Supplementary material

Pentoxifylline as a therapeutic option for preeclampsia: a study on its placental effects.

Michelle Broekhuizen[1,2,3], Rene de Vries[2], Marja AW Smits[4], Willem A Dik[4], Sam Schoenmakers[5], Birgit CP Koch[6], Daphne Merkus[3], Irwin KM Reiss[1], AH Jan Danser[2], Sinno HP Simons[1], Emilie Hitzerd[1,2]

[1] Division of Neonatology, department of Paediatrics, Erasmus University Medical Center, Rotterdam, the Netherlands;
[2] Division of Pharmacology and Vascular Medicine, Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands;
[3] Division of Experimental Cardiology, department of Cardiology, Erasmus University Medical Center, Rotterdam, the Netherlands;
[4] Laboratory Medical Immunology, Department of Immunology, Erasmus MC University Medical Center, Rotterdam, the Netherlands;
[5] Department of Obstetrics and Gynaecology, Erasmus University Medical Center, Rotterdam, the Netherlands;
[6] Department of Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands;

Table S1. Summary table of the compounds used for wire myography experiments.

Compound	CAS-number	Vender	Concentration
Agonists			
U46619 (9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$)	56985-40-1	Sigma Aldrich	10 nmol l^{-1} – 30 nmol l^{-1}
Pentoxifylline (Trental®)			1 nmol l^{-1} – 300 µmol l^{-1}
M1 ((±)-Lisofylline)	6493-06-7	Cayman Chemical Company	1 nmol l^{-1} – 100 µmol l^{-1}
M4 (1-(3'-Carboxypropyl)-3,7-dimethylxanthine)	6493-07-8	Chemodex	1 nmol l^{-1} – 100 µmol l^{-1}
M5 (1-(4'-Carboxybutyl)-3,7-dimethylxanthine)	38975-44-9	Chemodex	1 nmol l^{-1} - 100 µmol l^{-1}
SNP (sodium nitroprusside)	13755-38-9	Sigma Aldrich	1 nmol l^{-1} – 100 µmol l^{-1}
Forskolin	66575-29-9	Sigma Aldrich	1 nmol l^{-1} – 30 µmol l^{-1}
BAY 60-2770	1027642-43-8	Bayer	0.1 nmol l^{-1} - 10 µmol l^{-1}
Inhibitors			
Pentoxifylline (Trental®)			10 µmol l^{-1} or 100 µmol l^{-1}
SQ 22,536	17318-31-9	Sigma Aldrich	100 µmol l^{-1}
L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride)	51298-62-5	Sigma Aldrich	100 µmol l^{-1}
Rp-8-Br-PET-cGMPS (Rp-8-Bromo-β-phenyl-1,N2-ethenoguanosine 3',5'-cyclic monophosphorothioate sodium salt)	185246-32-6	Sigma Aldrich	3 µmol l^{-1}
Rp-cAMPS (Adenosine 3',5'-cyclic Monophosphorothioate, Rp-Isomer, Triethylammonium Salt)	151837-09-1	Sigma Aldrich	10 µmol l^{-1}
DPCPX (8-Cyclopentyl-1,3-dipropylxanthine)	102146-07-6	Sigma Aldrich	10 µmol l^{-1}
ZM 241385	139180-30-6	Sigma Aldrich	3 µmol l^{-1}
MRS1706	264622-53-9	Cayman Chemical Company	10 µmol l^{-1}

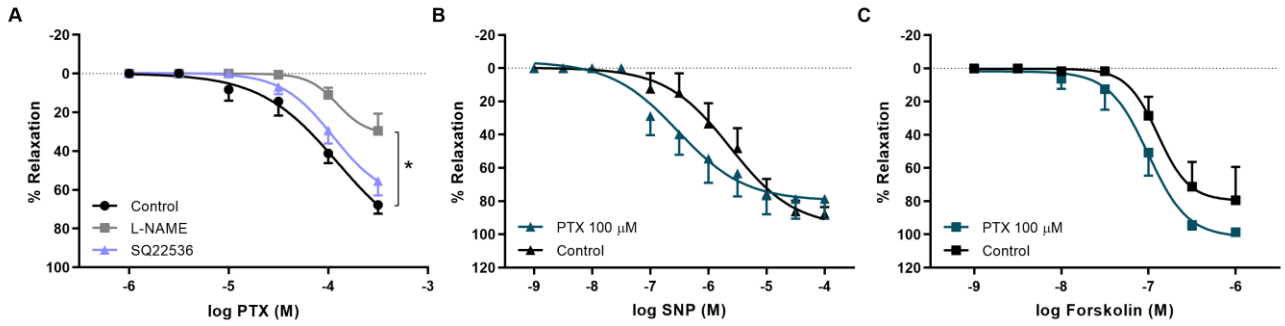

Figure S1. Results of the wire myography experiments using porcine coronary arteries. Concentration-response curves (CRC) from wire myography experiments displayed as % relaxation of U46619 preconstriction. **A)** CRC of pentoxifylline (PTX) in control segments (circles) or after incubation with SQ22536 (triangles) or L-NAME (squares, n = 9). **B)** CRC of sodium nitroprusside (SNP) in control segments (black triangles) or after incubation with 100 µmol l^{-1} pentoxifylline (cyan triangles). Pentoxifylline itself had no significant effect, but the interaction between the SNP concentration and % relaxation was significantly different (n = 5). **C)** CRC of forskolin in control segments (black squares) or after incubation with 100 µmol l^{-1} (cyan squares, n=5-6). Curves with antagonist were compared to curves without antagonist (control) using GLM-RM and data are depicted as mean ± SE % relaxation of U46619 preconstriction. * $P < 0.05$.

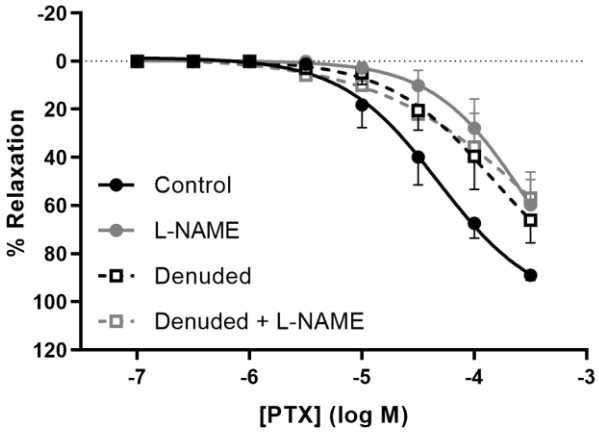

Figure S2. Exploratory data: Pentoxifylline-induced vasodilation is endothelium dependent in healthy placenta arteries. Concentration-response curves of pentoxifylline constructed using wire myography experiments in intact segments as well as in segments of which the endothelium was removed (Denuded), and depicted as percentage relaxation of preconstriction by U46619. Denudation attenuated vasodilation to pentoxifylline (black open squares, n = 4), similar to L-NAME (grey filled circles, n = 3), when compared to intact artery segments (black filled squares, n = 4). L-NAME did not further inhibit vasodilation in denuded arteries (grey open squares, n = 4). Data are depicted as mean ± SE % relaxation of U46619 preconstriction.

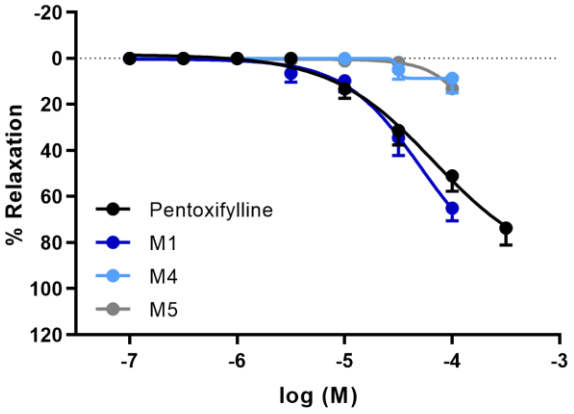

Figure S3. The effects of pentoxifylline metabolites in healthy chorionic plate arteries. Concentration-response curves of pentoxifylline and its metabolites M1 (lisofylline), M4 and M5 from wire myography experiments with human chorionic plate arteries (n = 6-12). Data are depicted as mean ± SE % relaxation of U46619 preconstriction. Comparisons of curves with antagonist to curves without antagonist (control) in GLM-RM did not result in statistically significant results.

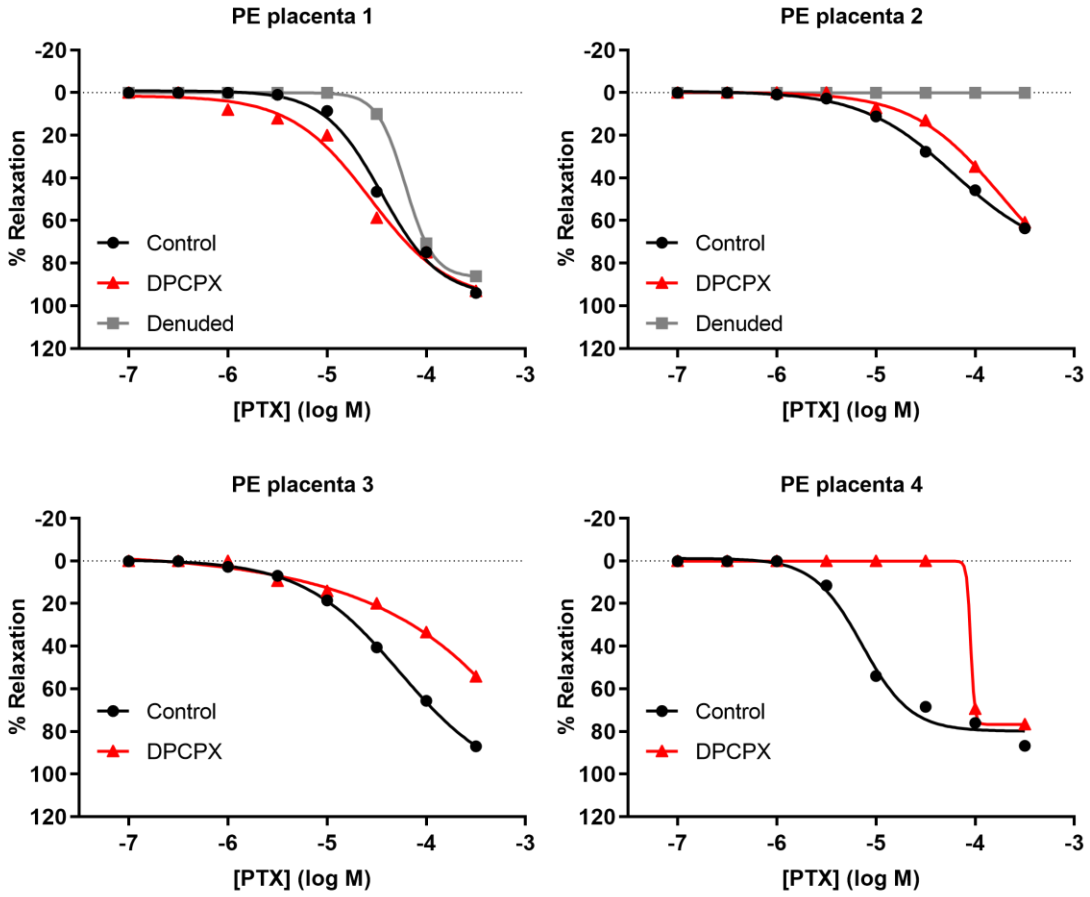

Figure S4. The effects of DPCPX and endothelial denudation on pentoxifylline-induced vasodilation in four individual experiments with chorionic plate arteries from pregnancies with preeclampsia (PE). Data are depicted as % relaxation of U46619 preconstriction and each data point represents the average value from two duplicate experiments.

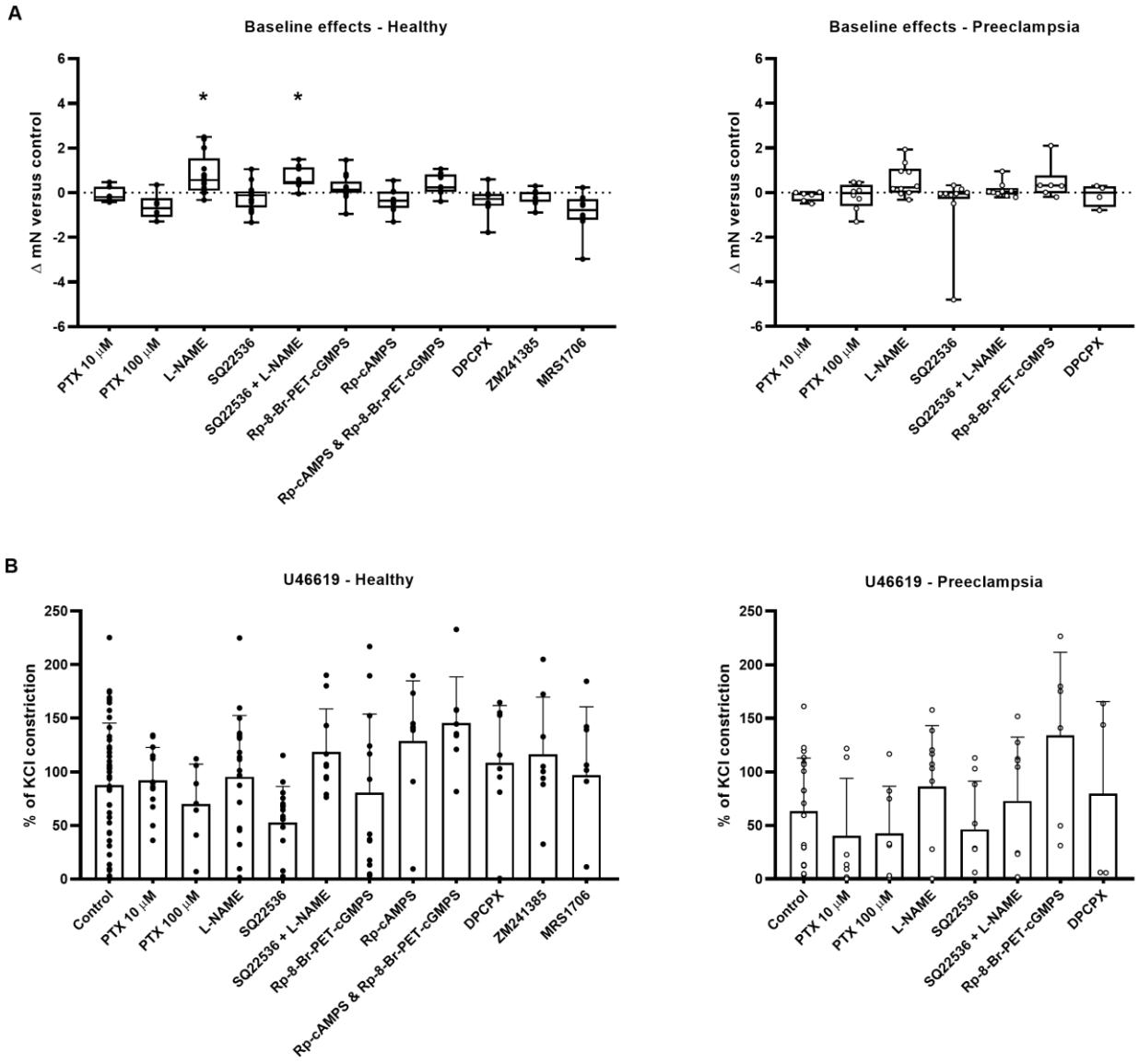

Figure S5. The baseline effects of antagonists on healthy and preeclamptic chorionic plate arteries in wire myography experiments. A) Incubation with L-NAME, alone or in combination with SQ22536 elevated the baseline tension when compared to the vessels without antagonist (control) in healthy placental arteries only. **B)** None of the antagonist altered the contractile response to 10 nmol l^{-1} U46619. * $P < 0.05$ in one sample t-tests with Benjamini-Hogbergh correction versus 0.