Raloxifene relaxes rat intrarenal arteries by inhibiting Ca\(^{2+}\) influx

Fung Ping Leung, Xiaqiang Yao, Chi-Wai Lau, Wing-Hung Ko, Limin Lu, and Yu Huang

1Department of Physiology, Chinese University of Hong Kong, Hong Kong; and 2Department of Physiology and Pathophysiology, Fudan University Shanghai Medical College, Shanghai, China

Submitted 16 September 2004; accepted in final form 27 January 2005

Leung, Fung Ping, Xiaqiang Yao, Chi-Wai Lau, Wing-Hung Ko, Limin Lu, and Yu Huang. Raloxifene relaxes rat intrarenal arteries by inhibiting Ca\(^{2+}\) influx. *Am J Physiol Renal Physiol* 289: F137–F144, 2005. First published February 15, 2005; doi:10.1152/ajprenal.00353.2004.—Raloxifene may confer vascular benefits without causing estrogen-related side effects. However, its precise mechanism is unknown. Thus conventional hormone replacement therapy (HRT) is no longer suitable for prevention of cardiovascular diseases in healthy menopausal women (27). Despite this evidence, the results from recent randomized clinical trials of HRT for primary or secondary prevention of heart disease have found no overall therapeutic benefit (12, 20). Instead, HRT resulted in higher incidences of strokes, heart attacks, and thrombosis (20), although the precise mechanism is unknown. Thus conventional HRT is no longer suitable for prevention of cardiovascular disease (19). Therefore, in recent years, a surge of research has focused on more selective agents that retain beneficial properties of estrogen in bone, lipids, and the cardiovascular system but with antiestrogenic activity in the breast and uterus. Such unique compounds are categorized as selective estrogen receptor modulators (SERMs). SERMs manifest selective agonistic or antagonistic activities in a multitude of estrogen target tissues. Although some SERM members have been known for decades, their tissue specificity has only recently gained recognition.

Treatment with raloxifene, a second generation SERM in healthy postmenopausal women, enhanced flow-mediated vasodilatation (5, 21, 23, 24), increased plasma nitric oxide (NO) concentrations (21), and decreased plasma endothelin-1 levels (21). However, raloxifene therapy did not improve vascular function in postmenopausal women with coronary heart disease, whose arteries had been affected by advanced atherosclerosis (10). Raloxifene also reduced the expression of vascular cell adhesion molecule-1 in human endothelial cells (25), improved lipid profile (16, 32) and homeostatic parameters (2, 6), and lowered systemic blood pressure and arterial stiffness in postmenopausal women (7). Nongenomic signaling through estrogen receptors accounts for part of estrogen-mediated vascular actions in vitro. Raloxifene relaxed mammalian arteries (8, 28) and veins (3, 4) via both endothelium-dependent and -independent mechanisms. The former was inhibited by the classic estrogen receptor antagonist ICI-182, 780 (8), and the latter was due to direct inhibition of voltage-sensitive Ca\(^{2+}\) channels, which were insensitive to ICI-182, 780 (4, 28). Raloxifene therapy attenuated hypertension-associated endothelial dysfunction, and the underlying mechanisms may involve increased activity of endothelial nitric oxide (NO) synthase and a reduction in production of reactive oxygen species (33).

Both clinical and animal studies suggest that raloxifene and other SERMs have potentials as novel alternatives to estrogen for the treatment of menopause-related cardiovascular diseases (34). Chronic estrogen treatment augmented endothelium-dependent relaxation in perfused kidneys to a greater extent in ovariecotomized female compared with male rats (17). Acute administration of estrogen lowered the elevated renovascular tone in hypertensive female rats by enhancing NO-mediated relaxation with little effect on male rats (35). These observations suggest sex-specific acute effects of estrogen on renal arteries. However, it is yet to be determined whether SERMs could exert effects similar to estrogen on renal artery tone regulation. To this end, we investigated the effects of raloxifene on the tone of isolated rat intralobar renal arteries compared with that of exogenous estrogen. Thus we specifically examined whether raloxifene-induced vascular action involved 1) the endothelium, 2) estrogen receptors, 3) inhibition of Ca\(^{2+}\) influx through Ca\(^{2+}\) channels, or 4) a sex difference.
The second series of experiments examined the effects of raloxifene in rings contracted by elevated extracellular K⁺ to assess its ability to modulate Ca²⁺ influx via voltage-sensitive Ca²⁺ channels. Each ring was contracted twice with 80 mM K⁺ at 30-min intervals. Rings were washed three times in Ca²⁺-free, 80 mM K⁺ solution containing 30 μM Na²-EGTA, then incubated in Ca²⁺-free, 80 mM K⁺ solution (with or without raloxifene, 30-min incubation) before cumulative addition of CaCl₂. In some experiments, rings were treated with 10 μM ICI-182, 780 for 10 min before addition of raloxifene. The effect of nifedipine was tested as the control.

The last set of experiments tested the influence of nifedipine on raloxifene-induced relaxation in rings without endothelia. Addition of 10 nM nifedipine caused partial and sustained reduction (by 64.9 ± 4.8% in male rats and 71.6 ± 8.2% in female rats) of U-6619 (100 nM)-induced contractions, and raloxifene was then cumulatively applied to reduce the remaining tension in nifedipine-treated rings. Raloxifene was also examined in the control rings, but a lower concentration of U-6619 (20 nM) was used to produce vessel tone comparable to that in the presence of nifedipine (U-6619, base-evoked tension ± SEM: 28 ± 4.3 mN without nifedipine vs. 23 ± 2.8 mN with nifedipine in male rats; 30 ± 1.42 mN without nifedipine and 2.05 ± 1.06 mN with nifedipine in female rats, n = 4, P > 0.05).

Measurement of vascular smooth muscle calcium levels. Intracellular Ca²⁺ concentration ([Ca²⁺]i) was measured in fura 2-loaded artery rings without endothelia using the fluorescence ratio imaging. Rings were fluorescently labeled for 1 h by incubating them with 10 μM fura 2-AM and 0.025% Pluronic F-127 in Krebs solution at room temperature. Extracellular fura 2-AM was washed off in Krebs solution. Artery rings were then perfused with 20 min with Krebs solution (37°C) at a rate of 2 ml/min to allow cleavage of intracellular fura 2-AM into active fura 2 by esterases. Because of the photosensitivity of the fura 2 molecule, precautions were taken to avoid extensive photobleaching, and the excitation light was blocked by a shutter when no fluorescence measurement was recorded.

The basic [Ca²⁺]i imaging setup was modified from that described by Huang et al. (11). After fura 2 loading, each artery ring was cut open along its longitudinal axis and pinned onto a block of silicone elastomer (Sylgard) with the lumen side upward, which was fixed onto a base plate of the custom-made flow chamber. The base plate was then covered with a gasket and coverglass (24 × 32 mm, thickness no. 1; Menzel-Glaser, Braunschweig, Germany), and affixed by screws. There was a 1-mm gap between the vessel and coverglass to allow flow passage. This arrangement allowed free vessel movement in response to drug application. After vessel mounting, the flow chamber was placed on an inverted microscope and perfused with Krebs solution (37°C) at 2 ml/min, aided by a six-channel perfusion pump (205S; Watson-Marlow) and a custom-made basic minivalve, multichannel perfusion system.

The fura 2-loaded vessels were visualized through a Nikon CF Fluo ×20 objective (numerical aperture 0.45) on an inverted Nikon Eclipse TE300 microscope. The fura 2 was excited using a collimated beam of light from a 75-W xenon arc lamp and passed through a microscope photometer D-104 (Photon Technology International) that altered wavelengths from 340 to 380 nm using an optical chopper (OC-4000, Photon Technology International). The emitted light at 510 nm was collected by a photomultiplier tube. Instrument control, data acquisition, and analysis were performed using FELIX 1.21 software (Photon Technology International). Fluorescence intensities were recorded as a function of time.

After being mounted, the arterial tissues were allowed to recover for 30 min at 37°C and then exposed for 30 min to Ca²⁺-free, 80 mM K⁺ solution containing 30 μM Na²-EGTA. Thereafter, they were perfused with the same high-K⁺ solution supplemented with 0.1, 0.3, 1, and 3 mM CaCl₂. Tissues were then washed several times in Ca²⁺-free 80 mM K⁺ solution until baseline level was restored. Following 30 min-incubation with raloxifene (0.3–5 μM), cumulative perfusion of CaCl₂ induced a second concentration-dependent in-
increases in [Ca^{2+}]. The effect of 10 μM ICI-182, 780 was also tested on the raloxifene (1 μM)-induced inhibition of a rise in [Ca^{2+}].

Data analysis. Data are means ± SD of rings from n rats. Increases in contractile force were expressed as a percentage of the mean value of two consecutive responses to 80 mM K^+. Cumulative concentration-response curves were analyzed by nonlinear curve fitting using GraphPad software (version 3.0). The negative logarithm of the dilator (or constrictor) concentration that caused half (pD2 or pEC50) of the maximal response (E_max) was obtained. For statistical analysis, a two-tailed Student’s t-test or one-way analysis of variance followed by Bonferroni posttests. Statistical significance was accepted when P < 0.05.

RESULTS

Raloxifene-induced relaxation. Contraction with the thromboxane A2 mimetic U-46619 or 80 mM K^+ was insignificantly greater in male than in female arteries (4.5 ± 0.64 mN in female and 5.9 ± 0.53 mN in male, n = 8–10, for U-46619; 4.8 ± 0.73 mN in female and 6.3 ± 0.50 mN in male, n = 5–6 for high K^+, P > 0.05).

In U-46619-contracted rings with endothelia, raloxifene induced concentration-dependent relaxation with pD2 of 6.23 ± 0.14 (female) or 5.97 ± 0.17 (male), and the relaxation was unaltered on endothelial denudation (pD2: 6.22 ± 0.19, P > 0.05 for female, Fig. 1A and 5.94 ± 0.13, P > 0.05 for male, Fig. 1D). The relaxant effects of raloxifene in rings from both sexes were unchanged after exposure to L-NAME, CTX plus apamin (Fig. 1, B and E, Table 1), indomethacin (Fig. 1, C and F, Table 1), or ICI-182, 780 (Fig. 1, C and F, Table 1).

Although the relaxant effect of raloxifene on U-46619 pre-contraction was slightly more in female (pD2: 6.23 ± 0.14) than in male rings (pD2: 5.97 ± 0.17, P < 0.05, Fig. 2A), raloxifene was equally effective in relaxing 80 mM K^+-contracted rings from both sexes (pD2: 6.45 ± 0.11 in female and 6.45 ± 0.15 in male, P > 0.05, Fig. 2B). Similar to raloxifene-induced relaxation, 17β-estradiol-mediated relaxation of U-46619-contracted rings was independent of the endothelium (Fig. 2C, Table 1), and 17β-estradiol induced almost identical relaxation in 80 mM K^+-contracted rings without endothelia (Fig. 2D, Table 1). The threshold concentration for relaxation was lower for raloxifene (>10 nM) than for 17β-estradiol (≥100 nM) (Fig. 2). In a comparison of pD2 values, raloxifene was more effective than 17β-estradiol in relaxing renal arteries contracted by either U-46619 or 80 mM K^+ (Table 1). Nifedipine, an L-type Ca^{2+} channel blocker at 100 nM, abolished the high-K^+ contraction (n = 4, data not shown).

In U-46619-contracted rings, the contractility was largely restored after 1 μM raloxifene was repetitively washed out for 60 min (86 ± 4 and 89 ± 12% recovery for male and female rings, respectively, n = 4), whereas the relaxing effect of 1 μM raloxifene was repeatable (51.6 ± 7.8 and 44.8 ± 7.4% for the first and second relaxations, respectively, P > 0.05, n = 4) in male rings and (55.4 ± 7.4 and 44.5 ± 10.0% for the first and second relaxations, respectively, P > 0.05, n = 4) in female rings.

Fig. 1. Raloxifene-induced relaxations in female (A–C) and male (D–F) rat renal arteries. Concentration-response curves in rings with and without endothelia (Endo; A and D), in the presence of N^G-nitro-L-arginine methyl ester (l-NAME) or charybdotoxin (CTX) plus apamin (B and E), and in the presence of indomethacin or ICI-182, 780 (C and F) are shown. Values are means ± SD of 6–8 experiments.
Raloxifene inhibition of CaCl$_2$-induced contraction. In artery rings bathed in Ca$^{2+}$-free, 80 mM K$^+$ solution, cumulative additions of CaCl$_2$ induced contractions (pEC$_{50}$: 3.21$\pm$0.29 in female and 3.34$\pm$0.11 in male rings, $P<0.05$). Treatment with raloxifene (0.1–5 M) diminished contractions with a progressive reduction in the maximal contraction (Fig. 3A) without affecting the contractile sensitivity to CaCl$_2$ in female rings without endothelia (Table 2). Similarly, the similar inhibitory effects of raloxifene were obtained in male rings (Fig. 3C, Table 2). It appears that raloxifene at concentrations higher than 1 M was slightly more effective in suppressing CaCl$_2$-induced contractions in female than in male rings (Table 2). Treatment with 10 M ICI-182, 780 did not antagonize raloxifene (1 M)-induced inhibition of contractions to CaCl$_2$ in both female (Fig. 3B) and male (Fig. 3D) rings.

Influence of nifedipine on raloxifene-induced relaxation. To examine whether partial inhibition of L-type voltage-sensitive Ca$^{2+}$ channels could attenuate raloxifene-induced relaxation, rings were first contracted with U-46619 and then exposed to 10 nM nifedipine for 30 min before cumulative additions of raloxifene. Figure 4A shows that raloxifene-induced relaxation was significantly reduced in nifedipine-treated rings compared with control rings from both sexes. Figure 4B summarizes the pD$_{2}$ values for raloxifene-induced relaxation in the absence and presence of nifedipine.

### Table 1. pD$_{2}$ values for raloxifene- and 17β-estradiol-induced renal artery relaxation

<table>
<thead>
<tr>
<th></th>
<th>U-46619 Tone</th>
<th>80 mM K$^+$ Tone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Control (+ Endo)</td>
<td>6.23$\pm$0.14 (8)</td>
<td>5.97$\pm$0.17*(8)</td>
</tr>
<tr>
<td>l-NAME</td>
<td>6.44$\pm$0.22 (6)</td>
<td>5.83$\pm$0.15*(6)</td>
</tr>
<tr>
<td>CTX/apamin</td>
<td>6.35$\pm$0.10 (6)</td>
<td>5.95$\pm$0.15*(6)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6.11$\pm$0.36 (6)</td>
<td>5.84$\pm$0.29*(6)</td>
</tr>
<tr>
<td>Control (– Endo)</td>
<td>6.22$\pm$0.19 (8)</td>
<td>6.00$\pm$0.12*(6)</td>
</tr>
<tr>
<td>Control (+ Endo)</td>
<td>5.54$\pm$0.22† (5)</td>
<td>5.35$\pm$0.11† (5)</td>
</tr>
<tr>
<td>Control (– Endo)</td>
<td>5.52$\pm$0.20† (5)</td>
<td>5.07$\pm$0.31† (5)</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD with the no. of experiments in parentheses. Endo, endothelium; l-NAME, N$^G$-nitro-l-arginine methyl ester; CTX, charybdotoxin. *Significant difference between female and male rings under the identical treatment ($P<0.05$). †Significant difference between raloxifene and 17β-estradiol treatment groups ($P<0.05$).

Fig. 2. Comparison of relaxation induced by raloxifene (A and B) and 17β-estradiol (C and D) in female and male renal arteries. Relaxations in U-46619-contracted rings (A and C) and relaxations in 80 mM K$^+$-contracted rings (B and D) are shown. Values are means $\pm$ SD of 6 experiments.
treatment with raloxifene (0.3 and 5 μM). The cumulative addition of CaCl₂ caused stepwise increases in [Ca²⁺]ᵢ, and a 30-min treatment with raloxifene reduced [Ca²⁺]ᵢ elevation (Fig. 5, A and B). Similarly, raloxifene suppressed the CaCl₂-stimulated [Ca²⁺]ᵢ rise in male vessels without endothelium (Fig. 5, D and E). Treatment with 10 μM ICI-182, 780 did not influence the raloxifene (1 μM)-mediated inhibition of the CaCl₂-stimulated [Ca²⁺]ᵢ rise (Fig. 5, C and F). In control experiments, nifedipine at 100 nM abolished the CaCl₂-induced rise in [Ca²⁺]ᵢ (data not shown).

**DISCUSSION**

This study investigated the vascular effect of raloxifene in isolated rat intralobar renal arteries, with and without a functional endothelium. There are several new findings of this study. First, raloxifene- or 17β-estradiol-induced renovascular relaxation was independent of the presence of the endothelium, and raloxifene was approximately fivefold more effective than 17β-estradiol. Second, the raloxifene-induced acute effect was unrelated to ICI-182, 780-sensitive estrogen receptors. Third, raloxifene reduced CaCl₂-mediated contractions and inhibited Ca²⁺ influx probably through L-type Ca²⁺ channels, which were insensitive to ICI-182, 780. Last, there is little or no sex difference in renovascular responses to raloxifene or 17β-estradiol.

Raloxifene inhibited high-K⁺-induced contraction in rat renal arteries with similar effectiveness (estimated IC₅₀ of 355 nM) as observed in rat cerebral arteries (estimated IC₅₀ of 360 nM) (28). These results suggest that raloxifene exerts direct muscle relaxation, probably by functioning as a Ca²⁺ channel blocker. Indeed, raloxifene inhibited Ca²⁺ influx via Ca²⁺ channels that were also sensitive to the L-type Ca²⁺ channel blocker nifedipine, as revealed by [Ca²⁺]ᵢ measurement in fura 2-loaded arterial rings. The potency was similar for raloxifene between relaxing CaCl₂-induced tension and inhibiting the [Ca²⁺]ᵢ increases. Like the L-type Ca²⁺ channel blocker nifedipine, raloxifene at 5 μM nearly prevented CaCl₂-induced increases in both vessel tone and [Ca²⁺]ᵢ. Besides, there was a slight or no difference in either the potency or sensitivity of the raloxifene-induced effect in rings contracted by U-46619 or high K⁺. Finally, partial inhibition of L-type Ca²⁺ channels by nifedipine attenuated raloxifene-induced relaxation in U-46619-contracted rings from both sexes. These data indicate that inhibition of Ca²⁺ entry via L-type Ca²⁺ channels is likely to account for raloxifene-mediated renovascular relaxation. The present study has thus provided evidence showing that raloxifene, like nifedipine, may act as an antagonist of Ca²⁺ channels in renal artery smooth muscle cells. However, it is unclear how raloxifene may directly act on Ca²⁺ channels in VSM cells if its effect is not mediated by estrogen receptors. Estrogen was demonstrated to activate Ca²⁺-activated K⁺ channels by direct interaction with the β-subunit of the channel protein (29). It is yet to be elucidated whether the Ca²⁺ channel could provide such an interactive site for raloxifene. Besides, it remains to be examined whether raloxifene lowers [Ca²⁺]ᵢ, via other Ca²⁺-signaling pathways, e.g., stimulation of plasma membrane Ca²⁺-ATPase activity in VSM.

**Table 2. CaCl₂-induced contraction in control and in the presence of raloxifene in renal arteries**

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC⁵₀</td>
<td>Eₘₐₓ (%)</td>
<td>pEC⁵₀</td>
<td>Eₘₐₓ (%)</td>
<td></td>
</tr>
<tr>
<td>Raloxifene, μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.21±0.29</td>
<td>149.8±33.5</td>
<td>3.34±0.11</td>
<td>111.6±2.5</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2.95±0.16</td>
<td>121.3±13.2</td>
<td>3.12±0.18</td>
<td>93.4±11.6*</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>2.80±0.40</td>
<td>87.2±15.8*</td>
<td>2.85±0.27</td>
<td>85.2±11.2*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.87±0.22</td>
<td>39.2±6.3*</td>
<td>2.83±0.38</td>
<td>51.0±16.1*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.95±0.58</td>
<td>14.5±10.5*</td>
<td>2.64±0.40*</td>
<td>23.7±15.4*</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means ± SD of 5–6 experiments. Eₘₐₓ, maximal response.
**Significant difference between raloxifene-treated and control groups (P < 0.05).
The clinical importance of the endothelial NO pathway is well accepted. NO protects against the development of atherosclerosis (30), and selective endothelial dysfunction is believed to be an early and pathogenic event in atherosclerosis (18). Treatment with raloxifene augmented flow-mediated vasodilation (5, 21, 23, 24) in menopausal women probably through increasing NO production (21). Raloxifene acutely relaxed rabbit coronary arteries (8) and improved coronary perfusion in the ischemic rat heart (15) by involving NO, and it also triggered nontranscriptional signaling pathways, leading to stimulation of endothelial NO synthase in human endothelial cells (26). However, our study did not show involvement of the endothelium because the relaxant effects of raloxifene or 17β-estradiol were identical in rings with and without endothelium.

Inhibition of NO synthase by L-NAME did not influence relaxation. Furthermore, relaxation responses to raloxifene remained unchanged after treatment with CTX plus apamin or with indomethacin. Similarly, the endothelium did not contribute to estrogen- or raloxifene-mediated cerebrovascular relaxation (22, 28). Estrogen failed to affect NO release and NO synthase activity in nonpregnant ewe renal arteries (31). Last, acute treatment with raloxifene did not modify endothelial NO-dependent renal artery relaxation responses to acetylcholine (data not shown). Although chronic raloxifene treatment upregulated the NO function (21, 33), the present results suggest that endothelium-derived relaxing factors do not contribute to the acute relaxing responses to raloxifene, at least in rat renal arteries.

Fig. 4. Influence of nifedipine on raloxifene-induced renovascular relaxation. A: concentration-response curves for raloxifene in U-46619-contracted rings without endothelium in the absence and presence of 10 nM nifedipine. M, male; F, female; Nif, nifedipine. B: pD₂ values for raloxifene-induced relaxation described in A. Rings were first contracted using U-46619, then partially relaxed by nifedipine, and finally exposed to raloxifene. Values are means ± SD of 5 experiments. *P < 0.05, **P < 0.01 between control and nifedipine-treated rings.

Fig. 5. Effect of raloxifene (Rf) on CaCl₂-stimulated rises in intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) in arterial tissues without endothelia bathed in 80 mM K⁺ solution. Raloxifene (0.3 and 5 μM)-induced inhibition of [Ca²⁺]ᵢ increases in female (A and B) or male (D and E) renal artery tissues is shown. Lack of effect of 10 μM ICI-182, 780 on raloxifene (1 μM)-induced inhibition CaCl₂-stimulated rises in [Ca²⁺]ᵢ, in rings from female (C) and male (F) rats is also shown. Values are means ± SD of 4 experiments. Traces are average of 4 recordings from 4 animals. Statistical significance was tested by 2-way ANOVA between curves. **P < 0.01, ***P < 0.001 compared with control.
Sex differences in blood vessel tone described in humans or experimental animals are likely caused by direct vascular effects of sex hormones. The present results show that there was a slight sex difference in the relaxing effects of raloxifene in rings contracted by a receptor-dependent constrictor (U-46619) but not by a receptor-independent constrictor (high K\(^+\)). This small discrepancy may be caused by a slight difference in initial tension induced by U-46619 between male (5.9 ± 0.53 mM) and female (4.5 ± 0.64 mM) rings. In the same preparations, 17\(\beta\)-estradiol induced similar relaxation in the rings from both sexes. Similarly, in rabbit coronary arteries, the relaxation response to estrogen (13) or raloxifene (9) showed no sex difference.

Nongenomic effects of raloxifene may be mediated through activation of estrogen receptors. The selective estrogen-receptor antagonist ICI-182, 780 did not antagonize raloxifene-induced relaxation or raloxifene-induced inhibition of contraction and the VSM [Ca\(^{2+}\)], increase in rings from either sex exposed to CaCl\(_2\). In contrast, this blocker attenuated endothelium-dependent coronary artery relaxation to raloxifene (8) without affecting the endothelium-independent effect (8, 22, 28). ICI-182, 780 also inhibited raloxifene-stimulated NO formation in human endothelial cells (26). Similarly, ICI-182, 780 antagonized only the endothelium-dependent relaxation to tamoxifen, another SERM (9). Taken together, it appears that the raloxifene-induced endothelium-mediated effect involves ICI-182, 780-sensitive estrogen receptors, whereas the direct muscle-relaxing action is probably unrelated to classic estrogen receptor stimulation.

In conclusion, we have provided experimental evidence to show a principal mechanism by which raloxifene relaxes the rat intralobar renal arteries. Both raloxifene and 17\(\beta\)-estradiol induced similar relaxation in the rings from both sexes. Similarly, in rabbit coronary arteries, the relaxation response to estrogen (13) or raloxifene (9) showed no sex difference.

In conclusion, we have provided experimental evidence to show a principal mechanism by which raloxifene relaxes the rat intralobar renal arteries. Both raloxifene and 17\(\beta\)-estradiol relax renal arteries through inhibiting Ca\(^{2+}\) entry mechanisms, probably via L-type Ca\(^{2+}\) channels. This effect is independent of a functional endothelium or ICI-182, 780-sensitive estrogen receptors, and it is equivalent in both sexes. Raloxifene induced significantly greater renovascular relaxation than 17\(\beta\)-estradiol at submaximal concentrations. Similar observations were also made in rabbit coronary arteries (8). However, chronic and in vivo genomic effects of raloxifene may differ. Raloxifene is clinically used to treat menopausal women, but the present data show that raloxifene is equally effective in causing renovascular relaxation in both sexes. However, chronic and genomic action of raloxifene on renovascularization may differ. Long-term oral intake of 60 mg/day of raloxifene in women is expected to result in a mean maximal plasma concentration of 1.36 \(\mu\)g/l raloxifene (\(\approx\)2.7 nM) (14). In the present study, the minimal concentration of raloxifene needed to cause vasorelaxation is >10 nM.

The efficacy of raloxifene-induced action would be reduced in in vitro studies that exclude the involvement of circulating hormones and dilator factors. Therefore, renal effects of raloxifene deserve further study in vivo where interactions with other circulating factors or its genomic effects may enhance its vascular action.

REFERENCES


