Supplemental Data

Size-dependent heterogeneity of contractile Ca^{2+}-sensitization in rat arterial smooth muscle

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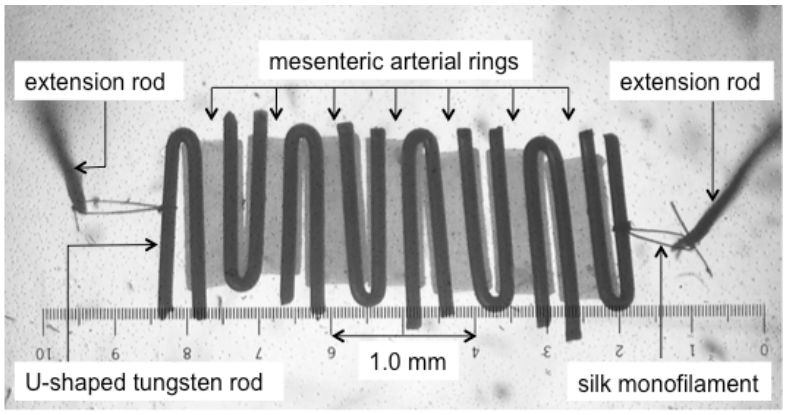
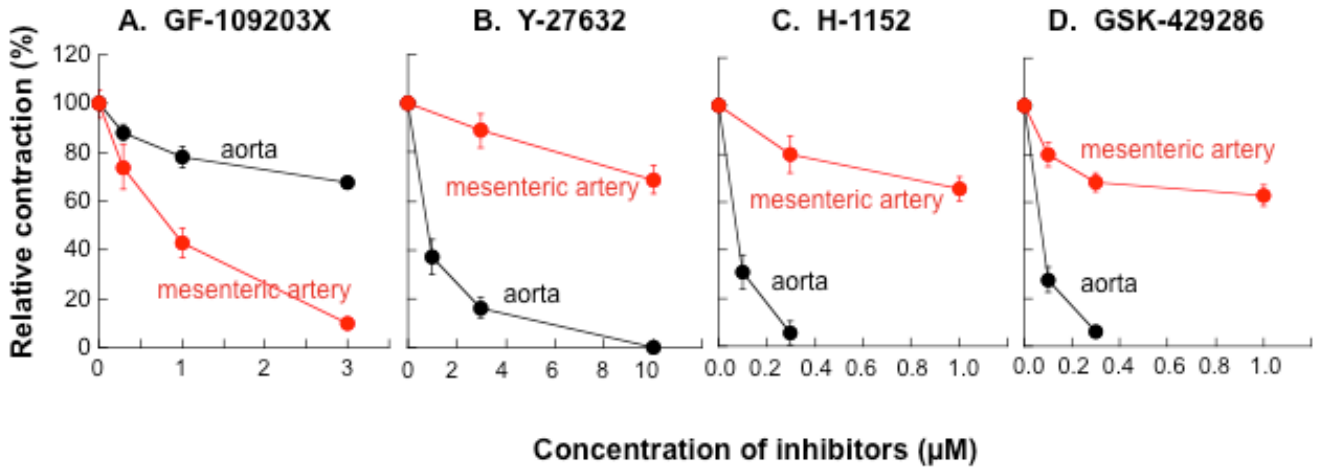
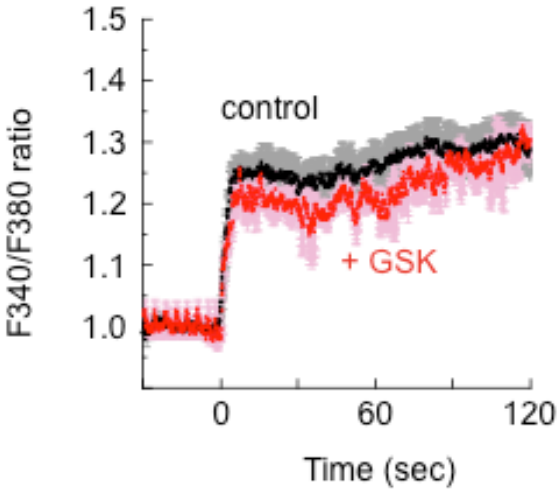


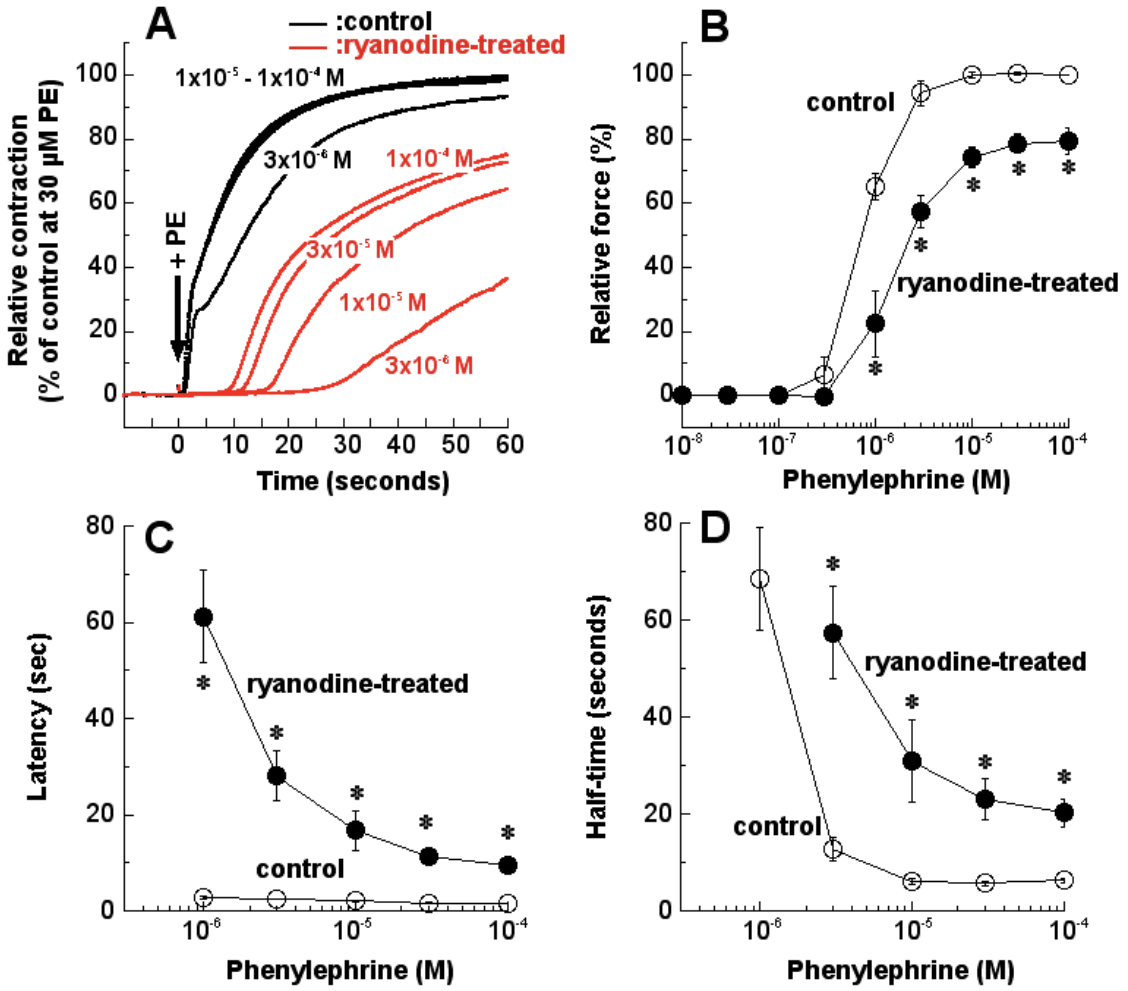
Supplemental Fig. S1. Representative photograph of a string of seven small mesenteric arterial tubes connected with U-shaped 0.1 mm-diameter tungsten clips for force measurement and quick-freezing. Each side of the string was hooked to a tungsten extension needle with a ring of monofilament silk thread. The arterial strings were stretched to about 1.2 mN of passive tension followed by a spontaneous decrease to a lower steady level, where the arteries produced a maximum force.



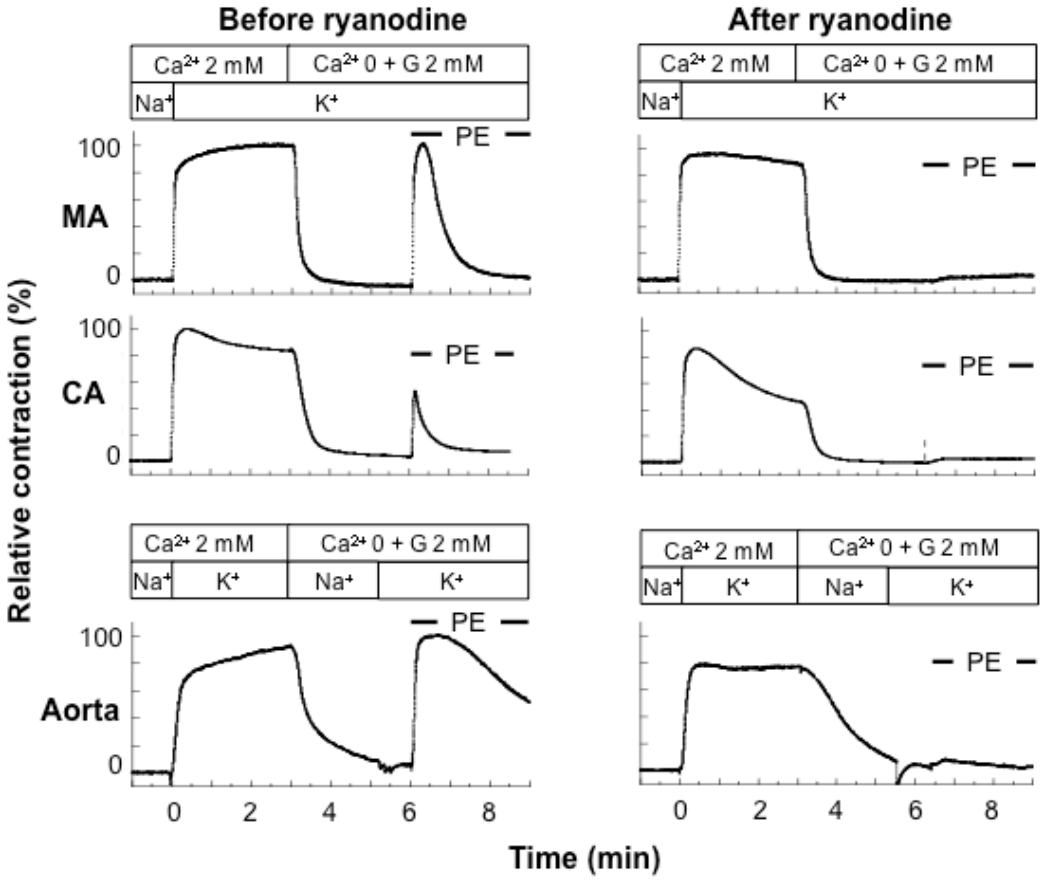
Supplemental Fig. S2. Relationship between concentration of inhibitors and steady-state levels of contraction in response to 30 μM PE in small mesenteric artery and aorta. n=4.



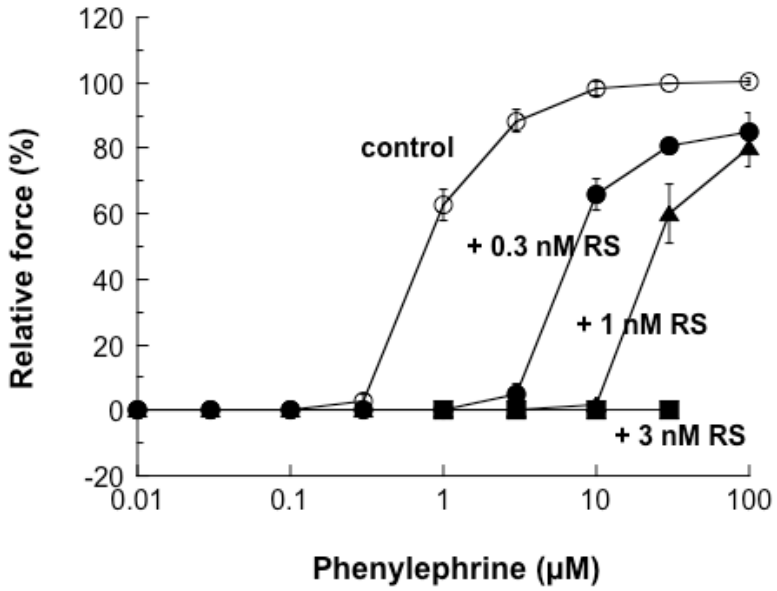
Supplemental Fig. S3. Effect of 1 μM GSK-429286 on the time course of PE-induced Ca^{2+} rise in rat aorta. n=3.



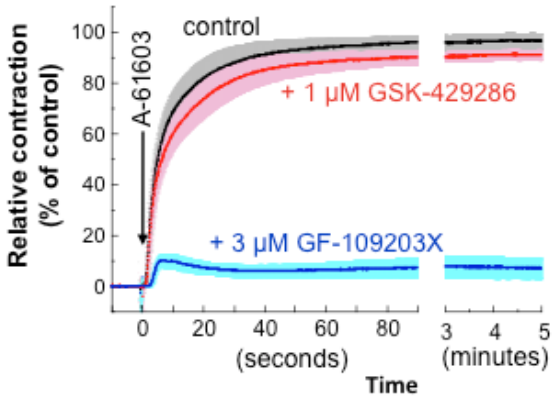
Supplemental Fig. S4. Effect of ryanodine pretreatment on PE-induced contraction in small mesenteric arteries. **A:** Contraction time courses in response to various PE concentrations in rat small mesenteric arteries with and without ryanodine pretreatment. **B:** effect of ryanodine pretreatment on the steady-state concentration-response relationship of PE-induced contraction in a cumulatively-increased fashion. In this series of experiments, the sustained level of contraction after the treatment was significantly lower (20%) compared to control. **C:** effect of ryanodine pretreatment on onset latency of PE-induced contraction. **D:** effect of ryanodine pretreatment on PE-induced contraction development half-time. n=4.



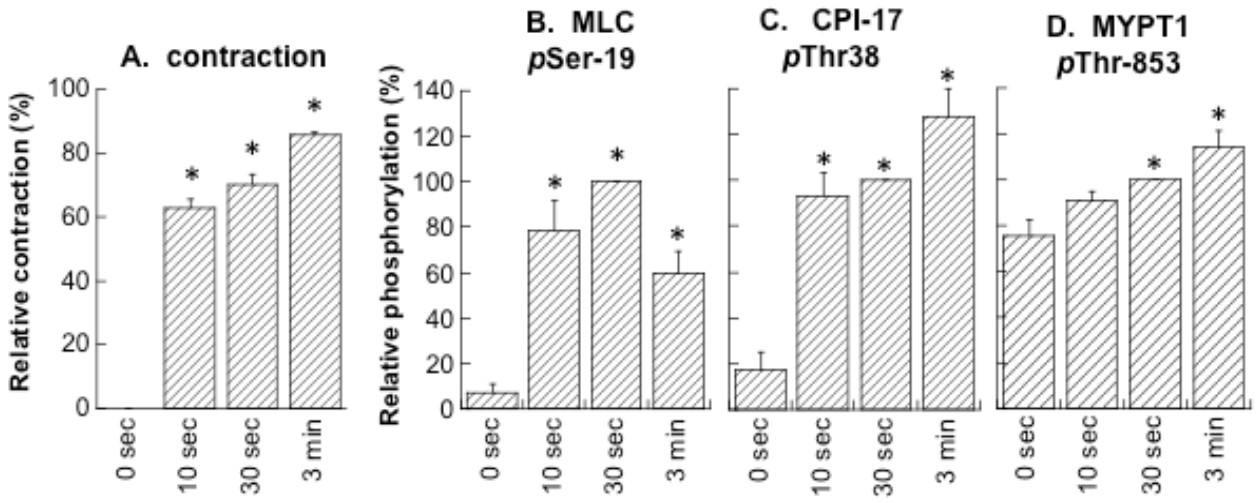
Supplemental Fig. S5. Effect of ryanodine SR Ca^{2+} depletion on PE-induced contraction in the absence of extracellular Ca^{2+} under depolarized conditions in small mesenteric artery (MA), caudal artery (CA), and aorta rings. MA (upper panel) and CA (middle) rings were stimulated with high K^+ solution containing 2 mM Ca^{2+} for 3 min. Then, Ca^{2+} was removed and 2 mM EGTA added to the high K^+ solution. MA and CA rings quickly relaxed even under high K^+ conditions. Thereafter, 30 μM PE was added to monitor the contractile response in the absence of Ca^{2+}. For aorta (lower), the high K^+ solution containing Ca^{2+} was replaced with Na^+ solution containing 2 mM EGTA to accelerate relaxation. The Ca^{2+}-free Na^+ solution was replaced with Ca^{2+}-free K^+ solution and 30 μM PE was added. After depleting SR Ca^{2+} with ryanodine (see the Methods), PE no longer produced a significant contraction in the absence of Ca^{2+} in MA, CA and aorta, suggesting that the Ca^{2+}-sensitizing mechanism in response to α_1-agonist is unable to produce significant contraction without Ca^{2+}-rise in rat arteries of varying sizes.



Supplemental Fig. S6. Effect of α_{1A} subtype-selective antagonist RS-100329 on steady-state concentration-response relationship of PE-induced contraction in small mesenteric artery. PE was cumulatively added in the presence and absence of RS-100329. RS; RS-100329. n=4.



Supplemental Fig. S7. Effect of 1 μM GSK-429286 or 3 μM GF-109203X on 30 nM A-61603-induced contraction in small mesenteric artery. GSK-429286; a ROCK inhibitor, A-61603; an α_{1A} subtype-specific agonist. n=3-6.



Supplemental Fig. S8. Time courses of phosphorylation of MLC (B), CPI-17 (C), and MYPT1 Thr853 (D) during PE-induced contraction (A) in rat aorta. The level of contraction was normalized with a maximum. The level of phosphorylation was normalized with that at 30 sec after PE stimulation. n = 3-5.