Hemodynamic effects of acute and repeated exposure to raloxifene in ovariectomized sheep

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Zoma, Willie D., R. Scott Baker, John L. Mershon, and Kenneth E. Clark. Hemodynamic effects of acute and repeated exposure to raloxifene in ovariectomized sheep. Am J Physiol Heart Circ Physiol 291: H1216–H1225, 2006. First published March 3, 2006; doi:10.1152/ajpheart.00666.2005.—We hypothesize that administration of acute and daily doses of raloxifene will have significant effects on ovine coronary and uterine hemodynamics and that these changes are estrogen receptor dependent. Eleven ovariectomized sheep were instrumented to measure mean arterial pressure, heart rate (HR), cardiac output (CO), and coronary (CBF) and uterine artery blood flows (UBF). A dose-response curve was generated for raloxifene (1, 3, and 10 μg/kg) and compared with a standard dose of estradiol-17β (1 μg/kg) given intravenously. In a second group of animals, raloxifene (10 μg·kg−1·day−1) was administered intravenously for 14 consecutive days, and cardiovascular responses were compared with a group of animals administered estradiol-17β (10 μg/kg) daily for the same period. To determine whether raloxifene-related vascular responses were estrogen receptor (ER) mediated, the animals were pretreated with estrogen antagonist ICI-182,780 given intravenously. Finally, RT-PCR was performed to determine the presence of ERα and ERβ mRNA in ovine coronary and uterine vessels. Raloxifene increased CBF and UBF dose dependently with a parallel decrease in the associated vascular resistances. Acute cardiovascular responses to daily doses of raloxifene and estradiol-17β were sustainable. In contrast to estradiol-17β, which significantly increases CO by increasing HR but not stroke volume, raloxifene significantly increased stroke volume without a significant parallel increase in HR. ICI-182,780 abolished raloxifene-induced hemodynamic responses, and ERα and ERβ mRNA are present in both ovine coronary and uterine vessels. Hence, the hemodynamic effects of raloxifene are dose dependent, sustainable, and estrogen receptor mediated.

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For cardioprotection in postmenopausal women. It is also important for the cardiovascular effects of raloxifene to be evaluated because raloxifene is currently approved in the United States for prevention and treatment of postmenopausal osteoporosis (11). The initiation of the osteoporosis therapy may well go beyond menopause and mimic the study populations in WHI, a period characterized by a significant rise in cardiovascular diseases.

It appears that raloxifene may contribute to cardiovascular health by exerting positive effects on lipid profile (10) and vascular endothelium (12, 36, 38, 47). Both animal and human studies show that raloxifene decreases total and low-density lipoprotein cholesterol without any significant impact on high-density lipoprotein cholesterol and triglycerides (10). Homocysteine and C-reactive protein are independent risk factors for cardiovascular events. A reduction in homocysteine concentrations after treatment with either raloxifene or estrogens is well documented. In contrast to estrogens, which increase C-reactive protein, raloxifene has no significant influence on C-reactive protein (44). Although the mechanism by which raloxifene produces its effects in vascular tissues remains largely unknown, some evidence points to a direct action on cardiovascular tissues (36, 39).

Studies from our laboratory have shown that vaginally administered raloxifene increases both coronary and uterine blood flow in the sheep through a nitric oxide (NO)-dependent mechanism (47), a pathway that we have also demonstrated with estrogen (20, 41). Our in vivo studies in nonpregnant sheep agree with those of Ogita et al. (31) in which raloxifene produced coronary vasodilation in ischemic hearts in anesthetized dogs and those in vitro studies of Figtree et al. (12) that showed raloxifene acutely relaxes coronary artery rings from rabbits by NO-dependent mechanisms. Both of these studies (12, 31) showed immediate vasodilator responses to raloxifene within the first 5 min, whereas in our study in unanesthetized sheep (47) raloxifene took significantly longer to produce vasodilation. Because these studies (12, 31, 47) examined the acute effects of raloxifene, it remains unknown whether raloxifene-induced vasorelaxation is sustainable with daily or chronic administration. It is important to characterize the raloxifene-induced vasomotor response because it is not uncommon for vasoactive compounds to lose their therapeutic efficacy over time as a result of vasomotor tolerance (7, 19, 30). A sustainable vascular relaxation with repeated exposure to raloxifene combined with the lipid-lowering effects may translate clinically to cardioprotection.

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Studies from our laboratory and others have shown that estrogens increase NO synthesis in cardiovascular tissues and induce vasodilation (20, 26, 33, 41). The estrogen-induced vasodilation is mediated through a classical estrogen receptor (ER) pathway: transcriptional activation of NO synthase (NOS), the enzymes that synthesize NO (26). Recent studies also suggest a nongenomic mechanism involving rapid activation of endothelial NOS through an estrogen receptor and mitogen-activated protein kinase (6, 31). It is yet to be determined in vivo whether raloxifene-induced cardiovascular changes are solely estrogen receptor mediated as demonstrated in vitro earlier (12).

Two distinct estrogen receptors are currently known: ERα and ERβ. ERβ, the newest estrogen receptor, was identified and cloned from humans (29). Both ERα and ERβ are expressed in the rat, primate, and human vasculature (25, 32, 35). Recently, we have shown that ovine brain contains both ERα and ERβ (9). Estrogen receptors are nuclear receptors that transduce extracellular signals, resulting in transcriptional responses (35), and the levels of the two forms are tissue and species specific (14, 15, 25, 32, 35). The tissue-specific effects of estrogen and SERMs such as raloxifene may be because of the differential distribution of ERα and ERβ. ERα and ERβ have been recently identified in the ovine uterine vasculature (5, 6, 21) and in the ovine coronary endothelium (21). Therefore, the present study was designed to 1) establish a dose-response curve for the cardiovascular effects of intravenously administered raloxifene, 2) determine whether raloxifene directly dilates the uterine artery, 3) determine whether coronary and uterine vascular responses to raloxifene are sustainable and comparable to those of estradiol-17β, 4) determine whether raloxifene-related vasorelaxation can be blocked in vivo by the estrogen receptor antagonist ICI-182,780 (42, 43), as we and others have shown with estradiol-17β (22, 26), and finally 5) confirm the presence and types of estrogen receptors in the nonpregnant ovine coronary and uterine vasculature. We chose the ewe as an animal model for predicting cardiovascular responses to estrogenic compounds because human and ovine cardiovascular responses to hormone replacement therapy are similar (16).

Methods

Surgical Procedures

Eleven nonpregnant sheep of mixed breed weighing between 55 and 67 kg (60 ± 3) underwent a left lateral thoracotomy by previously described methods (20, 26, 47). Briefly, food and water were withheld 24 h before surgery, and ewes were sedated with pentobarbital sodium (15 mg/kg) before induction of general anesthesia with a combination of isoflurane (2–3%) and oxygen. Animals were placed on their right side. The left chest and shoulder were shaved, cleansed, and draped in a sterile fashion. An incision was made in the fourth intercostal space and developed into the pericardial cavity, and the pulmonary artery and left circumflex coronary artery were isolated and fitted with Doppler flow probes (24 and 4 mm, respectively; Transonic Systems, Ithaca, NY). A chest tube attached to a Hemovac was in place for drainage of any pleural collection, and the chest incision was closed in layers. Cables and the chest tube were exteriorized on the ewe’s left flank were shaved, cleansed, and draped under sterile conditions. The femoral artery and vein were cannulated after exposure via a 3- to 4-cm incision in the left groin, and catheters were advanced to the level of the distal aorta and vena cava, respectively. The abdomen was opened through a 10- to 15-cm midline incision, and ovarioectomy was performed to prevent fluctuations in estrogen. Both right and left uterine arteries in the broad ligament were fitted with 2- to 3-mm Doppler flow probes (Transonic Systems). The lateral branch of each uterine artery was cannulated with a polyvinyl catheter (0.040 × 0.070 in.) and advanced proximally to the bifurcation for the local administration of compounds to be tested. The abdomen was closed in layers, and the catheters and cables were exteriorized on the left flank through a subcutaneous tunnel. Catheters were wrapped in alcohol-soaked sponges placed in a canvas pouch. All catheters were flushed daily with heparin (1,000 U/ml) to maintain patency. Postoperative care was the same as described previously. Animals were allowed at least 1 wk to recover from surgery before initiation of experiments. All procedures described above were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati and were performed in an American Association for the Accreditation of Laboratory Animal Care- and United States Department of Agriculture-approved facility.

Four additional animals underwent the same preoperative, anesthetic, and postoperative procedures described above. In these animals, the femoral artery and vein were catheterized and animals underwent ovarioectomy as described above. These animals received a daily bolus of saline for 7 days and were then killed for tissue procurement to determine coronary and uterine artery mRNA expression of ERα and ERβ.

Physiological Measurements

Mean arterial pressure was measured with a Micron MP-15 blood pressure transducer connected to the femoral artery catheter and coupled to a SensorMedics cardiocachometer to measure heart rate. Cardiac output and left circumflex coronary blood flows were monitored with the pulmonary and left coronary flow probes, respectively. Uterine blood flow was measured via the flow probes on the right and left main uterine arteries. All measurements were recorded continuously on a SensorMedics R-612 (SensorMedics, Yorba Linda, CA) eight-channel physiological recorder. Vascular resistances were calculated as mean arterial pressure divided by the appropriate measured blood flow (not corrected for venous pressure) to derive coronary, uterine, and systemic vascular resistances.

Drug Preparations

Raloxifene (Eli Lilly, Indianapolis, IN) was dissolved in absolute ethyl alcohol (3 mg/ml), and then the required dose was diluted to a final volume of 5 ml with normal saline and administered intravenously immediately. Estradiol-17β (Sigma Chemical, St. Louis, MO) was prepared as a stock solution of 1 mg/ml in absolute ethyl alcohol and diluted immediately before intravenous administration with saline solution to a final volume of 5 ml. Identical vehicles for estradiol-17β and raloxifene (alcohol and saline solution) were also evaluated.

ICI-182,780 (Tocris, Baldwin, MO) is a high-affinity estrogen receptor antagonist commonly referred to as a pure anti-estrogen and was dissolved first in absolute ethanol before titrating the solution back to 30% ethanol with saline and was then administered intravenously immediately as a 10-min infusion, as described previously (26). Identical vehicles for ICI-182,780 were also tested.
Experimental Protocol

Mean systemic arterial pressure, heart rate, cardiac output, coronary blood flow, and uterine blood flow were measured continuously in all experiments. A baseline for all variables was recorded for 60 min before the administration of a test agent and for 6 h thereafter. Estradiol-17β was given intravenously via the femoral vein catheter. Initially, ewes received 1.0 μg/kg body wt of estradiol-17β bolus each day until cardiovascular responses were stable and reproducible (20, 26, 47) and were used as a comparison for raloxifene responses and with saline-alcohol controls. No experiments were initiated unless all variables had returned to baseline.

In the first set of experiments, six animals (n = 6) were used to generate a dose-response curve to 1, 3, and 10 μg/kg iv raloxifene. The compound was administered intravenously, and cardiovascular parameters were recorded continuously for 6 h and then recorded again at 24 h to determine whether cardiovascular parameters had returned to baseline.

The second set of experiments was designed to determine if raloxifene could act locally to dilate the local uterine vasculature (n = 3). A dose of 10 μg raloxifene was administered in a branch of the uterine artery via a uterine artery catheter, and the systemic and local cardiovascular responses were monitored continuously for 6 h and then again at 24 h.

In the third set of experiments, sheep received a bolus dose of 10 μg/kg raloxifene daily for 14 days (n = 5), and this group was compared with another group of ewes (n = 5) that received a 10 μg/kg−2 day−1 bolus of estradiol-17β intravenously daily for 14 days via the femoral vein. The basal through-peak cardiovascular responses were continuously recorded for 6 h on days 1, 7, and 14 and then again 24 h postdose to evaluate any residual cardiovascular responses in both groups.

In the fourth set of experiments (n = 6), the estrogen receptor antagonist ICI-182,780 was administered systemically to determine if it could block raloxifene-induced cardiovascular changes. The dose of ICI-182,780 was chosen based on previous work from our laboratory (26). After a 1-h baseline, ewes were treated with ICI-182,780 dissolved in 30% ethanol-saline solution infused at 10 μg·kg−1·min−1 for 10 min in the femoral vein catheter, which is advanced to the distal vena cava (total alcohol = 0.09 ml/min into average cardiac output of 5 l/min). Immediately thereafter, raloxifene (10 μg/kg) was administered via the femoral vein, and cardiovascular responses were recorded continuously for 6 h.

RNA Extraction and RT-PCR Reaction

The methods and primers used in the present study were identical to those of Deitch et al. (9). Briefly, total RNA was extracted with Trizol reagent (GIBCO-BRL) and underwent reverse transcription into cDNA (Superscript; GIBCO-BRL) with oligodeoxythymidine primer. PCR was optimized and carried out with 5 μl of the RT reaction for 35 cycles. Reactions for ERα, ERβ, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were carried out on each coronary and uterine artery mRNA sample. Gel electrophoresis with 1% agarose-ethidium bromide gel was used to identify the desired DNA product and was confirmed on the basis of the expected size and sequence.

Statistical Analysis

Data are reported as means of absolute values or mean percentage changes from baseline values ± SE. Data were normally distributed. Paired Student’s t-test was used to examine changes from baseline with Bonferroni correction applied where appropriate. The differences between groups and over time were analyzed using repeated-measures ANOVA. P < 0.05 was considered significant. Data were analyzed in the Department of Biostatistics at the University of Cincinnati with the use of SAS software.

RESULTS

Pharmacological Studies

Time course of coronary vasodilation. The time course of the estradiol-17β-related increase in coronary blood flow differed from the raloxifene-related response (Fig. 1). Whereas the estradiol-17β-related response began after a lag period of 30–45 min and peaked at 120 min, the raloxifene-related response began after 45–60 min and peaked at 3–5 h (Fig. 1). In these studies, coronary blood flow was increased significantly over baseline (Table 1). Both raloxifene and estradiol-17β showed an initial peak at 15 min followed by a much longer sustained peak at the later times.

Systemic Dose Response to Raloxifene

Raloxifene significantly increased coronary blood flow dose dependently by 9, 12, and 24% with a parallel significant decrease in coronary vascular resistance by 8, 15, and 23% from baseline (Table 1) at the doses of 1, 3, or 10 μg/kg body wt respectively (Fig. 2A). All responses were measured at the respective peaks (120 or 240 min). There were no changes in mean arterial pressure or heart rate related to the studied doses. At a dose of 1, 3, or 10 μg/kg body wt, raloxifene increased cardiac output over baseline (Table 1) by 7, 8, and 12% (Fig. 2B), with a parallel decrease in systemic vascular resistance by

Coronary Blood Flow

Fig. 1. Time course of coronary blood flow in %change in response to raloxifene (10 μg/kg) and estradiol-17β (10 μg/kg). Compounds were administered iv at time point 0. Both compounds increased coronary blood flow in the nonpregnant sheep (P ≤ 0.01 for both by paired t-test). The estradiol-17β response began earlier and peaked sooner than that of raloxifene (P ≤ 0.01 by ANOVA). Each point represents the mean ± SE for 5 raloxifene- and 5 estradiol-17β-treated animals.
6, 9, and 15%, respectively (Fig. 2B). Only the highest dose of raloxifene significantly lowered systemic vascular resistance. Cardiac output was increased significantly by estradiol-17β, whereas systemic vascular resistance was significantly decreased (Fig. 2B).

Uterine Vascular Responses to Raloxifene

Uterine vascular response to estradiol-17β began after a lag time of ~30 to 45 min and peaked at 120 min, producing the expected response of 250 –300 ml/min (Fig. 3A). Raloxifene required 45–60 min to begin the vasoilation and increased uterine blood flow dose dependently by 36, 160, and 294 ml/min from a baseline value of 17 ml/min, with a parallel decrease in uterine vascular resistance (not shown). Raloxifene-induced cardiovascular effects peaked between 4 and 5 h independent of the dose administered. All raloxifene- and estradiol-17β-induced changes returned to baseline within 24 h. There were no vehicle-related cardiovascular effects.

Local Uterine Artery Administration

To determine whether raloxifene acts directly on the uterine circulation, raloxifene (10 μg) was administered directly into one of the uterine arteries. Raloxifene elicited an increase in ipsilateral uterine blood flow from a baseline of ~12 ml/min to a peak of 75 ± 14 ml/min within 4 h (Fig. 3B) after an initial lag period of 45 min. There was no effect on contralateral uterine blood flow or any other measured cardiovascular parameters. Flows returned to baseline within 24 h.

Chronic Daily Administration

Mean arterial pressure. Neither raloxifene (10 μg·kg⁻¹·day⁻¹) nor estradiol-17β (10 μg·kg⁻¹·day⁻¹) altered systemic arterial blood pressure over the 14-day study period (not shown).

Heart rate. Raloxifene-treated animals displayed no changes in heart rate over the 14-day period. In contrast, estradiol-17β acted differently, consistently increasing heart rate over baseline by 18, 24, and 25% on days 1, 7, and 14, respectively (Fig. 4A). There was no significant time-related increase in heart rate in either raloxifene- or estradiol-17β-treated animals during the 14-day period.

Cardiac output. Estradiol-17β-treated animals increased cardiac output by 21, 17, and 22% on days 1, 7, and 14, respectively; raloxifene increased it by 15, 18, and 16% (Fig. 4B). These values were significant on days 7 and 14 for both compounds. Both compounds acted similarly, without displaying any significant time-related changes in cardiac output.

Table 1. Baseline parameters

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
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<tr>
<td>Mean arterial pressure, mmHg</td>
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</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>Systemic vascular resistance, mmHg·1⁻¹·min</td>
<td>11.8 ± 0.9</td>
</tr>
<tr>
<td>Stroke volume, ml/beat</td>
<td>56.8 ± 6.9</td>
</tr>
<tr>
<td>Circumflex blood flow, ml/min</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Coronary vascular resistance, mmHg·ml⁻¹·min</td>
<td>1.01 ± 0.23</td>
</tr>
<tr>
<td>Total uterine blood flow, ml/min</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Fig. 2. A: peak %change in coronary blood flow and coronary vascular resistance from baseline (Table 1). Treatment with 1, 3, and 10 μg/kg body wt iv raloxifene increased coronary blood flow by 9 ± 3% (P = 0.022), 12 ± 2% (P = 0.008), and 24 ± 4% (P = 0.005), respectively. Overall, there was a dose-dependent effect of raloxifene on coronary blood flow at 240 min (P = 0.021). There were also concomitant decreases in coronary vascular resistance of 8 ± 2% (P = 0.040), 15 ± 2% (P = 0.003), and 23 ± 3% (P = 0.002) in response to 1, 3, and 10 μg raloxifene/kg, respectively. Estradiol-17β increased coronary blood flow by 22 ± 5% (P = 0.006) while decreasing coronary vascular resistance by 22 ± 3% (P = 0.005). The response to estradiol-17β reached its maximum at 120 min. Each bar represents mean ± SE of the same 6 animals. *P ≤ 0.05 and **P ≤ 0.01 by paired t-test. B: peak %change in cardiac output and systemic vascular resistance from baseline (Table 1). Administration of 1, 3, and 10 μg/kg raloxifene iv resulted in a nonsignificantly increased cardiac output of 7 ± 5% (P = 0.308), 8 ± 5% (P = 0.278), and 12 ± 5% (P = 0.080), respectively. Systemic vascular resistances concomitantly decreased by 6 ± 5% (P = 0.379), 9 ± 4% (P = 0.093), and 15 ± 5% (P = 0.041) in response to 1, 3, and 10 μg raloxifene/kg, respectively. Raloxifene did not produce a dose-dependent effect on either of these measured parameters (P > 0.10). Estradiol-17β (1 μg/kg) increased cardiac output by 14 ± 3% (P = 0.021) with a concomitant decrease in systemic vascular resistance by 17 ± 4% (P = 0.012). These responses peaked at 120 min after estradiol-17β administration and 240 min after raloxifene. Each bar represents the mean ± SE of the same 6 animals. *P ≤ 0.05 by paired t-test.
respectively (Fig. 4C), estradiol-17β had no significant impact on stroke volume. There was no time-related change in stroke volume for either compound over the 14-day period.

**Coronary blood flow.** Whereas raloxifene increased coronary blood flow by 27, 19, and 15%, estradiol-17β increased it by 20, 27, and 27% on days 1, 7, and 14, respectively (Fig. 5). Neither raloxifene nor estradiol-17β produced any time-related change in coronary blood flow over the 14-day period. The onset of coronary response was variable among raloxifene-treated animals beginning at 1–2 h and peaking at 3–5 h. The estradiol-17β-related-response was consistent beginning at 30–45 min and peaking at 120 min.

**Coronary vascular resistance.** Coronary vascular resistance decreased in raloxifene-treated animals by 27, 17, and 17% and in estradiol-17β treated animals by 18, 23, and 22% on days 1, 7, and 14, respectively (Fig. 5). Similar to coronary blood flow, no time-related changes in coronary vascular resistance occurred in response to raloxifene or estradiol-17β during the 14-day study period.

**Uterine blood flow.** Uterine blood flow in raloxifene-treated animals increased by 285, 310, and 314 ml/min from an average baseline value of 19 ml/min, whereas estradiol-17β-treated animals increased by 327, 418, and 445 on days 1, 7, and 14, respectively, (Fig. 6). The time course of uterine blood flow response to raloxifene, estradiol-17β, or vehicle control in ml/min. Treatment with 1, 3, and 10 μg raloxifene/kg resulted in dose-dependent increases in uterine blood flow of 36 ± 9 (P = 0.012), 160 ± 47 (P = 0.005), and 294 ± 39 (P = 0.003) ml/min, respectively. Raloxifene produced a dose-dependent hemodynamic effect from hours 1 through 6 (P = 0.004). Uterine blood flow increased significantly in 1 μg/kg estradiol-17β-treated animals from a baseline of 17 ± 3 to 293 ± 52 ml/min (P = 0.008). Uterine vascular resistances decreased dose dependently and for each dose of raloxifene and estradiol-17β (P < 0.01 for all, not shown). The ethanol/saline vehicle control elicited no response. Each curve represents the mean ± SE of the same 6 animals.

**Systemic vascular resistance.** Raloxifene decreased systemic vascular resistance by 18, 16, and 18%, whereas estradiol-17β treatment decreased it by 18, 15, and 22% on days 1, 7, and 14, respectively (Fig. 4B). Similar to cardiac output, there were no time-related changes in systemic vascular resistance with either raloxifene or estradiol-17β.

**Stroke volume.** Whereas raloxifene increased calculated stroke volume by 13, 15, and 12% on days 1, 7, and 14, respectively (Fig. 4C), estradiol-17β had no significant impact on stroke volume. There was no time-related change in stroke volume for either compound over the 14-day period.

**Effect of Anti-estrogen ICI-182,780**

 Pretreatment with systemically administered ICI-182,780 abolished all raloxifene-induced changes in coronary and uterine blood flow (Fig. 7). The raloxifene-induced increases in cardiac output were eliminated completely, as were changes in systemic, coronary, and uterine vascular resistance (not shown). ICI-182,780 alone (Fig. 7) or its vehicle had no effect on baseline cardiovascular parameters in this study, as demonstrated by Mershon et al. (26).

**RT-PCR for Ovine ERα and ERβ mRNA in Coronary and Uterine Arteries**

Ovine coronary and uterine arteries expressed mRNA for both ERα and ERβ, confirming the work of Byers et al. (5) and Liao et al. (21). The signal for ERα was consistently stronger than that for ERβ in both types of arteries. ERα bands appeared at 598 base pairs (bp), ERβ at 410 bp, and GAPDH at 400 bp, all as expected.

**DISCUSSION**

The current study examined the cardiovascular effects of acute and daily doses of raloxifene in conscious, nonpregnant, ovariectomized ewes and compared the effects with those of estradiol-17β. The doses of raloxifene used in the study were within the therapeutic range (27) and induced cardiovascular changes dose dependently. To our knowledge, this is the first study that examined the relationships between doses and raloxifene-induced cardiovascular changes over a time course in
Fig. 5. Peak % change in coronary blood flow (top) and coronary vascular resistance (bottom) from baseline on days 1, 7, and 14 of treatment. Whereas raloxifene significantly increased coronary blood flow by 27% ($P = 0.001$), 19% ($P = 0.004$), and 15% ($P = 0.016$), estradiol-17β increased it by 20% ($P = 0.005$), 27% ($P = 0.014$), and 27% ($P = 0.004$) on days 1, 7, and 14, respectively. Although there appears to be a time-related decrease in coronary blood flow with raloxifene treatment, it was not significant ($P = 0.220$), and there were no observed time-related change in either parameter ($P = 0.726$). The time course profiles of both compounds are as described in the dose-response studies. Peak coronary vascular resistance decreased in raloxifene-treated animals by 27% ($P = 0.001$), 17% ($P = 0.001$), and 17% ($P = 0.011$), whereas estradiol-17β decreased it by 18% ($P = 0.009$), 23% ($P = 0.013$), and 22% ($P = 0.003$) on days 1, 7, and 14, respectively. Both compounds behaved similarly over the study period ($P = 0.265$), and there were no observed time-related changes in either parameter ($P = 0.586$ for all by ANOVA). Both raloxifene- and estradiol-17β-induced cardiovascular changes returned to baseline within 24 h.

Bars represent means ± SE for 5 raloxifene- and 5 estradiol-17β-treated animals. *$P \leq 0.05$ and **$P \leq 0.01$ by paired $t$-test.

Fig. 4. $A$: peak % change from baseline for heart rate on days 1, 7, and 14 of treatment. Raloxifene-treated animals showed no significant changes in heart rate on days 1, 7, and 14, respectively. In contrast, estradiol-17β significantly increased heart rate from baseline, peaking at 18% ($P = 0.034$), 24% ($P = 0.009$), and 25% ($P = 0.004$) on days 1, 7, and 14, respectively. There was no time-related change in heart rate with either compound ($P = 0.152$). Both compounds behaved similarly over the study period ($P = 0.186$) where there were no observed time-related changes for either compound. Bars represent means ± SE for 5 raloxifene- and 5 estradiol-17β-treated animals. *$P \leq 0.05$ and **$P \leq 0.01$ by paired $t$-test.

$B$: peak % change in cardiac output and systemic vascular resistance from baseline on days 1, 7, and 14 of treatment. Raloxifene increased cardiac output by 15% ($P = 0.176$), 18% ($P = 0.034$), and 16% ($P = 0.042$), whereas estradiol-17β-treated animals increased by 21% ($P = 0.053$), 17% ($P = 0.042$), and 22% ($P = 0.016$) on days 1, 7, and 14, respectively. Concomitantly, systemic vascular resistance decreased by 18% ($P = 0.056$), 16% ($P = 0.050$), and 18% ($P = 0.033$) in raloxifene-treated animals, whereas it decreased by 18% ($P = 0.031$), 15% ($P = 0.114$), and 22% ($P = 0.004$) in estradiol-17β-treated animals on days 1, 7, and 14, respectively. Both compounds behaved similarly over the study period ($P = 0.726$), and there were no observed time-related changes in either parameter ($P = 0.340$ for all by ANOVA).

$C$: peak % change in stroke volume from baseline on days 1, 7, and 14 of treatment. Raloxifene increased stroke volume by 13% ($P = 0.044$), 15% ($P = 0.146$), and 12% ($P = 0.034$). In contrast, estradiol-17β-treated animals were quite variable, being 3% ($P = 0.827$), −6% ($P = 0.291$), and 7% ($P = 0.308$) on days 1, 7, and 14, respectively. As a group, raloxifene stroke volume responses were significantly greater than those of estradiol-17β over the 14-day period ($P = 0.027$, by ANOVA). There were no time-related changes for either compound. Bars represent means ± SE for 5 raloxifene- and 5 estradiol-17β-treated animals. *$P \leq 0.05$ by paired $t$-test.
conscious, ovariectomized, nonpregnant sheep. This study also demonstrated for the first time that blockade of estrogen receptors with ICI-182,780 abolishes raloxifene-induced uterine and coronary vasodilation.

The effects of intravenously administered raloxifene on cardiovascular parameters (cardiac output, coronary blood flow, urinary blood flow, systemic vascular resistance, coronary vascular resistance, and urinary vascular resistance) differed from those related to vaginally administered raloxifene in their time lag and duration of action (47). The cardiovascular response to vaginally administered raloxifene occurred after a lag period of 6 h, and coronary blood flow was still elevated at 24 h. All raloxifene-induced cardiovascular changes in that study returned to baseline within 48 h. In contrast, the cardiovascular responses to intravenously administered raloxifene began after a lag period of 45 min, peaked at 300 min, and by 24 h all parameters had returned to baseline. The differences observed in the time lag and duration of action in the two studies may in part be explained by drug availability inherent in the two different routes of administration and perhaps in some part by the “first uterine pass effect” often associated with administration by the vaginal route. It has been shown that steroids can pass from the vagina to the uterus before increasing in the systemic circulation, thus leading to a uterine response (4). Both raloxifene- and estradiol-17β-induced coronary and uterine hemodynamic changes in the present study occurred over a 120- to 240-min period; this delayed response is suggestive of a genomic mechanism for vasodilation rather than a nongenomic action that occurs rapidly.

We also determined whether raloxifene exhibits vasomotor tolerance in vivo, a property that has not been adequately investigated to date. Unlike agents with vasomotor properties that exhibit tachyphylaxis (7, 19, 30), neither raloxifene nor estradiol-17β lost vasomotor response over the 2-wk treatment period. The maintenance of vasomotor response to raloxifene is reassuring and may be important therapeutically because a sustained coronary and uterine vasodilation is expected to promote better tissue nutrition and oxygenation. Whereas raloxifene-related coronary vasodilation and previously re-
ported positive effects on lipid profile may translate to cardioprotection, the increase in uterine blood flow may be beneficial in diseases the underlying pathology of which is characterized by uterine underperfusion.

Except for the difference in potency, there were no significant differences between estradiol-17β and raloxifene-induced changes on mean arterial pressure, systemic vascular resistance, coronary blood flow, coronary vascular resistance, and uterine blood flow. This suggests that raloxifene shares most of the hemodynamic benefits of estrogen. Cardiac output is a product of heart rate and stroke volume. Both estradiol-17β and raloxifene significantly increased cardiac output. Estradiol-17β-related increases in cardiac output were associated with a parallel increase in heart rate but without a significant change in stroke volume. In contrast to estradiol-17β, raloxifene significantly increased stroke volume without a parallel increase in heart rate. The ability of raloxifene to increase stroke volume may be because of its reported ability to increase fractional shortening in the heart (31). The ability of raloxifene to increase stroke volume without a parallel increase in heart rate suggests that raloxifene may be a better promoter of cardiac efficiency. To our knowledge, this is the first study to demonstrate this difference in stroke volume response between raloxifene and estradiol-17β.

Studies from our laboratory and others have shown that local administration of estradiol-17β in the uterine artery produces local uterine vasodilation that is mediated by NO (41) and that is estrogen receptor dependent (22). In the present study, we demonstrated for the first time that administration of raloxifene locally in one of the uterine arteries causes vasodilation and increases blood flow in the ipsilateral uterine artery without changes in the contralateral artery. The response occurred over several hours, suggesting a genomic response. We have previously shown that administration of estradiol-17β through an indwelling catheter in the ovine circumflex coronary artery produces significant localized and dose-dependent vasodilation without an increase in heart rate or cardiac output (2). The effects of intracoronary administration of raloxifene were not evaluated in the present study.

We have previously reported inhibition of the estradiol-17β-induced increase in coronary blood flow with ICI-182,780 (26), and others have reported inhibition of the estradiol-17β-related increase in uterine blood flow with ICI-182,780 in ovariectomized ewes (22). In the present study, we have demonstrated for the first time in vivo that systemic application of the pure estrogen receptor antagonist ICI-182,780 abolishes raloxifene-induced coronary, systemic, and uterine vasodilatation. Raloxifene-induced coronary vasodilation was completely blocked by ICI-182,780 in both the anesthetized dog (31) and isolated coronary artery vessels from rabbits (11), clearly demonstrating that raloxifene-induced cardiovascular changes are an estrogen receptor-mediated mechanism.

The expression of ERα and ERβ mRNA has been shown in several different tissues and species. Our study confirms the reports of Byers et al. (5) and Liao et al. (21) that showed the presence of ERα and ERβ mRNA in ovine coronary and uterine arteries. In our study, ERα was identified as the predominant estrogen receptor expressed at the mRNA level in both ovine coronary and uterine arteries. Currently, it is not clear whether raloxifene acts through ERα, ERβ, or both receptors to produce its hemodynamic effects in the uterine and coronary vasculatures.

The presence of ERα and ERβ receptors in human coronary vasculature has been reported (18, 32), and the mechanisms by which estrogen exerts its influence on blood vessels include upregulation of NOS (8, 20, 34, 45). Human vascular smooth muscle (coronary, iliac, and aorta) has higher levels of ERβ expression compared with ERα, and higher concentrations of ERβ have been reported in women compared with men (15). The higher levels of ERα mRNA in our animals may be due in part to the fact that these animals had been ovariectomized and thus not exposed to estrogen for 1 wk. A recent study suggests that ERβ may play a more important cardioprotective role compared with ERα (17). It remains unclear whether SERMs and heterogeneous estrogens currently approved for postmenopausal estrogen replacement therapy selectively bind to one of these two estrogen receptors in the coronary vascular bed. An understanding of the binding pattern of these compounds to ERα and the subtypes of ERβ may facilitate prediction of their hemodynamic influence in reproductive and nonreproductive tissues. Modulation of ERα and ERβ responses by raloxifene in the ovine model remains the focus of ongoing research in our laboratory.

In summary, the present study shows that the vascular relaxing effect of raloxifene in ovariectomized sheep is dose dependent and sustainable. The inhibition of raloxifene-related cardiovascular changes in vivo by pure estrogen receptor antagonist ICI-182,780 strongly suggests that raloxifene actions are estrogen receptor dependent. The clinical significance of our findings and those reported earlier on lipid profile, homocysteine, and C-reactive protein may not be known until the ongoing Raloxifene Use for The Heart trial is completed (28).

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DISCLOSURES
J. L. Mershon is currently a stockholder of Eli Lilly and Company.

REFERENCES
H1224 HEMODYNAMIC RESPONSES TO RALOXIFENE IN SHEEP


