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

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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 action on renal vascular circulation is unknown. This study aimed to examine the sex difference and roles of the endothelium and Ca\(^{2+}\) channels in rat renovascular relaxation to raloxifene. On isolated intralobar renal artery rings mounted in a myograph and contracted by U-46619, concentration-relaxation curves were constructed for raloxifene and contractions to CaCl\(_2\) were studied. Changes in intracellular Ca\(^{2+}\) concentration levels ([Ca\(^{2+}\)]\text{i}) of vascular smooth muscle (VSM) were measured by fura 2 fluorescence. Raloxifene or 17β-estradiol was equally effective in relaxing renal arteries from both sexes, with raloxifene being more potent than 17β-estradiol. Endothelial denudation did not affect raloxifene- or 17β-estradiol-induced relaxation. N\textsuperscript{\textcircled{-}}-nitro-l-arginine methyl ester, charybdootoxin plus apamin, indomethacin, or ICI-182, 780 did not modify the effect of raloxifene. Raloxifene caused similar relaxations in rings contracted by U-46619 and high K\textsuperscript{+}. Nifedipine attenuated the potency of raloxifene. Raloxifene reduced CaCl\(_2\)-induced contractions. K\textsuperscript{+} (80 mM) stimulated an increase in VSM [Ca\(^{2+}\)]\text{i}, and raloxifene attenuated this effect. Raloxifene-induced reduction of contraction and increase in VSM [Ca\(^{2+}\)]\text{i} was insensitive to ICI-182, 780. In summary, raloxifene causes relaxation in rat renal arteries; this effect is independent of a functional endothelium and is not mediated by ICI 182, 780-sensitive estrogen receptors. Raloxifene inhibited both contractions and VSM [Ca\(^{2+}\)]\text{i} in response to CaCl\(_2\), indicating that raloxifene relaxes rat renal arteries primarily through inhibiting Ca\(^{2+}\) influx via Ca\(^{2+}\) channels. There is little sex difference in raloxifene-induced relaxation.

17β-estradiol; relaxation; rat renal artery

SEX-RELATED DIFFERENCES in the risk for cardiovascular diseases are generally recognized (1). In pooled analysis, observational studies report that hormone replacement therapy (HRT) reduced the primary risk of cardiovascular disease 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 ovariectomized 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.
Methods

Chemicals. Acetylcholine, U-46619, N\(^{\text{2}}\)-nitro-L-arginine methyl ester (l-NAME), indomethacin, charybdotoxin (CTX), apamin, nifedipine, and 1β,estradiol were purchased from Sigma (St. Louis, MO). ICI-182, 780 was purchased from Tocris. Raloxifene was a gift from Lilly Corporate Center (Indianapolis, IN). U-46619, raloxifene, 17β-estradiol, indomethacin, and nifedipine were dissolved in DMSO and others in distilled water. Further dilution was made from a stock solution.

Blood vessel preparation. This study was approved by the Experimental Animal Ethics Committee at the Chinese University of Hong Kong. Sprague-Dawley rats of both sexes (~300 g) were killed by cervical dislocation. After the abdominal cavity was opened, the kidneys were removed and placed in ice-cold Krebs solution (in mM): 119 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1 MgCl\(_2\), 25 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), and 11 t-glucose. The intralobar renal arteries (mean external diameter of 366 μm) were dissected from both kidneys, and each artery was cleaned of adhering fatty tissues and cut into two ring segments, ~2 mm in length. Each segment was mounted in a Multi Myograph System (Danish Myo Technology, Aarhus, Denmark), and changes in arterial tone were recorded. Briefly, two tungsten wires (each 40 μm in diameter) were inserted through the segment’s lumen, and each wire was fixed to the jaws of a myograph. The organ chamber was filled with 5 ml Krebs solution and oxygenated with a 95% O\(_2\)-5% CO\(_2\) gas mixture. Krebs solution in the chamber was maintained at 37°C using a built-in heat-exchanger device to give a pH value of between 7.3 and 7.5. Each ring was stretched initially to 2 mN, an optimal tension, and then allowed to stabilize at this baseline tone for 90 min before the start of each experiment. Each experiment was conducted using rings from different rats. In total, this study used 43 rings from different rats. In 30-min incubations using a high-K\(_{\text{a}}\) solution supplemented with 0.1, 0.3, 1, and 3 mM CaCl\(_2\), the rings were exposed to Ca\(^{2+}\)-free, 80 mM K\(^{+}\) solution containing 30 μM N\(_2\)-EGTA, then incubated in Ca\(^{2+}\)-free, 80 mM K\(^{+}\) solution (with or without raloxifene, 30-min incubation) before cumulative addition of CaCl\(_2\). 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-46619 (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-evoked tension 3.28 ± 0.56 mN with nifedipine in male rats; 3.03 ± 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 Ca\(^{2+}\) concentration ([Ca\(^{2+}\)_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 for 20 min with Krebs solution (37°C) at a rate of 2 ml/min to allow clearance 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 excessive photobleaching, and the excitation light was blocked when no fluorescence measurement was recorded.

The basic [Ca\(^{2+}\)_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, Dow Corning, Midland, MI) 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 glass 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 Fluor×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 Ca\(^{2+}\)-free, 80 mM K\(^{+}\) solution containing 30 μM Na\(_2\)-EGTA. Thereafter, they were perfused with the same high-K\(^{+}\) solution supplemented with 0.1, 0.3, 1, and 3 mM CaCl\(_2\). Tissues were then washed several times in Ca\(^{2+}\)-free 80 mM K\(^{+}\) solution until baseline level was restored. Following 30 min-incubation with raloxifene (0.3–5 μM), cumulative perfusion of CaCl\(_2\) induced a second concentration-dependent in-
creases in [Ca\(^{2+}\)]. The effect of 10 \(\mu\)M ICI-182, 780 was also tested on the raloxifene (1 \(\mu\)M)-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 (p\(D_2\) or pEC\(_{50}\)) of the maximal response (\(E_{\text{max}}\)) 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 A\(_2\) 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 p\(D_2\) of 6.23 ± 0.14 (female) or 5.97 ± 0.17 (male), and the relaxation was unaltered on endothelial denudation (p\(D_2\): 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 (p\(D_2\): 6.23 ± 0.14) than in male rings (p\(D_2\): 5.97 ± 0.17, \(P < 0.05\), Fig. 2A), raloxifene was equally effective in relaxing 80 mM K\(^+\)-contracted rings from both sexes (p\(D_2\): 6.45 ± 0.11 in female and 6.45 ± 0.15 in male, \(P > 0.05\), Fig. 2B). Similar to raloxifene-induced relaxation, 17\(\beta\)-estradiol-mediated relaxation of U-46619-contracted rings was independent of the endothelium (Fig. 2C, Table 1), and 17\(\beta\)-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 17\(\beta\)-estradiol (>100 nM) (Fig. 2). In a comparison of p\(D_2\) values, raloxifene was more effective than 17\(\beta\)-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 \(\mu\)M 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 \(\mu\)M 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.
Raloxifene inhibition of CaCl2-induced contraction. In artery rings bathed in Ca2+-free, 80 mM K+ solution, cumulative additions of CaCl2 induced contractions (pEC50: 3.21 ± 0.29 in female and 3.34 ± 0.11 in male rings, P < 0.05). Treatment with raloxifene (0.1–5 μM) diminished contractions with a progressive reduction in the maximal contraction (Fig. 3A) without affecting the contractile sensitivity to CaCl2 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 μM was slightly more effective in suppressing CaCl2-induced contractions in female than in male rings (Table 2). Treatment with 10 μM ICI-182, 780 did not antagonize raloxifene (1 μM)-induced inhibition of contractions to CaCl2 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 Ca2+ 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 pD2 values for raloxifene-induced relaxation in the absence and presence of nifedipine.

Raloxifene inhibition of high K+-stimulated rise in vascular smooth muscle [Ca2+]. To ascertain that raloxifene relaxes renal arteries principally by inhibiting Ca2+ influx through L-type Ca2+ channels as suggested by tension measurement, the effect of raloxifene on vascular smooth muscle (VSM) [Ca2+]i was examined in arterial tissues without endothelia. CaCl2 induced a rise in VSM [Ca2+]i in Ca2+-free, 80 mM K+ solution, and the first and second concentration-dependent responses were similar. Traces in Fig. 5, A and B, show changes in VSM [Ca2+]i, measured as the fluorescence ratio in female renal vessels in response to CaCl2 in 80 mM K+ solution before and after...
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Fig. 3. Effects of raloxifene on CaCl2-induced contraction in male rat renal arteries in 80 mM K+ solution. Concentration-response curves in the absence and presence of different concentrations of raloxifene in rings without endothelium from female (A) and male rats (C) are shown. Lack of effect of 10 μM ICI-182, 780 (ICI) on raloxifene (1 μM)-induced inhibition of CaCl2-induced constriction in rings from female (B) and male rats (D) is also shown. Values are means ± SD of 5–6 experiments. Statistical significance was tested by 2-way ANOVA between curves. *P < 0.05 and ***P < 0.01 compared with control (without raloxifene treatment).

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

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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 endothelia. 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 mN) and female (4.5 ± 0.64 mN) rings. In the same preparations, 17β-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 [Ca2+]i increase in rings from either sex exposed to CaCl2. 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 17β-estradiol relax renal arteries through inhibiting Ca2+ entry mechanisms, probably via L-type Ca2+ 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 17β-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 μg/l raloxifene (≈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.

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