sequential series of experiments were conducted. In *series-1*, 18 rats were randomized 1:1 to receive 1 mg of 17β-estradiol (β-estradiol 3, 17-disulfate dipotassium salt, Sigma #E1636) or an equivalent volume of 0.9% NaCl as bolus dose into the right atrium immediately before starting chest compression. The dose was chosen because it had been shown to elicit cardioprotective effects in a rat model of trauma-hemorrhage by preventing decreases in PI3K and Akt activity [12]. 17β-estradiol was dissolved in 0.9% NaCl to a concentration of 1 mg/ml (1.96 mM). Lack of a beneficial and possible detrimental effect of 17β-estradiol in *series-1* prompted us to examine the possibility of a dose dependency effect of 17β-estradiol [20]. Hence, a second series of experiments (*series-2*) was conducted in which 40 rats were randomized 1:1:1:1 to

receive a continuous infusion of 0.9% NaCl as control or 17β-estradiol at concentrations aimed at achieving steady plasma levels of 10^0 nM, 10^2 nM, or 10^4 nM during chest compression. Each of these concentrations had shown in studies to have myocardial beneficial effects; the 10^0 nM concentration stimulated mitochondrial function by activating F_0F_1-ATPase in isolated rat heart mitochondria [5] and diminished myocardial injury in a rat model of coronary occlusion by inhibiting the phosphorylation of PKCα [21]; the 10^2 nM concentration benefited isolated ischemic rat heart by preserving mitochondrial structure and function with evidence of reduced cytochrome *c* release and less inhibition of mitochondrial respiration after stop-flow ischemia in isolated hearts [6]; and the 10^4 nM dose decreased infarct size in an isolated rat heart model of regional ischemia by activating the PI3K and PKC pathways [22]. The 17β-estradiol or control infusion was delivered into the right atrium concurrently with chest compression and was maintained for the initial 15 minutes post-resuscitation. To achieve the desired 17β-estradiol plasma level in the coro-

Table 3. Hemodynamic effects of chest compression

	Series-1 (bolus dose)		Series-2 (infusion)				Series-3 (dobutamine)	
	Saline	E_b	Saline	E_{10}^0	E_{10}^2	E_{10}^4	Saline	E_{10}^2
Depth (mm)	16.8 ± 0.2	16.6 ± 0.5	16.9 ± 0.4	16.9 ± 0.1	16.9 ± 0.3[8]	16.9 ± 0.1[9]	16.9 ± 0.1	16.8 ± 0.2
Coronary perfusion pressure (mmHg)	28 ± 5	25 ± 7	28 ± 4	31 ± 8	31 ± 9	31 ± 5	32 ± 10	26 ± 6
CPP/Depth (mmHg/mm)	1.7 ± 0.3	1.5 ± 0.4	1.7 ± 0.2	1.9 ± 0.5	1.8 ± 0.6	1.8 ± 0.3	1.9 ± 0.6	1.6 ± 0.4
Mean aortic pressure (mmHg)	38 ± 6	35 ± 8	44 ± 11	45 ± 12	43 ± 11	40 ± 5	47 ± 6	44 ± 8

The data shown correspond to the average of 8 second recording obtained at the end of every minute averaged from minute 5 of chest compression until the last minute of chest compression. In brackets, numbers of rats that completed 8 minutes of chest compression; there were three instances of spontaneous defibrillation in series 2. E denotes groups treated with 17β-estradiol indicating when it was bolus dose (i.e., b) or the target plasma concentration (nM) of the infusion. Values are mean ± SD. *Series-1* and *series-3* were analyzed using t-test; *series-2* was analyzed using one-way ANOVA noting no statistically significant differences in any of the series.
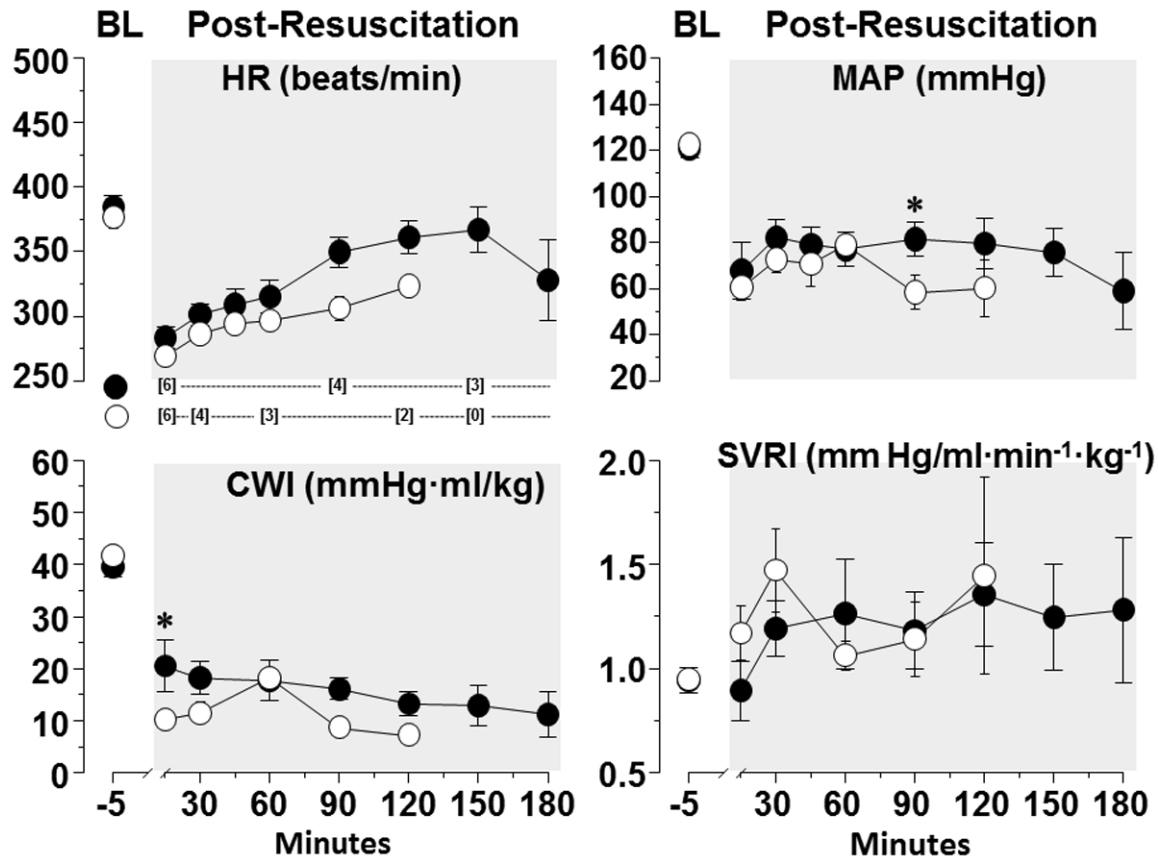
Figure 3. Effects of 1 mg/kg 17β-estradiol (○, n = 9) compared with saline control (●, n = 9) on heart rate (HR), mean aortic pressure (MAP), cardiac work index (CWI), and systemic vascular resistance index (SVRI) in *series-1*. Measurements were obtained at baseline (BL, 5 min before inducing ventricular fibrillation) and post-resuscitation. Numbers in brackets denoted number of surviving rats during the post-resuscitation interval. Values are mean ± SEM. Differences between groups were analyzed by two-way repeated measures ANOVA. *$p \leq 0.05$ denote statistically significant difference between estradiol and saline at the specified time points.

nary circuit, we assumed the infusion would be diluted in the bloodstream in proportion to the cardiac output. We used data from previous experiments in the same rat model in which the cardiac output measured with fluorescent microspheres during chest compression was approximately 5 ml/min [19]. The 17β-estradiol infusion was delivered at 0.1 ml/min yielding a dilution factor of 50. Thus, to achieve plasma levels close to the target concentrations noted above, the solutions contained 17β-estradiol at concentrations of 50 nM, 5×10^3 nM, and 5×10^5 nM. The 17β-estradiol solutions were prepared freshly before each experiment. In this series, 17β-estradiol also failed to favor resuscitability and survival. We finally asked whether 17β-estradiol could exert an adjunctive role on post-resuscitation myocardial function in the presence of dobutamine as previously reported

for erythropoietin. Thus, a third series of experiments (*series-3*) was conducted in which 12 rats were randomized 1:1 to receive either 0.9% NaCl or 17β-estradiol infusion to achieve a plasma concentration of 10^2 nM (as in *series-2*). The 17β-estradiol dose was chosen because it was associated in *series-2* with the highest post-resuscitation aortic pressure. A total of 0.9 ml of dobutamine HCl (2000 μg/ml; Hospira Inc.) was dissolved in 48 ml of 0.9% NaCl yielding a dobutamine concentration of 36.8 μg/ml in 0.88% NaCl. The solution was infused into the right atrium at 0.4 ml/kg·min⁻¹ using a syringe pump (PHD 2000 programmable, Harvard apparatus) to deliver dobutamine at 15 μg/kg·min⁻¹. The infusion was started immediately before delivering electrical shocks or immediately after return of spontaneous circulation in instances of spontaneous defibrilla-
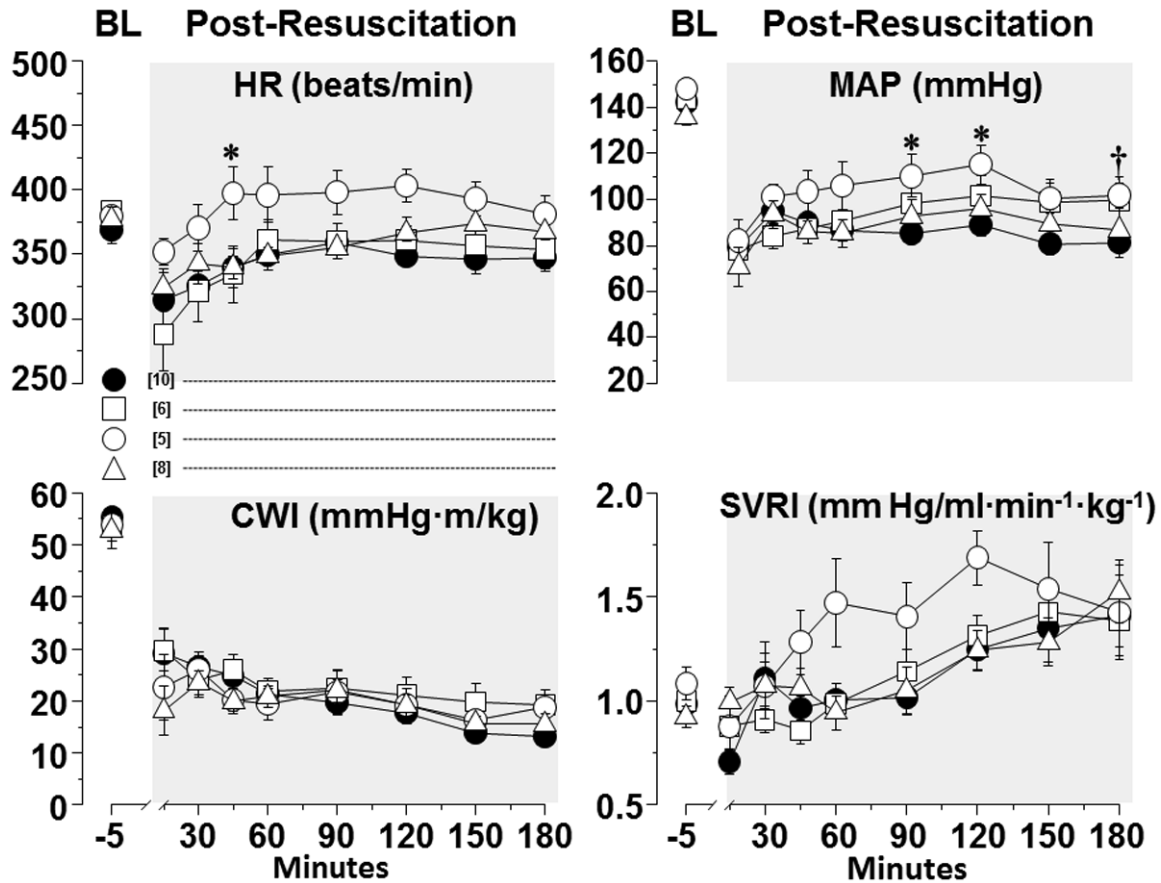
Figure 4. Effects of different concentrations of 17β-estradiol 100 nM (□, n = 10), 10^2 nM (○, n = 10), and 10^4 nM (Δ, n = 10) compared with saline control (●, n = 10) on heart rate (HR), mean aortic pressure (MAP), cardiac work index (CWI), and systemic vascular resistance index (SVRI) in *series-2*. Measurements were obtained at baseline (BL, 5 min before inducing ventricular fibrillation) and post-resuscitation. Numbers in brackets denoted number of surviving rats during the post-resuscitation interval. Values are mean ± SEM. Differences between groups were analyzed by two-way repeated measures ANOVA. There was an overall statistically significant difference among different interventions for HR ($p = 0.037$); *$p < 0.05$, 17β-estradiol 10^2 nM vs saline, †$p < 0.05$, 17β-estradiol 100 nM vs saline at the specified time points.

tion and was maintained throughout the post-resuscitation interval of 180 minutes.

The investigators performing the experiments were blind to the treatment assignment, which was revealed at the completion of each series only after the corresponding experiments had been completed and the descriptive data analyzed.

Measurements

Signals were processed using signal conditioners (BIOPAC Systems), sampled at 250 scans/s, and digitized using a 16-bit data-acquisition board (model AT-MIO-16XE-50; National Instruments). Pressures were measured using disposable pressure transducers (Maxxim Medical) calibrated to zero at midchest level. The-

rmodilution cardiac output (ml/min) was measured using the thermocouple catheter after bolus injection of 200 μl 0.9% NaCl solution into the right atrium. Cardiac index (ml/min·kg^{-1}) corresponded to cardiac output divided by the rat weight. Stroke volume index (ml/kg) corresponded to the cardiac index divided by the heart rate. Cardiac work index (mmHg·m/kg) corresponded to the difference between mean aortic pressure and mean right atrial pressure multiplied by the stroke volume index. Systemic vascular resistance index (mmHg/ml·min^{-1}·kg^{-1}) corresponded to the difference between mean aortic pressure and mean right atrial pressure divided by cardiac index. Chest compression depth was measured using a displacement transducer (LVDT, Omega Engg) attached to the piston device. The coronary perfusion pressure
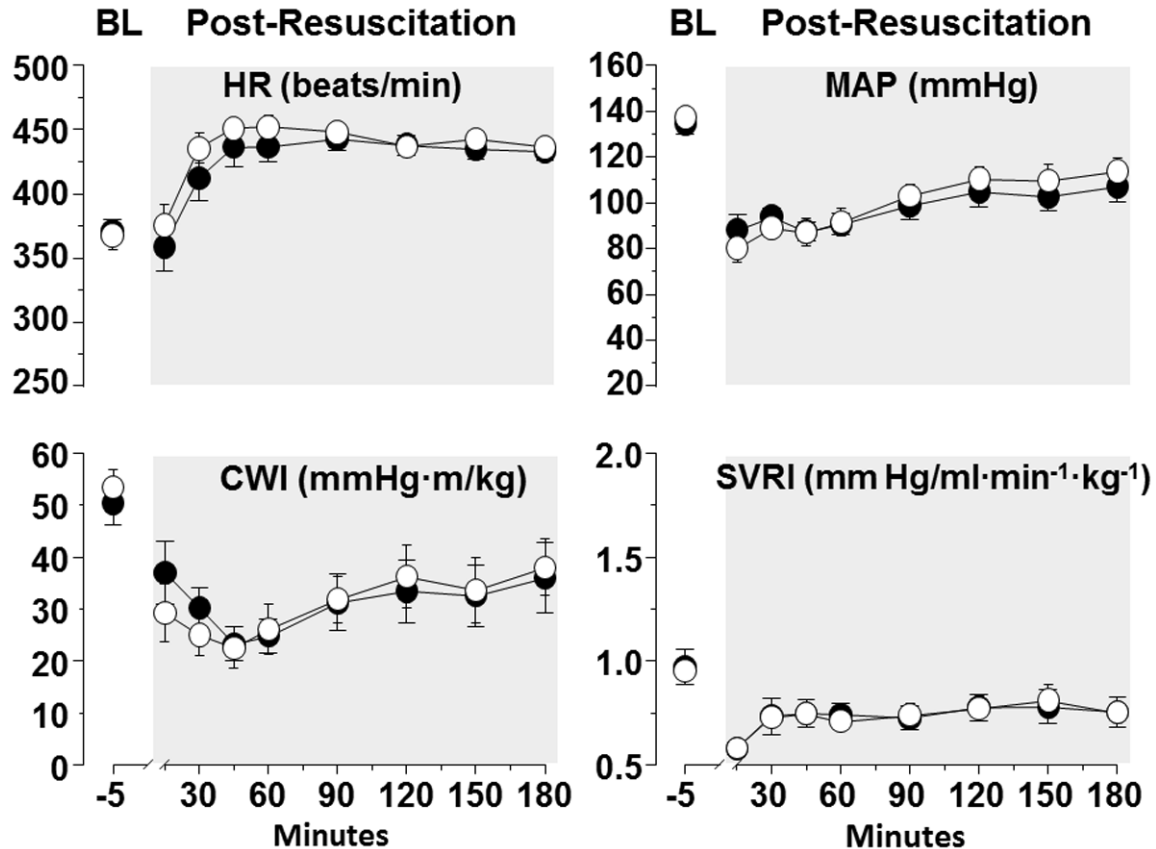
Figure 5. Effects of 10^2 nM 17β-estradiol (○, n = 6) compared with saline control (●, n = 6) on heart rate (HR), mean aortic pressure (MAP), cardiac work index (CWI), and systemic vascular resistance index (SVRI) in *series-3*. Measurements were obtained at baseline (BL, 5 min before inducing ventricular fibrillation) and post-resuscitation. Values are mean ± SEM. Differences between groups were analyzed by two-way repeated measures ANOVA. No statistically significant differences were found between the two groups in HR, MAP, CWI and SVRI.

during resuscitation was calculated by subtracting the aortic minus the right atrial pressure measured between compressions at the end of chest re-expansion.

Statistical analysis

For continuous and repetitive variables (e.g., hemodynamic variables), two-way repeated measures ANOVA was used to test for treatment effect between groups and their interaction over time identifying differences at specified time points when present. For continuous but non-repetitive variables, Student's *t*-test was used when comparing two groups and one-way ANOVA with the Holm-Sidak test for multiple comparisons when comparing more than two groups. Alternative nonparametric tests were used if the data failed tests for normality or equal variance. Kaplan-Meier survival analysis was performed using the Gehan-Breslow

method to assess differences in resuscitability and survival. The data were presented as means ± SD unless otherwise stated. A two-tail value of $p < 0.05$ was considered significant.

Results

Baseline characteristics and hemodynamic parameters were comparable within each series (**Table 1**).

Resuscitation effort, resuscitability, and survival

The resuscitation efforts within each of the 3 series are summarized on **Table 2**. There were no statistically significant differences with regards to the duration of VF or number of electrical shocks and cumulative energy delivered to terminate VF or to treat episodes of recurrent VF. The outcome of the resuscitation effort and

subsequent survival for *series-1* and *series-2* are shown in **Figures 1** and **2**; the outcome for *series-3* is not shown as all rats were resuscitated and survived the post-resuscitation interval. In *series-1*, more rats in the 17β-estradiol group had refractory VF and non-sustained spontaneous circulation throughout the post-resuscitation interval but the differences were not statistically significant. In *series-2*, a similar adverse trend was observed in the 17β-estradiol groups attaining statistically significant lower survival when all three 17β-estradiol groups combined were compared with saline controls (**Figure 2**).

Cardiac and hemodynamic function

Chest compression: The hemodynamics of chest compression including the coronary perfusion pressure generated, its relationship to compression depth, and the mean aortic pressure were comparable between 17β-estradiol and control rats within each of the 3 series (**Table 3**), failing to support an effect on left ventricular distensibility.

Post-resuscitation: Rats in all series developed the well-established phenomenon of post-resuscitation myocardial dysfunction without a beneficial effect associated with the use of 17β-estradiol (**Figures 3** to **5**). In *series-1*, rats from the 17β-estradiol group had a lower cardiac work index at post-resuscitation 15 minutes and a lower mean aortic pressure at post-resuscitation 90 minutes. In *series-2*, however, a higher mean aortic pressure was observed in the 10^2 nM 17β-estradiol group at post-resuscitation 90 and 120 minutes and in the 10^0 nM 17β-estradiol group at post-resuscitation 180 minutes but without differences in cardiac work index consistent with an effect on systemic vascular resistance (**Figure 4**). In *series-3*, inotropic support with dobutamine helped to partially reverse post-resuscitation myocardial dysfunction but without an additional effect promoted by 17β-estradiol (**Figure 5**).

Discussion

The present study demonstrated in a rat model of VF that 17β-estradiol not only failed to preserve the hemodynamic efficacy of chest compression but also adversely affected the subsequent post-resuscitation interval independent of dose and mode of administration without added benefit (or detriment) when dobutamine was used for inotropic stimulation.

Effects of 17β-estradiol during chest compression

As the blood returns to the heart during the relaxation phase of chest compression, distensible ventricles are critically important to secure adequate preload for the subsequent compression. However, ventricular distensibility progressively decreases during chest compression as a manifestation of mitochondrial injury leading to diminishing ability to generate ATP [2]. Reductions in distensibility negatively impact the hemodynamic efficacy of chest compression and eventually preclude reestablishing cardiac activity [23]. In previous studies in our laboratory, using rat and pig models of VF and resuscitation, protection of mitochondrial bioenergetic function by selective inhibition of the Na^+-H^+ exchanger isoform-1 [17] enabled preservation of left ventricular distensibility yielding higher coronary perfusion pressures for a given compression depth [19]. A similar effect was attained with administration of erythropoietin in rats [24]. However, administration of 17β-estradiol in the present studies failed to elicit such effect given that the same coronary perfusion pressure as that of the control rats was generated for a given depth of compression, regardless of the mode of administration or dose.

Effects of 17β-estradiol on resuscitability and survival

We further hypothesized that 17β-estradiol could help to reestablish cardiac activity and ameliorate post-resuscitation myocardial dysfunction. However – and contrary to our hypothesis - 17β-estradiol regardless of mode of administration or dose, had no favorable effect on return of spontaneous circulation and post-resuscitation myocardial dysfunction, but instead exerted a detrimental effect on survival consequent to further decline in myocardial function. This adverse effect of 17β-estradiol was puzzling and in conflict with previously reported favorable myocardial effects in acute settings other than cardiac arrest (e.g., hemorrhagic shock, coronary occlusion), prompting us to review possible reasons for this adverse effect.

Adverse effects of 17β-estradiol

As discussed earlier, we had hypothesized that 17β-estradiol could elicit effects beneficial for

cardiac resuscitation given the reported protective effects on mitochondrial function. However, in further review of the literature, we found that 17β-estradiol could also elicit effects potentially detrimental to cardiac resuscitation. For example, in one study in isolated liver mitochondria in which 17β-estradiol was added into standard respiratory medium before initiating respiration reactions, 17β-estradiol impaired mitochondrial function by decreasing state-3 respiration [25]. And in another study, 17β-estradiol rapidly inhibited mitochondrial F_0F_1-ATP synthase activity in human osteoclasts and preosteoclasts [26]. Despite the above observations, identification of the underlying mechanisms responsible for the lack of effect and potential detrimental effects were beyond the scope of our study.

Limitations and implications of the study

Gender and age of the rat used in the present model should be considered before extrapolating the findings to other settings. In one study, the responsiveness of 17β-estradiol was shown to be age dependent in the uterus and adipose tissue of mice [27]. In another study, 17β-estradiol showed a gender-related difference in estrogen receptor expression in growth plate of spine and limb of rats [28]. Also epidemiological studies in humans have shown gender and age related cardiovascular benefits of estrogen, which could be associated with gender and age related variation in estrogen receptor expression in the cardiovascular system [29]. Accordingly, age, gender, and species could modulate the effects 17β-estradiol yielding different effects; i.e., in younger or older female rats or in humans. However, the rat model used in the present study has been shown to be useful in identifying effects that are applicable to other animal models and species.

More importantly, the present study examined only the effects of 17β-estradiol on immediate resuscitation, hemodynamic stabilization after return of cardiac activity, and short-term survival without excluding delayed beneficial effects evident days after resuscitation in surviving animals. Noppens and colleagues showed neuroprotective effects in a mice model of cardiac arrest when 17β-estradiol was given continuously after cardiac arrest over three days [30].

Conclusions

The present study failed to provide evidence that 17β-estradiol could elicit beneficial myocardial effect during resuscitation from cardiac arrest and impact initial survival and cautions on possible detrimental effects which could compromise survival from cardiac arrest, while showing no additional beneficial effects in the presence of dobutamine.

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