2016-BJP-0574-RP.R1

Online Supporting information

Role of inducible nitric oxide synthase in endothelium-independent relaxation to raloxifene in rat aortas

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Running title: Inducible nitric oxide synthase in raloxifene-induced vasorelaxation

FIGURE LEGENDS FOR ONLINE SUPPORTING INFORMATION

Figure S1. A, Concentration-dependent inhibition of U46619-induced contractions in endothelium-intact rat aortic rings by raloxifene (Rf) (n=6-8). B, pEC_{50} values for U46619-induced contraction in the absence and presence of raloxifene (0.1-10 μM, n=6-8). C, Similar inhibitory effects of 1 μM raloxifene on U46619-contracted endothelium-intact (+Endo) and –denuded aortic rings (-Endo) (n=7-9). D, pEC_{50} values obtained in (C). Concentration-response curves for U46619-induced contractile responses in rat endothelium-intact aortic rings. Raloxifene reduced the U46619 contraction in the absence and presence of 100 μM L-NAME (E) or 3 μM ODQ (G). Results are mean ± SEM of 6-9 rings from different rats. F & H, pEC_{50} values obtained in E & G. Contractions were normalized as percentage of 60 mM K^+-induced tension. Statistical differences are indicated by between different treatment groups (*

$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). NS indicates no significant difference.

Figure S2. The steady-state maximum relaxation to raloxifene (Rf, 1 μM) in the U46619-contracted endothelium-intact (+endo) and endothelium-denuded (-endo) aortic rings. Statistical differences ($P < 0.001$) are indicated by [a] between vehicle control and raloxifene group in endothelium-intact rings, and [b] between vehicle control and raloxifene group in endothelium-denuded rings. Results are mean ± SEM of 6-7 rings from different rats.

Figure S3. A, Recordings showing time-dependent relaxant responses to 10 μg/ml LPS in endothelium-denuded aortic rings in control and in the presence of 1 μM ICI 182,780. B, No inhibitory effects of ICI 182,780 on LPS-induced aortic relaxation. C, The maximal relaxant effect of LPS in the absence and presence of ICI 182,780. Statistical differences are indicated by ** ($P < 0.01$) or *** ($P < 0.001$) between vehicle control and treatment groups. Results are mean ± SEM of 6 rings from different rats. NS indicates no significant difference.

Figure S4. A, Recordings showing time-dependent relaxant responses to 10 μg/ml LPS in endothelium-denuded aortic rings in control and in the presence of 3 μM PDTC. B, Inhibitory effects of PDTC on LPS-induced aortic relaxation. C, The maximal relaxant effect of LPS in the absence and presence of PDTC. Statistical differences are indicated by *** ($P < 0.001$) between vehicle control and LPS group, and [a] ($P < 0.05$) between LPS and treatment group. Results are mean ± SEM of 6 rings from different rats.

Figure S5. A, Inhibitory effects of 100 μM L-NAME or 100 μM AMT-HCl on LPS (10 μg/ml)-induced relaxation. B, Inhibitory effect of 10 μM actinomycin D or 10 μM cycloheximide on LPS-induced relaxation. C, The maximal relaxant effect of LPS in the absence and presence of L-NAME, AMT-HCl, actinomycin D and cycloheximide. Statistical differences are indicated by *** ($P < 0.001$) between vehicle control and LPS group, and [a] ($P < 0.05$) between LPS and other treatment groups. These experiments were performed on endothelium-denuded aortic rings. Results are mean ± SEM of 5-6 rings from different rats.

Figure S6. Comparison of relaxations induced by 1 μM raloxifene (A) and 100 nM SNP (B) in endothelium-denuded aortic rings following different pharmacological treatments. Statistical differences are indicated by * ($P < 0.001$) between control and treatment groups. Results are mean ± SEM of 6-11 rings from different rats.

Figure S7. A, The time-course for the relaxant response induced by raloxifene (1 μM), 17β-estradiol (E_2, 1 and 10 μM) and tamoxifen (Tam, 1 and 10 μM) in the U46619-contracted endothelium-denuded aortic rings. B, The steady-state maximum relaxation to raloxifene, 17β-estradiol and tamoxifen. Statistical differences are indicated by between vehicle control and other treatment groups (*** $P < 0.001$). Results are mean ± SEM of 5-6 rings from different rats. NS indicates no significant difference.

Figure S8. A, Inhibitory effects of 100 μM AMT-HCl or 10 μM cycloheximide on 17β-estradiol (10 μM)-induced relaxation in the U46619-contracted endothelium-denuded aortic rings. B, The maximal relaxant effect of 17β-estradiol in the absence and presence of AMT-HCl and cycloheximide. C, Inhibitory effect of 100 μM AMT-HCl or 10 μM cycloheximide on tamoxifen (10 μM)-induced relaxation. D, The maximal relaxant effect of tamoxifen in the absence and presence of AMT-HCl and cycloheximide. Statistical differences are indicated by *** ($P < 0.001$) between vehicle control and 17β-estradiol or tamoxifen group, and ^{a} ($P < 0.05$) between 17β-estradiol or tamoxifen and other treatment groups. Results are mean ± SEM of 5-6 rings from different rats.