Raloxifene relaxes porcine coronary arteries via blockade of L-type calcium channels. Involvement of the p38 MAPK pathway

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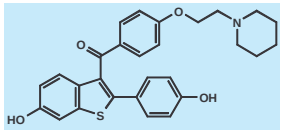
Introduction

Selective estrogen receptor modulators (SERMs) such as raloxifene interact with estrogen receptors, behaving as agonists in bone and vascular tissue or antagonists in mammary gland and uterus [1]. It has not been completely clarified whether raloxifene exert vasorelaxant effects similar to those induced by estrogen. In the present study we investigated the vasoactive responses to raloxifene in rings of isolated porcine coronary arteries (PCAs).



Raloxifene

Methods

Rings of PCAs with and without endothelium were mounted isometrically under an initial tension of 20 mN in organ baths filled with Krebs-Henseleit solution continuously aerated with 95% O_2/5% CO_2 (pH 7.4). To inhibit vascular eicosanoid production by cyclooxygenase, experiments were conducted in the continuous presence of indomethacin (6 µM). Following an equilibration period of 1 h, tissues were stimulated once with KCl (30 mM) and twice with $PGF_{2\alpha}$ (3 µM) or KCl (30 mM). The presence or absence of endothelium was assessed functionally by measuring the extent of endothelium-dependent relaxation following application of substance P (10 nM).

After an incubation period of 1 h, the rings were contracted by KCl (30 mM) or $PGF_{2\alpha}$ (3 µM). When the contractile response had reached a plateau, the rings were relaxed by raloxifene. Antagonists were incubated 1 h before the contraction with KCl or $PGF_{2\alpha}$. SB 203580 (10 µM) and PD 98059 (10 µM) were incubated 30 minutes before the contraction with $PGF_{2\alpha}$. BAPTA-AM (10-20 µM) was added when the contractile response to $PGF_{2\alpha}$ had reached the plateau. After an incubation period of 30 minutes the relaxation with raloxifene followed.

In other experiments, a transient contraction was induced by caffeine (10 mM). After incubation of raloxifene (3 µM) for 30 min, a second contraction to caffeine (10 mM) was established in each arterial ring.

In separate experiments, cumulative concentration-response curves to $CaCl_2$ were established in Ca^{2+}-free, high K^+ (60 mM) depolarizing Krebs-Henseleit solution [2] in the absence and presence of raloxifene, (*S*)-(–)-Bay K 8644, and (*S*)-(–)-Bay K 8644 plus raloxifene.

References

[1] Snyder, KR et al. (2000). Raloxifene hydrochloride. Am. J. Health-Syst. Pharm. 57:1669-1675.

[2] Ebeigbe, AB et al. (1988). Effects of calcium channel blockade in canine saphenous veins after storage at –190°C. Br. J. Pharmacol. 94:381-388.

Results

If vascular rings were precontracted with KCl (30 mM) or $PGF_{2\alpha}$ (3 µM), raloxifene (0.1–3 µM) elicited relaxation in an endothelium-independent manner (Fig. 1).

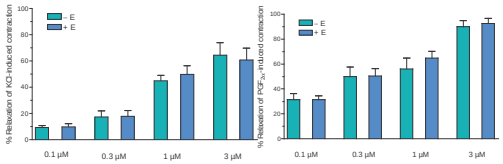


Fig. 1 Relaxant responses to raloxifene (0.1-3 µM) after 120 min in KCl-precontracted PCAs (*left panel*) and $PGF_{2\alpha}$-precontracted PCAs (*right panel*) with (+E) and without (–E) endothelium. Data are mean values ± SEM from 4 animals.

The estrogen receptor antagonist ICI 182,780 (10 µM) failed to inhibit the relaxant response to raloxifene (1 µM) (Fig. 2).

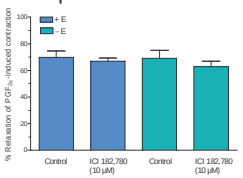


Fig. 2 Effect of incubation (60 min) with ICI 182,780 (10 µM) on raloxifene (1 µM)-induced relaxation (120 min) in PCAs with (+E) and without (–E) endothelium. Data are mean values ± SEM from 4 animals.

SB 203589 (10 µM), an inhibitor of p38 MAPK, diminished raloxifene-induced relaxation in endothelium-denuded vessels (–E), whereas the ERK1/2 kinase kinase inhibitor PD 98059 (10 µM) was ineffective (Fig. 3).

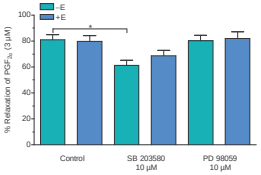


Fig. 3 Effect of incubation (30 min) with SB 203580 (10 µM) or PD 98059 (10 µM) on raloxifene (3 µM)-induced relaxation (120 min) in PCAs with (+E) and without (–E) endothelium. Data are mean values ± SEM from 6 animals. **P*<0.05.

Preincubation (60 min) of vascular rings with raloxifene (1–3 µM) diminished the contractile response to KCl (30 mM) and $PGF_{2\alpha}$ (3 µM), respectively (Fig. 4).

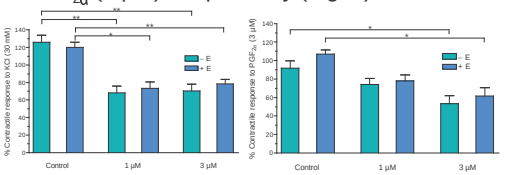


Fig. 4 Inhibition of the contractile response to KCl (*left panel*) and $PGF_{2\alpha}$ (*right panel*) by raloxifene in PCAs with (+E) and without (–E) endothelium. Data are mean values ± SEM from 4 - 10 animals. **P* < 0.01. ***P* < 0.001.

Caffeine (10 mM) which activates Ca^{2+}-release from intracellular stores caused a transient contraction in PCAs that was not inhibited by raloxifene (3 µM; Fig. 5).

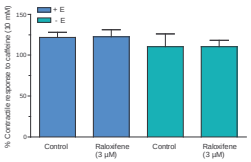


Fig. 5 Transient contractions induced by caffeine (10 mM) in the absence and presence of raloxifene (3 µM) in PCAs with (+E) and without (–E) endothelium. Data are mean values ± SEM from 4 animals.

Raloxifene (3 µM)-induced relaxation was not influenced by the intracellular calcium chelator BAPTA-AM (10-20 µM; Fig. 6).

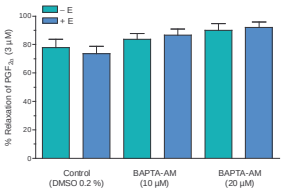


Fig. 6 Effect of incubation (30 min) with BAPTA-AM on raloxifene (3 µM)-induced relaxation (120 min) in PCAs with (+E) and without (–E) endothelium. Data are mean values ± SEM from 6 animals.

Calcium-induced contractions in K^+-depolarized arteries were concentration-dependently inhibited by raloxifene (0.3–3 µM) (Fig. 7, *left panel*). The inhibition by raloxifene (1–3 µM) was not affected by ICI 182,780 (1–10 µM) (data not shown).

If arterial rings were incubated with the L-type Ca^{2+}-channel activator (*S*)-(–)-Bay K 8644 (0.1 µM), cumulative concentration-response curves to Ca^{2+} were shifted to the left. Raloxifene (0.3–3 µM) inhibited the effect of (*S*)-(–)-Bay K 8644 (0.1 µM) in a concentration-dependent manner (Fig. 7, *right panel*).

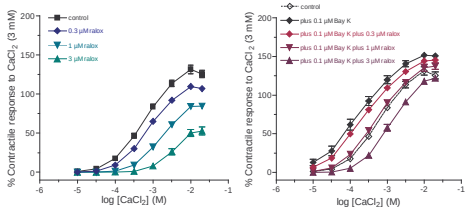


Fig. 7 Inhibition of the contractile response to $CaCl_2$ by raloxifene (*left panel*) and to $CaCl_2$ after (*S*)-(–)-Bay K 8644 incubation (*right panel*) in PCAs without (–E) endothelium. Data are mean values ± SEM from 3 - 6 animals.

Conclusions

- Raloxifene has an inhibitory effect on voltage-gated and receptor-operated L-type calcium channels in PCAs, thus achieving vascular relaxation independent of the endothelium
- Raloxifene does not inhibit the release of Ca^{2+} from intracellular stores
- ICI 182,780-sensitive estrogen receptors are not involved in the vasorelaxant response to raloxifene in PCAs
- p38 MAPK seems to be involved in the relaxant response to raloxifene

Acknowledgements

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