Supplemental Data

Endothelium-dependent contractions of isolated arteries to thymoquinone require biased activity of sGC with subsequent cIMP production

Charlotte M. Detremmerie, Zhengju Chen, Zhuoming Li, Khalid M. Alkharfy, Susan W.S. Leung, Aimin Xu, Yuansheng Gao, Paul M. Vanhoutte

Journal:
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS (JPET)

Legends:

Supplementary Table 1 – Parameters for UPLC-MS/MS measurement of cyclic nucleotides in isolated arteries

Protonated mass, quantifier, qualifier [in mass to charge ratio (m/z)], quantifier/qualifier ratio and retention times (in minutes) are given for measurement of cyclic AMP, cyclic GMP, cyclic IMP and the internal standard, tenofovir.

Supplementary Table 2 – Effect of L-NAME or ODQ on relaxation phase of response to thymoquinone in isolated arteries

Effect of L-NAME/ODQ on the relaxation phase of the response to thymoquinone in isolated rat aortae (n=4), mesenteric arteries (n=4) and porcine coronary arteries (n=5) with endothelium precontracted with serotonin (porcine arteries, 10^{-8} -10^{-5} mol/L) or with phenylephrine (rat arteries, 10^{-8} - 10^{-6} mol/L). Data are presented as maximal relaxation [as % of the reference contraction to KCl (60 mmol/L)] to 10^{-3} mol/L thymoquinone in rat arteries and 10^{-4} mol/L thymoquinone in porcine coronary arteries. Data shown as means ± standard error of the mean; n represents the number of rings of different animals (i.e. individual observations).

Supplementary Figure 1 - Tracings of isometric tension recordings in isolated arteries

Tracings of isometric tension recording in isolated rat aortae **(A)**, rat mesenteric arteries **(B)** and porcine coronary arteries **(C)** with endothelium. The arteries were exposed to 60 mmol/L KCl prior to precontraction to phenylephrine ($3x10^{-7}$ mol/L in rat aortae and 10^{-6} mol/L in rat mesenteric arteries) or serotonin (10^{-6} mol/L, porcine coronary arteries) and exposure to thymoquinone ($3x10^{-5}$ mol/L in rat arteries and 10^{-5} M in porcine coronary arteries).

Supplementary Figure 2 - Effect of COX inhibitors, NADPH oxidase inhibitors and endothelin-receptor antagonists on the response to thymoquinone in isolated arteries

(A-B) Effects of indomethacin (10^{-5} mol/L), apocynin (10^{-4} mol/L) and bosentan (10^{-6} mol/L) on thymoquinone-induced augmentations in rings with endothelium of rat aortae (n=4-5, **A**) and of porcine coronary arteries (n=4-5, **B**), contracted with phenylephrine (rat arteries, 10^{-8} -10^{-6} mol/L) or with serotonin (porcine arteries, 10^{-8} - 10^{-5} mol/L). In all graphs, the control group includes untreated preparations with endothelium.

The augmentations are shown as areas under curve of the contraction phase of the corresponding concentration-response graphs. Data shown as means ± standard error of the mean; n represents the number of rings of different animals (i.e. individual observations). "*" indicates statistically significant differences from controls ($P \leq 0.05$).

Supplementary Figure 3 - Effect of sGC-products/product analogues or protein kinase inhibitors on the response to thymoquinone in isolated arteries

(A) Effect of ODQ (10^{-5} mol/L), 8-Br-cGMP (10^{-5} M, in rings treated with ODQ) and PPi (10^{-5} M, in rings treated with ODQ) in porcine coronary arteries (n=4-9) with endothelium precontracted with serotonin (10^{-8} - 10^{-5} mol/L) on thymoquinone ($3x10^{-5}$ mol/L)-induced augmentations.

(B) Effect of KT-5720 ($3x10^{-7}$ mol/L) and KT-5823 (10^{-6} mol/L) in rat aortae (n=4-8) with endothelium precontracted with phenylephrine (10^{-8} -10^{-6} mol/L) on thymoquinone (10^{-5} mol/L)-induced augmentations. In all graphs, the control group includes untreated preparations with endothelium.

Contractions are expressed as percentage of the precontraction to serotonin in porcine coronary arteries (**A**) or as percentage of the reference contraction to KCl (60 mmol/L) in rat arteries (**B**). Data shown as means ± standard error of the mean; n represents the number of rings of different animals (i.e. individual observations). "*" indicates statistically significant differences from controls ($P \leq 0.05$).

Supplementary Figure 4 - Effect of thymoquinone on cyclic GMP levels in isolated porcine coronary arteries

Effect of $3x10^{-5}$ mol/L thymoquinone on intracellular cyclic GMP levels in isolated porcine coronary

arteries (n=3) with endothelium precontracted with serotonin (10^{-8} - 10^{-5} mol/L).

The results are presented as a ratio of the cyclic GMP level in pmoles to the dry weight of the respective samples. Data shown as means ± standard error of the mean; n represents the number of rings of different animals (i.e. individual observations).

Supplementary Figure 5 - Effect of thymoquinone on intracellular cyclic nucleotide levels in isolated porcine coronary arteries

(A-B) Effect of thymoquinone ($3x10^{-6}$ – $3x10^{-5}$ mol/L) on the intracellular levels of cyclic GMP **(A)** and cyclic AMP **(B)** in isolated porcine coronary arteries (n=4-5) with endothelium precontracted with serotonin (10^{-8} - 10^{-5} mol/L) and treated with or without $3x10^{-5}$ M ODQ.

Data shown as concentration in pmol per mg protein tissue and presented as means ± standard error of the mean; n represents the number of experiments for which at least 4 rings were pooled from 4 different animals (i.e. pooled observations). "*" indicates statistically significant differences from the control group with endothelium ($P \leq 0.05$).

Supplementary Table 1 - Parameters for UPLC-MS/MS measurement of cyclic nucleotides in isolated arteries

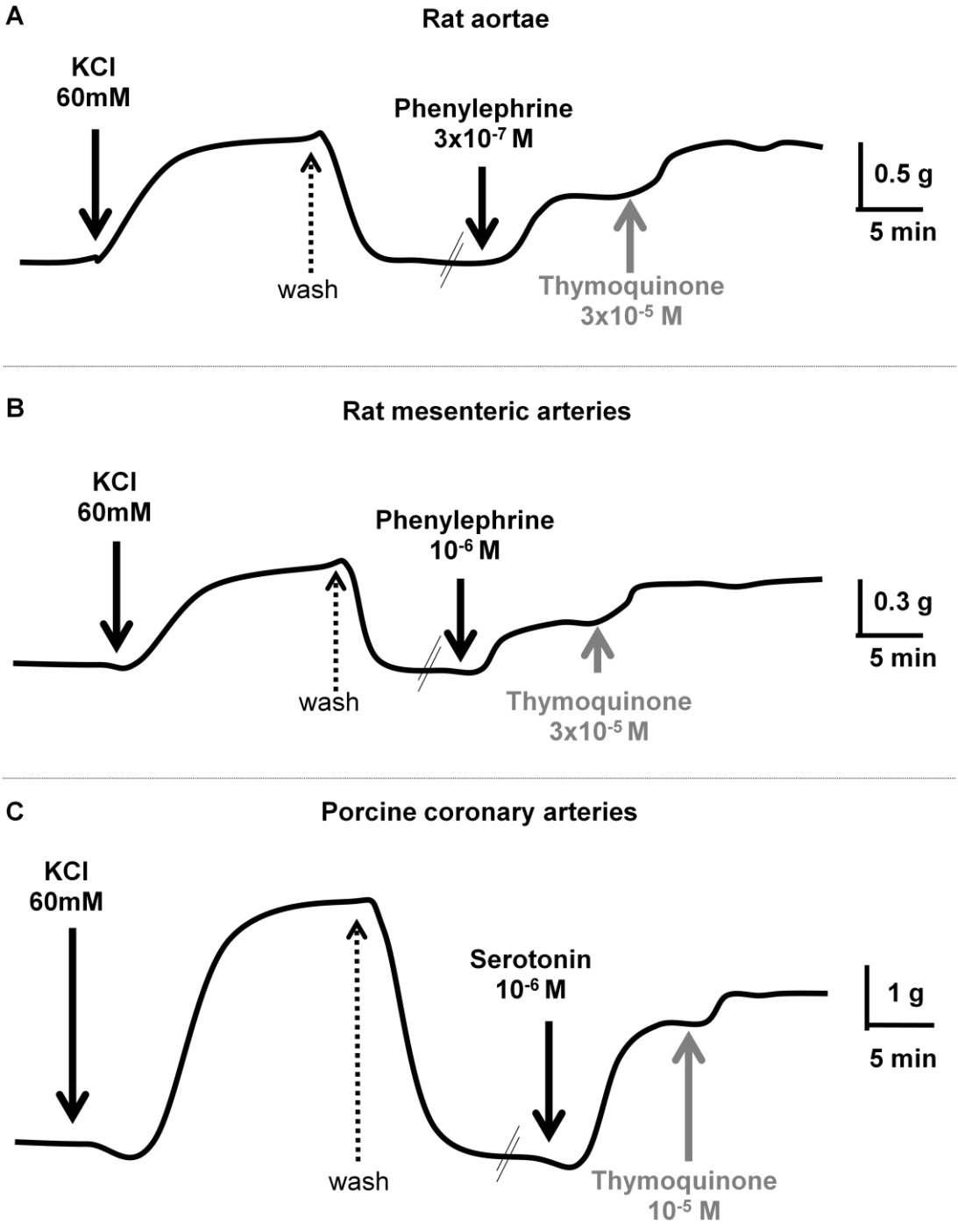
Parameters	cAMP	cGMP	cIMP	Tenofovir
$[M+H]^+$ (m/z)	330	346	331	288
Quantifier (m/z)	136	152	137	176
Qualifier (m/z)	119	135	110	270
Quantifier/qualifier ratio	16.7	27.7	55.6	4.08
Retention Time (min)	3.68	2.39	2. 64	2.36

Supplementary Table 2 – Effect of L-NAME or ODQ on relaxation phase of response to thymoquinone in isolated arteries with endothelium

Species/Vascular bed	Control (%)	L-NAME 10^{-4} M (%)	ODQ 10^{-5} M (%)
Rat aorta (n=6)	-66.1+/-19.8	-75.9+/-11.2	-63.2+/-9.1
Rat mesenteric artery (n=4)	-87.2+/-15.5	-93.1+/-10.7	-90.8+/-5.1
Porcine coronary artery (=6)	-70.5+/-36.8	-92.1+/-12.1	-90.8+/-13.8

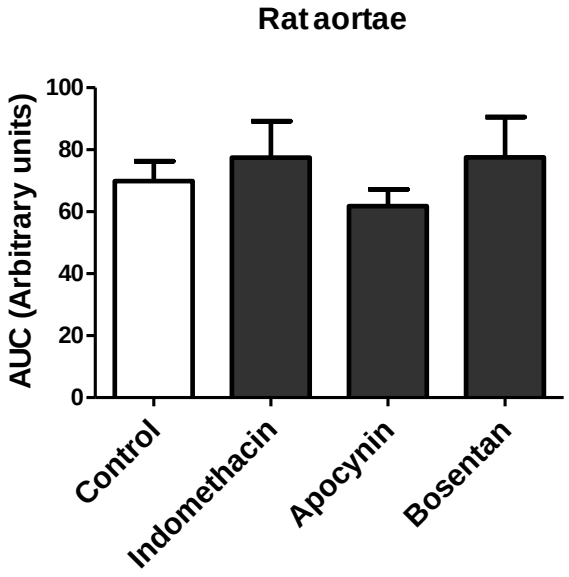
Results presented as maximal relaxation calculated as % of reference contraction to 60 mM KCl

Supplementary Figure 1 – Tracings of isometric tension recording in isolated arteries

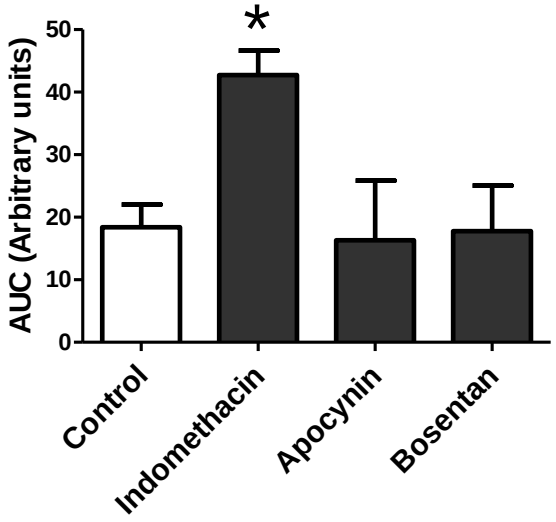


Supplementary Figure 2 – Effect of COX inhibitors, NADPH oxidase inhibitors and endothelin-receptor antagonists on the response to thymoquinone in isolated arteries

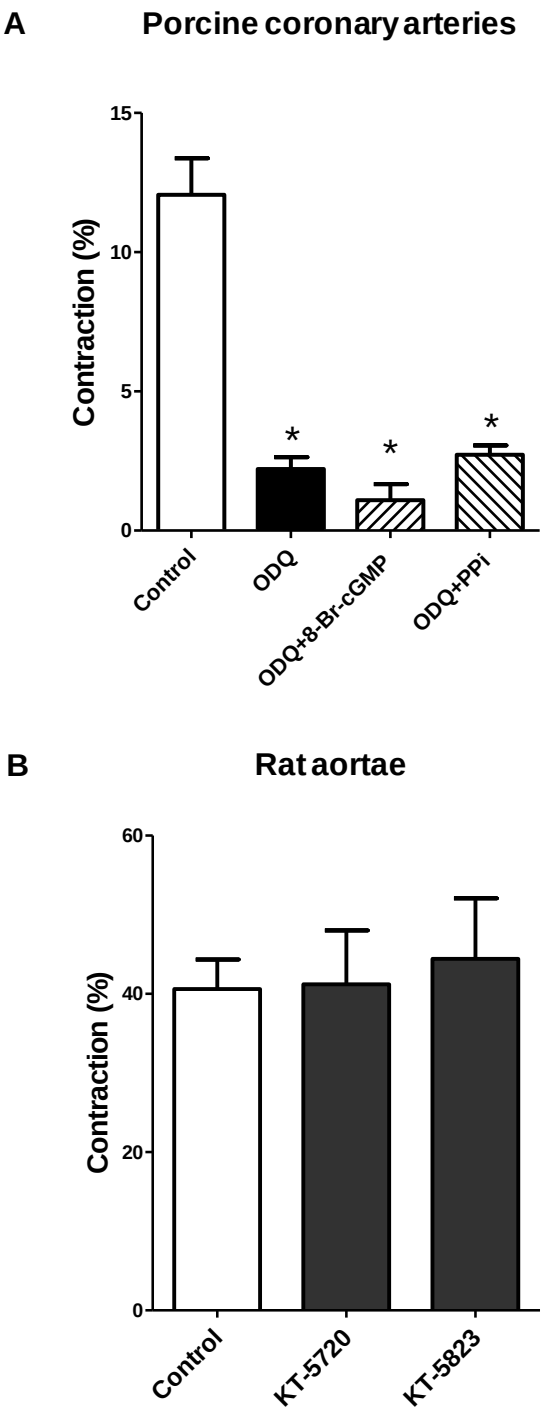
A



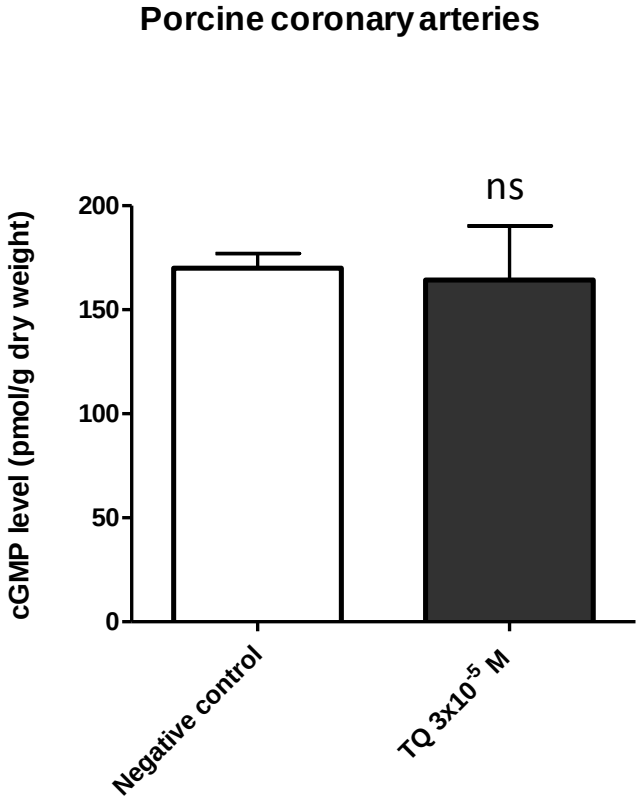
B



Supplementary Figure 3 – Effect of sGC-products/product analogues or protein kinase inhibitors on the response to thymoquinone in isolated arteries



Supplementary Figure 4 – Effect of thymoquinone on cyclic GMP levels in isolated porcine coronary arteries



Supplementary Figure 5 – Effect of thymoquinone on intracellular cyclic nucleotide levels in isolated porcine coronary arteries