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

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McNulty, Patrick H., Dinesh Jagasia, 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 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
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 17β-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
group control. Male rats were similarly randomized to either
6 wk of 17β-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
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 dysrhyth-
mas 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 tachycar-
dia or fibrillation upon reperfusion was noted by visual
inspection of the heart and was documented by electrocardi-
ographic recording.

In the infarct protocol, eight rats from each group under-
went a 45-min left coronary artery occlusion designed to
produce an intermediate-sized myocardial infarction. During
a coronary occlusion of this duration, ventricular dysrhyth-
mas 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. Sus-
tained 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 coro-
nary occlusion was released, and the ischemic region was
allowed to reperfuse for 3 h.

Analytical methods. Plasma unconjugated β-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 reoccluded, 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 thick-
ness. The five exposed surfaces thus created were photo-
graphed, and 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 Bonfer-
oni 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 infact 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 β-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 ovariecto-
mized, estrogen-replaced females, seven of eight norm-
al 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).

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

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Ovariectomized</th>
<th>Estrogen Male</th>
<th>Male, Estragon</th>
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<tbody>
<tr>
<td>β-Estradiol, pg/ml</td>
<td>285 ± 28</td>
<td>&lt;20*</td>
<td>345 ± 31</td>
<td>&lt;20*</td>
</tr>
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</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>Reperfusion dysrhythmias, % of rats</th>
<th>Female</th>
<th>Ovariectomized</th>
<th>Estrogen</th>
<th>Male, Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic dysrhythmias, % of rats</td>
<td>88</td>
<td>88</td>
<td>100</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>
</tr>
</tbody>
</table>

Table 2. Occurrence of reperfusion and ischemic ventricular dysrhythmias

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).

To date, the latter two hypotheses have been tested mainly by examining the effect of estrogenic status on the outcome from experimental coronary occlusion in canines. Short-term (2 wk) estradiol treatment was shown to ameliorate contractile dysfunction and to prevent reperfusion dysrhythmias after a 15-min coronary occlusion (15). Acute administration by either the intramuscular or intracoronary route similarly prevented ischemic (20, 24) and reperfusion (20, 24) dysrhythmias and reduced infarct size (24). Importantly, however, estrogen also increased distal coronary perfusion pressure during both ischemia and reperfusion (20), suggesting that these protective effects may actually be mediated not by changes in intrinsic myocardial ischemic tolerance but rather by modification of blood flow into the threatened myocardial region in the collateral-rich canine heart.

Previous studies in the intact rat coronary occlusion model have demonstrated that both ischemic and reperfusion dysrhythmia frequency and infarct size can be
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, ovarioectomy and estrogen treatment were used to create a wide physiological spectrum of gender-estrogenic state conditions in this model. Although we did not examine for specific transcriptional effects of ovarioectomy 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 at the time of experimental coronary occlusion (Table 1). Groups exhibited closely matched ischemic zone size (Fig. 1A), 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 nevertheless 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 ovarioectomy does not increase the incidence of dysrhythmias or myocardial infarct size in females undergoing a coronary occlusion, relative to normal or ovarioectomized 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.

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