Effect of 6-wk estrogen withdrawal or replacement on myocardial ischemic tolerance in rats

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McNulty, Patrick H., Dinesh J agasia, Jennifer M. Whiting, and Teresa Caulin-glaser. Effect of 6-wk estrogen withdrawal or replacement on myocardial ischemic tolerance in rats. Am J Physiol Heart Circ Physiol 278: H1030–H1034, 2000.—Menopausal status is a risk factor for coronary artery disease death, but the mechanism underlying this association is uncertain. To test whether estrogen ameliorates the effects of acute myocardial ischemia in ways likely to translate into a mortality difference, we compared the response to brief (6-min) and prolonged (45-min) coronary occlusion in vivo in five groups (each n = 16) of rats: ovariectomized females; ovariectomized females after 6 wk 17β-estradiol replacement; male rats supplemented with estradiol for 6 wk; normal males; and normal females. Coronary occlusion produced a uniform ischemic risk area averaging 53 ± 3% of left ventricular volume. After a brief occlusion, reperfusion ventricular tachycardia/fibrillation occurred with >85% frequency in all groups. During a prolonged occlusion, ischemic ventricular tachycardia/fibrillation occurred in 100% and sustained tachycardia requiring cardioversion in >75% of rats in all groups. Myocardial infarct size averaged 52 ± 4% of the ischemic risk area and was similarly unaffected by gender or estrogen status. We conclude that neither short-term estrogen withdrawal, replacement, nor supplementation significantly affects the potentially lethal outcomes from acute coronary occlusion in this species.

menopause; gender; coronary artery disease

THE OBSERVATION THAT coronary artery disease mortality is low in premenopausal women and rises after menopause (5) has suggested that sex hormone replacement might reduce coronary mortality in postmenopausal women and perhaps also in men. This hypothesis is supported by a number of retrospective and observational studies demonstrating an inverse relationship between estrogen use and coronary end points such as myocardial infarction and death from ischemic heart disease (1, 9, 12, 17, 25, 35). Contrary to expectations, however, the only large prospective clinical trial of postmenopausal sex hormone therapy reported to date revealed no effect on coronary mortality at four years (13). This suggests the need to more closely examine the effects of estrogen withdrawal and replacement on mechanisms of coronary death.

Estrogen replacement would be expected to retard the development of coronary atherosclerosis over the long term by favorable effects on the lipoprotein phenotype (2, 3). Although this may constitute its primary mechanism of mortality reduction, studies in canines have reported that estrogen may also provide short-term or even acute protection from coronary death by ameliorating the potentially lethal effects of an acute coronary occlusion (15, 20, 24). These observations have been interpreted to indicate a direct effect of estrogen on myocardial ischemic tolerance, a hypothesis with important potential clinical implications. However, the canine heart is notoriously well collateralized, and the observed effects could alternatively reflect estrogen-mediated modulation of ischemic region size or blood flow during coronary occlusion or reperfusion.

In this study, we used an intact rat model of reversible coronary occlusion-reperfusion to examine the hypothesis that short-term estrogen withdrawal and replacement in vivo affects myocardial ischemic tolerance in ways likely to translate into a mortality difference. Rats were chosen because their coronary circulation is much less well collateralized than that of dogs (19), allowing creation of a uniform ischemic risk area during coronary occlusion. Estrogen-withdrawn and estrogen-replaced female rats were compared for their vulnerability to reperfusion ventricular dysrhythmias after a brief coronary occlusion and for ischemic dysrhythmias and relative infarct size after an intermediate-duration occlusion, all potentially mortal end points that we (21) and others (18, 30) have shown can be ameliorated in this model by interventions that improve myocardial ischemic tolerance. Male rats were also studied because current opinion supports both favorable (20) and unfavorable (27) effects of estrogen on the outcome from myocardial ischemia in males.

METHODS

Experimental animals. Studies were approved by the Animal Care Committee of the Veterans Affairs Connecticut Medical Center and were conducted in accordance with guidelines published by the American Physiological Society. Female Sprague-Dawley rats (n = 32 ovariectomized and n = 16 normal controls) weighing 200–250 g were purchased from Harlan Laboratories. Ovariectomized female rats had been subjected to bilateral oophorectomy by the breeder 2 wk before purchase. Male Sprague-Dawley rats (n = 32) were

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purchased from the same source. Rats were divided into five groups on the basis of gender and estrogen status.

Ovariectomized female rats were randomized to receive either subcutaneous implantation of 3.0 mg of 17b-estradiol in the form of biodegradable pellets (Innovative Research of America, Sarasota, FL) designed to release ~70 µg estradiol/day for 6 wk (n = 16) or no treatment (n = 16). Normal female rats (n = 16) received no specific treatment and served as a control group. Male rats were similarly randomized to either 6 wk of 17b-estradiol treatment (n = 16) or no treatment (n = 16).

Experimental protocol. At the end of the 6-wk treatment period, rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and mechanically ventilated with room air for coronary occlusion-reperfusion experiments. The surgical preparation has been described in detail (21–22). The heart was exposed by median sternotomy, and the left coronary was encircled with a 6–0 prolene suture-snare ~7 mm from its origin at the base of the heart. A heating lamp was used to prevent hypothermia. Needle electrodes were placed in the four limbs for recording the surface electrocardiogram (Gould strip-chart recorder). A blood sample was obtained from a central vein for measurement of plasma estradiol level, and rats were then assigned to one of two experimental protocols.

In the reperfusion protocol, eight rats from each of the five study groups underwent a 6-min left coronary occlusion followed by 10 min of open-artery reperfusion. Ischemia was confirmed by the appearance of cyanosis of the anterior left ventricle upon cinching the coronary snare, and reperfusion was confirmed by the appearance of regional hyperemia. This sequence reliably produces sustained ventricular dysrhythmias in this model during the first minute of reperfusion but not during the coronary occlusion (18, 21, 30). The occurrence of sustained (defined as >10 s duration) ventricular tachycardia or fibrillation upon reperfusion was noted by visual inspection of the heart and was documented by electrocardiographic recording.

In the infarct protocol, eight rats from each group underwent a 45-min left coronary artery occlusion designed to produce an intermediate-sized myocardial infarction. During a coronary occlusion of this duration, ventricular dysrhythmias invariably occur from the 10th through ~25th minute of ischemia (21). In most rats, these take the form of intermittent episodes of sustained (defined as >10 s duration or causing cardiac dilatation) ventricular tachycardia. Sustained dysrhythmias were terminated by tapping the surface of the heart with a metal instrument, and the number of individual occurrences was recorded. After 45 min, the coronary occlusion was released, and the ischemic region was allowed to reperfuse for 3 h.

Analytical methods. Plasma unconjugated b-estradiol was measured by RIA after extraction from thawed plasma samples.

Ischemic risk area and infarct size were estimated as previously described (21). First, the left coronary artery was briefly recouled, and a solution of methylene blue dye in water was injected in the left ventricle of the beating heart to define the ischemic risk area in vivo. Hearts were excised, and the portion of the left ventricle distal to the coronary occlusion was divided into three transverse sections of 2–3 mm thickness. The five exposed surfaces thus created were photographed. The blue dye exclusion zone representing the ischemic risk area of each was quantified by planimetry of magnified photographs. Sections were then stained at 37°C with 2% triphenyltetrazolium chloride and photographed again for measurement of infarct size. For each heart, infarct size is expressed as a percent of ischemic risk area, averaged over the three sections.

Data analysis. Comparisons of ischemic risk area size and infarct size as a percentage of ischemic risk area were made by one-way ANOVA. Individual post hoc comparisons were made with Student’s t-tests if needed, following the Bonferroni convention for repeated measures. For rats in the reperfusion protocol, the incidence of reperfusion ventricular dysrhythmias was compared by Chi-squared test. For rats in the infarct protocol, the frequency of sustained ischemic ventricular dysrhythmias was compared using one-way ANOVA, with post hoc comparisons between groups made as indicated by Bonferroni t-tests, assuming parametric data. Rats not exhibiting a sustained dysrhythmia were assigned a frequency value of zero. Comparisons were also made using the Kruskal-Wallis statistic, assuming nonparametric data. All data are expressed as means ± SD.

RESULTS

Plasma estradiol level. Results are shown in Table 1. Plasma estradiol levels averaged 285 ± 28 pg/ml in normal female rats, fell to <20 pg/ml 8 wk after bilateral ovariectomy, and returned to 345 ± 31 pg/ml after treatment for 6 wk with b-estradiol [P = not significant (NS) vs. normal females]. Estradiol levels in untreated male rats were <20 pg/ml and 6-wk estrogen treatment raised this to 320 ± 30 pg/ml.

Reperfusion dysrhythmias after brief coronary occlusion. In the reperfusion protocol, ventricular tachycardia and/or ventricular fibrillation upon releasing the coronary occlusion was a nearly uniform finding observed in seven of eight normal females, seven of eight ovariectomized females, eight of eight ovariectomized, estrogen-replaced females, seven of eight normal males, and seven of eight estrogen-treated male rats (all P = NS).

Ischemic dysrhythmias during sustained coronary occlusion. During 45-min coronary occlusions, frequent episodes of nonsustained ventricular tachycardia were observed in every rat in all five experimental groups. Sustained (>10 s) ventricular tachycardia requiring mechanical termination was observed in seven of eight normal females, six of eight ovariectomized females, six of eight ovariectomized, estrogen-replaced females, seven of eight normal males, and seven of eight estrogen-treated males (all P = NS). In those rats who exhibited sustained ventricular dysrhythmias, the number of episodes per animal averaged 8 ± 2 in normal females, 7 ± 2 in ovariectomized females, 9 ± 1 in ovariectomized, estrogen-replaced females, 8 ± 2 in normal males, and 8 ± 1 in estrogen-treated males (all P = NS). These results are summarized in Table 2.

Table 1. Plasma b-estradiol levels on the day of study

<table>
<thead>
<tr>
<th>Female Ovariectomized</th>
<th>Ovariectomized Estradiol Male</th>
<th>Male, Estradiol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>b-Estradiol, pg/ml</td>
<td>285 ± 28</td>
<td>&lt;20*</td>
<td>345 ± 31</td>
</tr>
</tbody>
</table>

Data are means ± SD for 16 rats in each group. *P < 0.01 vs. female.
Ischemic risk area and infarct size. Ischemic risk area and infarct size values for rats in the infarct protocol are shown in Fig. 1. Coronary occlusion produced an ischemic risk zone averaging 53 ± 3% of left ventricular volume, in agreement with previous experience in this model (21), and risk zone size was not affected by gender or estrogen status. Infarct size averaged 52 ± 4% of the ischemic risk zone and also did not differ significantly among the groups.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Ovariectomized</th>
<th>Ovariectomized, Estrogen</th>
<th>Male</th>
<th>Male, Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion dysrhythmias, % of rats</td>
<td>88</td>
<td>88</td>
<td>100</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Ischemic dysrhythmias, % of rats</td>
<td>88</td>
<td>75</td>
<td>75</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Ischemic dysrhythmias, episodes/rat</td>
<td>8 ± 2</td>
<td>7 ± 2</td>
<td>9 ± 1</td>
<td>8 ± 2</td>
<td>8 ± 1</td>
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Values are for 8 rats/group. Incidence (%) of sustained ventricular dysrhythmia upon reperfusion of a 6-min coronary occlusion or during a 45-min occlusion and number (means ± SD) of dysrhythmic episodes during the 45-min occlusion. There were no significant differences among the 5 groups.

DISCUSSION

Sex hormone replacement of postmenopausal women likely slows the development and progression of coronary atherosclerosis by increasing the plasma concentration of high-density lipoprotein cholesterol and inhibiting oxidation of low-density lipoproteins (2–3). That this long-term effect constitutes its primary mechanism for coronary mortality reduction is suggested by the recently demonstrated apparent inefficacy of short-term (~3–4 yr) estrogen-plus-progestin replacement for the secondary prevention of coronary death (13) and other data demonstrating a benefit after longer-duration treatment (12). Nevertheless, three more immediate mechanisms of protection from ischemic death have also been proposed. The first mechanism of protection is a possible salutary effect on coronary blood flow. Acute estrogen administration relaxes coronary arteries by both endothelium-dependent (8) and -independent (23) mechanisms, whereas chronic supplementation is reported to increase the expression of vascular nitric oxide (33) and prostacyclin (4) synthases and may augment myocardial blood flow by improving both coronary (34) and collateral artery (28) vasomotor tone. The second mechanism is estrogen's induction of the expression of an ischemia-tolerant myocardial phenotype via its ability, as a steroid hormone, to affect nuclear transcription of specific myocardial proteins (for review, see Ref. 26). Both cardiomyocytes and fibroblasts contain functional estrogen receptors (11), and estrogen has been reported to regulate the myocardial expression of, for example, immediate-early response genes (10) and genes coding for myosin ATPase (29), atrial natriuretic peptide (7), potassium channels (6), and elements of the ANG system (16). The third mechanism is that hyperestrogenemia per se might be protective by virtue of its acute, nongenomic actions on the ischemic myocardium, including reducing leukocyte-mediated cytokine production (32), superoxide (15) or nitric oxide (24) formation, or myeloperoxidase activity (32).

Fig. 1. Ischemic risk area as a percentage of left ventricular (LV) volume (A) and infarct size as a percentage of ischemic risk area (B) for rats in the five study groups. ○, Values for individual rats; ●, means ± SD for each group. FEMALE, untreated female rats; OVA, 8-wk ovariectomized females; OVA-EST, ovariectomized females after 6 wk of estrogen replacement; MALE, untreated male rats; MALE-EST, males after 6 wk of estrogen treatment.
reduced acutely by ischemic preconditioning (18, 21, 30) or its pharmacological equivalent (31). These same end points can be ameliorated in delayed fashion by stimuli that upregulate the expression of specific myocardial proteins [e.g., heat shock proteins (14) and superoxide dismutase (36)] associated with ischemic tolerance. In the present study, ovariectomy and estradiol treatment were used to create a wide physiological spectrum of gender-estrogenic status conditions in this model. Although we did not examine for specific transcriptional effects of ovariectomy or estrogen replacement, each experimental group’s hormonal status was maintained constant for 6 wk before experiments so that rats could reasonably be assumed to have achieved a steady state with respect to myocardial phenotype by the time of study. Measurements on the day of study furthermore confirmed that the various groups differed predictably in their circulating estradiol levels (Table 1), confirming the original expectation that estrogenic state would not affect this parameter in the collateral-deficient rat heart. The finding that, in this setting, there was no difference in ischemic or reperfusion dysrhythmia frequency or infarct size argues against the hypothesis that acute or short-term changes in estrogenic status produce major changes in myocardial ischemic tolerance by genomic or nongenomic mechanisms. Of course, it remains possible that more subtle changes might have been revealed with larger numbers of experiments. Also possible is that estrogen status may have been associated with differences in risk area size or risk area blood flow too subtle to have been demonstrated by the blue dye exclusion technique. However, considered together with evidence that estrogen may never improve the outcome from acute coronary occlusion in canines (15, 20, 24) and humans (1, 25) (two species with abundant coronary collaterals), the current results imply that, if indeed the high-estrogen state does confer a coronary outcome benefit independent of long-term effects on atherosclerosis, this is more likely attributable to effects on coronary arterial or coronary collateral tone and ischemic region blood flow (28).

Patients with established coronary atherosclerosis frequently experience brief coronary occlusions as a result of transient vasospasm and are at risk for more prolonged occlusion from thrombosis in situ. The main determinants of survival from such episodes are the propensity for reperfusion and ischemic ventricular dysrhythmias and the size of the resulting myocardial infarction. The results of this study demonstrate that a 6- to 8-wk period of estrogen withdrawal produced by surgical ovariectomy does not increase the incidence of dysrhythmias or myocardial infarct size in females undergoing a coronary occlusion, relative to normal or ovariectomized estrogen-replaced females. Estrogen supplementation of normal males is similarly without effect on these end points. These results imply that, in patients with established coronary atherosclerosis, short-term estrogen withdrawal, replacement, or supplementation would not be expected to significantly affect coronary mortality by direct effects on myocardial ischemic tolerance. These findings may partly explain the negative results of recent trials of short-term postmenopausal hormone replacement for secondary prevention of coronary death (13) and suggest that the relationship between estrogenic status and coronary mortality may instead be mediated by mechanisms operating over longer periods of time.

This work was supported by a Merit Review grant from the Department of Veterans Affairs.

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Received 8 January 1999; accepted in final form 25 October 1999.

REFERENCES


