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An Elevation in Physical Coupling of Type 1 IP_3 Receptors to TRPC3 Channels Constricts Mesenteric Arteries in Genetic Hypertension

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Abstract

Hypertension is associated with an elevation in agonist-induced vasoconstriction, but mechanisms involved require further investigation. Many vasoconstrictors bind to phospholipase C-coupled receptors, leading to an elevation in inositol 1,4,5-trisphosphate (IP_3) that activates sarcoplasmic reticulum (SR) IP_3 receptors (IP_3Rs). In cerebral artery myocytes, IP_3Rs release SR Ca^{2+} and can physically couple to canonical transient receptor potential 3 (TRPC3) channels in a caveolin-1-containing macromolecular complex, leading to cation current (I_{Cat}) activation that stimulates vasoconstriction. Here, we investigated mechanisms by which IP_3Rs control vascular contractility in systemic arteries and IP_3R involvement in elevated agonist-induced vasoconstriction during hypertension. Total and plasma membrane-localized TRPC3 protein was ~2.7- and 2-fold higher in mesenteric arteries of hypertensive spontaneously hypertensive rats (SHR) than in Wistar-Kyoto (WKY) rat controls, respectively. In contrast, IP_3R1, TRPC1, TRPC6, and caveolin-1 expression was similar. TRPC3 expression was also similar in arteries of pre-hypertensive SHR and WKY rats. Control, IP_3- and endothelin-1 (ET-1)-induced FRET between IP_3R1 and TRPC3 was higher in hypertensive SHR than WKY myocytes. IP_3-induced I_{Cat} was ~3-fold larger in SHR myocytes. Pyr3, a selective TRPC3 channel blocker, and CIRBP-TAT, an IP_3R-TRP physical coupling inhibitor, reduced IP_3-induced I_{Cat} and ET-1-induced vasoconstriction more in SHR than WKY myocytes and arteries. Thapsigargin, a SR Ca^{2+}-ATPase blocker, did not alter ET-1-stimulated vasoconstriction in SHR or WKY arteries. These data indicate that ET-1 stimulates physical coupling of IP_3R1 to TRPC3 channels in mesenteric artery myocytes, leading to vasoconstriction. Furthermore, an elevation in IP_3R1 to TRPC3 channel molecular coupling augments ET-1-induced vasoconstriction during hypertension.

Keywords

Hypertension; vasoconstriction; TRPC channels; IP_3 receptors; endothelin-1

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Conflicts of Interest/Disclosures
None.

Introduction

Hypertension is associated with arterial hypercontractility that alters vascular bed hemodynamics (1;2). Several mechanisms have been proposed to stimulate vasoconstriction in hypertension, including altered myocyte ion channel expression and function (3). An increase in myocyte voltage-dependent Ca^{2+} current density elevates intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), leading to vasoconstriction in hypertension (4;5). In hypertensive animal models, a reduction in expression and function of the β_1 subunit of the large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel increases vascular tone (6;7). Altered expression of several transient receptor potential (TRP) channels is also proposed to be associated with systemic and pulmonary hypertension (8). However, molecular mechanisms by which alterations in TRP channel expression result in vasoconstriction in hypertension are poorly understood.

Many vasoconstrictors bind to phospholipase C-coupled receptors, leading to an elevation in inositol 1,4,5-trisphosphate (IP_3) that activates endo/sarcoplasmic reticulum-localized IP_3 receptors (IP_3Rs) (9). Three structurally and functionally distinct IP_3R isoforms, designated IP_3R1, IP_3R2, and IP_3R3 have been identified (10). IP_3R1 is the predominant molecular and functional isoform expressed in vascular myocytes (10). Agonist-induced IP_3R activation results in sarcoplasmic reticulum (SR) Ca^{2+} release in cerebral artery myocytes that partially contributes to constriction (10). Close spatial proximity of IP_3R1 to plasma membrane canonical transient receptor potential 3 (TRPC3) channels also permits IP_3 to promote binding of the IP_3R1 N-terminus to the TRPC3 channel calmodulin and IP_3R binding (CIRB) domain (11). Isoform-selective physical coupling of IP_3R1 to TRPC3 channels activates a cation current (I_{Cat}) in cerebral artery myocytes (11;12). IP_3-induced I_{Cat} activation leads to membrane depolarization, voltage-dependent Ca^{2+} channel activation, an elevation in global $[Ca^{2+}]_i$ and vasoconstriction (13). This physical coupling mechanism is essential for IP_3-induced I_{Cat} activation and a major contributor to agonist-induced constriction in cerebral arteries (11;12;14). In contrast, mechanisms by which IP_3Rs control systemic artery contractility are poorly understood, with contributions of SR Ca^{2+} release and physical coupling of IP_3Rs to TRP channels unclear. Similarly, whether pathological alterations in IP_3R-mediated signaling contribute to elevated systemic vascular contractility during hypertension is poorly understood.

Here, we investigated physiological functions of IP_3Rs in myocytes of mesenteric arteries. We also tested the associated hypothesis that molecular and functional alterations in IP_3R signaling contribute to mesenteric artery vasoconstriction in hypertension. Our data indicate that IP_3 and endothelin-1 (ET-1) stimulate physical coupling of IP_3R1 to TRPC3 channels, leading to I_{Cat} activation in mesenteric artery myocytes and vasoconstriction. Data also indicate that hypertension is associated with an elevation in IP_3-induced physical coupling of IP_3R1 to TRPC3 channels, leading to vasoconstriction. In contrast, ET-1 does not induce vasoconstriction by stimulating SR Ca^{2+} release in arteries of normotensive or hypertensive rats. In summary, our findings indicate that an elevation in IP_3R1 to TRPC3 channel molecular coupling contributes to vasoconstriction during hypertension.

Materials and Methods

Expanded Materials and Methods are available as Supplemental Documentation.

Results

IP_3R1 interacts with TRPC3 and cav-1 in mesenteric arteries

IP_3R1, TRPC3 and cav-1 are located in the same macromolecular complex and physically interact in cerebral artery myocytes (12). Whether these proteins structurally organize in systemic arteries is unclear. Co-IP was used to examine whether IP_3R1 associates with TRPC3 channels and cav-1 in Wistar-Kyoto (WKY) rat mesenteric arteries. A monoclonal mouse anti-IP_3R1 antibody co-immunoprecipitated IP_3R1, TRPC3, and cav-1 from WKY rat mesenteric artery lysate (Fig. 1). These data suggest that IP_3R1, TRPC3 channels and cav-1 exist in the same macromolecular complex in mesenteric arteries.

Hypertension is associated with an elevation in TRPC3, but not TRPC1, TRPC6, IP_3R1 or cav-1, protein in mesenteric arteries

At 6 weeks of age, WKY and Spontaneously Hypertensive (SHR) rat blood pressures (tail cuff systolic, WKY vs. SHR: ~ 137 vs. 137 mmHg) are similar (15). In contrast, at 12 weeks of age, WKY and SHR blood pressures (~151 vs. 217 mmHg, respectively) are significantly different (15). Western blotting indicated that TRPC3 protein was ~2.7-fold higher in hypertensive (13 wk) SHR than in age-matched WKY rat mesenteric arteries (Fig. 2A,G; Data Supplement Fig. S1). In contrast, TRPC1, TRPC6, IP_3R1 and cav-1 proteins were similar (Fig. 2B–E,G). To examine the hypothesis that hypertension is a factor associated with elevated vascular TRPC3 expression, protein was compared in 6 week old pre-hypertensive SHR and WKY rat arteries. TRPC3 protein was similar in 6 week old SHR and WKY rat mesenteric arteries (Fig. 2F,G, Data Supplement Fig. S1). These data indicate that TRPC3 protein increases during the development of genetic hypertension, whereas TRPC1, TRPC6, IP_3R1 and cav-1 expression remain unaltered in mesenteric arteries.

Plasma membrane-localized TRPC3 channel protein is elevated in SHR arteries

Arterial surface biotinylation was performed to examine cellular distribution of TRPC3 channels in mesenteric arteries. Biotinylation indicated that ~ 99.6 and 98.8 % of total TRPC3 protein was present in the arterial plasma membrane in SHR and WKY rats, respectively (Fig. 3A,B). Surface TRPC3 protein was ~2-fold higher in SHR than WKY arteries (Fig. 3A,C). These data indicate that TRPC3 channels are predominantly membrane-localized in mesenteric arteries. Furthermore, during hypertension the increase in TRPC3 channel expression directly translates to an elevation in plasma membrane TRPC3 protein.

Hypertension is associated with increased spatial localization of TRPC3 channels nearby IP_3R1 in arterial myocytes

Next, we investigated spatial proximity between arterial myocyte IP_3R1 and TRPC3 in SHR and WKY mesenteric artery myocytes. Alexa 546- and 488-labeled secondary antibodies bound to primary antibodies targeting IP_3R1 and TRPC3, respectively, produced mean N-FRET of ~23.7% in isolated WKY rat arterial myocytes (Fig. 4A,B). In SHR myocytes, the same antibodies produced a higher mean N-FRET of ~29.1% (Figure 4A,B). Bt-IP_3, a membrane-permeant IP_3 analogue, and ET-1, a vasoconstrictor, both increased mean N-FRET to ~28% in WKY myocytes (Fig. 4B). Bt-IP_3 and ET-1 stimulated a larger increase in mean N-FRET to ~34 and 35 %, respectively in SHR myocytes (Fig. 4B). To test the hypothesis that hypertension is associated with an elevation in spatial localization of IP_3R1 and TRPC3 channels in arterial myocytes, N-FRET was measured in mesenteric artery myocytes from 6 week old pre-hypertensive SHR and WKY rats. Baseline and the ET-1-stimulated elevation in mean N-FRET were similar in 6 week old SHR and WKY myocytes (Fig. 4C). These data indicate that TRPC3 is located in close spatial proximity to IP_3R1, that IP_3 and ET-1 increase molecular localization between these proteins, and that hypertension

is associated with an elevation in the spatial localization of TRPC3 channels to IP_3R1 in mesenteric artery myocytes.

IP_3 stimulates larger cation currents (I_{Cat}) in SHR than WKY myocytes

The significance of an elevation in TRPC3 channel expression and IP_3R1 to TRPC3 molecular localization in SHR arterial myocytes was investigated by measuring I_{Cat} using patch-clamp electrophysiology. Control I_{Cat} density was similar in SHR and WKY arterial myocytes with mean values at ~6 pA/pF at −120 mV (Fig 5A,B). IP_3 increased mean I_{Cat} density by ~ 6 pA/pF in WKY myocytes and by ~ 18 pA/pF in SHR myocytes, or ~ 3-fold more (at −120 mV, Fig. 6A,B). Pyr3, a selective TRPC3 channel blocker (16), reduced IP_3-induced I_{Cat} in SHR and WKY rat arterial myocytes by ~ 90 and 76 %, respectively (Fig. 5A,B). CIRBP-TAT, a membrane-permeant peptide that blocks physical coupling of IP_3Rs to TRPC channels (11;17), inhibited IP_3-induced I_{Cat} in SHR and WKY rat myocytes similarly to Pyr3 (Fig. 5A,B). These data indicate that IP_3 stimulates physical coupling of IP_3R1 to TRPC3 channels, leading to I_{Cat} activation in mesenteric artery myocytes. Data also reveal that augmentation of this coupling mechanism elevates IP_3-induced TRPC3 currents in SHR myocytes during hypertension.

ET-1 stimulates larger vasoconstriction through an SR Ca^{2+} release-independent mechanism in SHR mesenteric arteries

The contribution of IP_3Rs and TRPC channel activation to ET-1-induced mesenteric artery constriction was examined. ET-1 stimulated concentration (1–100 nM)-dependent vasoconstriction in both WKY and SHR arteries. ET-1-induced vasoconstriction was larger at all concentrations studied, as was maximal force generation, in SHR arteries (Fig. 6A). In contrast, the half-maximal effective concentration (EC_{50}) of ET-1 was similar in SHR (3.05 nM) and WKY (2.40 nM) arteries. Depolarization (80 mM KCl)-induced constriction was also larger in SHR than WKY rat mesenteric arteries (Δ tension, SHR vs. WKY: 6.9 ± 1.3 mN vs., 3.5 ± 0.4; n=14 for each; $P<0.05$). Thapsigargin, a SR Ca^{2+}-ATPase inhibitor that abolishes sarcoplasmic reticulum Ca^{2+} release, did not alter baseline tension or ET-1-induced vasoconstriction in SHR and WKY arteries (Data Supplement Fig. S2, Fig. 6B). These findings indicate that ET-1-induced vasoconstriction occurs independently of SR Ca^{2+} release in mesenteric arteries of both SHR and WKY rats and is larger in SHR arteries. Data also suggest that ET-1 sensitivity is similar in SHR and WKY arteries.

Pathological vasoconstriction occurs due to an elevation in physical coupling of IP_3R1 to TRPC3 channels in myocytes

The functional significance of IP_3R1 to TRPC3 channel physical coupling in mediating ET-1-induced vasoconstriction was studied. 2-APB, an IP_3R and TRPC channel inhibitor, did not alter baseline arterial tension or depolarization-induced vasoconstriction (Data Supplement Figs. S2 and S3). In contrast, 2-APB reduced ET-1-induced vasoconstriction, doing so more effectively in SHR than WKY arteries (~34 versus 18 % reduction, respectively; Fig. 6C). Pyr3 and CIRBP-TAT did not alter baseline tension or depolarization-induced vasoconstriction, but both blockers inhibited ET-1-induced vasoconstriction (Data Supplement Figs. S2 and S3, and Figure 6C). Importantly, Pyr3 more effectively blocked ET-1-induced constriction in SHR than WKY arteries (~83 % versus 59 % reduction, respectively; Fig. 6C). CIRB-TAT also attenuated ET-1-induced constriction more in SHR than WKY arteries (~57 versus 39 % reduction, respectively; Fig. 6C). Taken together, these data indicate that an elevation in plasma membrane TRPC3 channels and physical coupling of these channels to IP_3R1 in myocytes augments ET-1-induced vasoconstriction during hypertension.

Discussion

Here, we studied the functional significance of IP_3Rs and TRPC3 channels to agonist-induced vasoconstriction in mesenteric arteries and the contribution of pathological alterations in IP_3R-mediated signaling mechanisms during hypertension. Our data show for the first time that IP_3R1 is located in close proximity to TRPC3 and is contained within a macromolecular complex containing TRPC3 and cav-1 in mesenteric arteries. Hypertension is associated with an increase in both total and plasma-membrane TRPC3 protein and IP_3R1 to TRPC3 spatial localization, leading to an elevation in IP_3-induced I_{Cat} in arterial myocytes. SR Ca^{2+} release does not contribute to ET-1-induced vasoconstriction in mesenteric arteries of either normotensive or hypertensive rats, suggesting that IP_3R activation stimulates vasoconstriction through alternate mechanisms. Our data indicate that physical coupling of IP_3R1 to TRPC3 channels contributes to ET-1-induced vasoconstriction in mesenteric arteries and that enhancement of this mechanism contributes to the elevation in ET-1-induced vasoconstriction during hypertension.

TRPC3 expression is altered in several cell types in hypertension. TRPC3 mRNA and protein were elevated in lung tissues and pulmonary artery myocytes from patients with idiopathic pulmonary hypertension (18). When compared with normotensive controls, TRPC3 protein was also higher in SHR and human monocytes, human renal arteriolar endothelium, kidney cortex of hypertensive Munich Wistar Frömter rats and SHR aorta, mesenteric, carotid and cerebral arteries (19–24). Our data indicating that TRPC3 protein is higher in mesenteric arteries of hypertensive SHR than age-matched WKY rats is consistent with these results from other cell types. Mechanisms that elevate arterial myocyte TRPC3 channels in SHR are unclear. Conceivably, the chronic elevation in blood pressure may stimulate transcriptional upregulation of TRPC3 channels. In addition, TRPC3 upregulation may be one mechanism that elevates systemic blood pressure. Functional and molecular alterations in IP_3R1 and cav-1 also occur during hypertension. Pulmonary hypertension was associated with an elevation in pulmonary artery IP_3R1 mRNA and protein, whereas IP_3R1 expression was lower in renal medulla of SHR than WKY rats (25;26). Although cav-1 knockout mice develop pulmonary hypertension, cav-1 protein was lower in SHR than WKY rat aorta (27;28). Data from this and our previous study indicate that IP_3R1, TRPC3, and cav-1 exist in a macromolecular signaling complex in both cerebral and mesenteric arteries (12). Cav-1 and IP_3R1 expression were similar in SHR and WKY mesenteric arteries, suggesting that altered cav-1 and IP_3R1 expression may be tissue-dependent in hypertension. TRPC3, TRPC1, and TRPC6 channels regulate membrane potential, $[Ca^{2+}]_i$ and contractility in myocytes from a wide variety of different blood vessels (29). Lower carotid artery and higher mesenteric arteriole TRPC1 protein has been reported in SHR rats (23;24). TRPC6 protein was also higher in mesenteric artery myocytes of Milan hypertensive rats and ouabain-induced hypertensive rats than in normotensive controls (30;31). Our data indicate that TRPC1 and TRPC6 protein is similar in SHR and WKY rat mesenteric arteries. These *contradictory data may result from differences in the arterial bed and/or animal models of hypertension studied*.

Hypertension is associated with pathological alterations in vascular myocyte contractility, morphology, gene expression, and proliferation (32). These changes *may occur due to altered* trafficking, cell surface expression, and spatial localization of signaling molecules, leading to dysregulation of physiological functions, including contractility. A proportion of TRPC3 protein is located intracellularly in a variety of cell types, including cerebral artery myocytes and cardiomyocytes (11;33). Data here show that essentially 100% of TRPC3 protein is located within the plasma membrane in mesenteric arteries, suggesting that TRPC3 distribution may differ depending on the cell type or anatomical origin of the vasculature. FRET experiments indicated that IP_3R1 and TRPC3 channels are located in

close spatial proximity and that ET-1 and IP_3 increase spatial proximity of these proteins in mesenteric artery myocytes, consistent with data in cerebral artery myocytes (11;12). IP_3R1 and TRPC3 antibodies generated a higher FRET signal in SHR myocytes that could originate from an increase in the number of TRPC3 channels nearby IP_3R1 or from a reduction in the distance between TRPC3 and IP_3R1, possibilities between which the FRET method cannot distinguish. Given that surface TRPC3 is higher and that IP_3R1 and TRPC3 are already in such close spatial proximity to permit physical coupling, it is highly likely that the higher FRET signal occurs, at least in part, due to a larger number of TRPC3 channels nearby IP_3R1.

ET-1 and UTP activate an IP_3R1-dependent TRPC3-mediated I_{Cat} in cerebral artery myocytes (13;14;34). UTP-induced whole cell currents were also larger in SHR carotid artery myocytes, although signaling mechanisms involved were not determined (24). Here, data indicate that IP_3 stimulates physical coupling between IP_3R1 and TRPC3 channels, leading to I_{Cat} in mesenteric artery myocytes. IP_3-induced I_{Cat} density was larger in SHR than WKY rat myocytes, likely due to an increased number of TRPC3 channels nearby IP_3R1 and an elevation in functional coupling. This conclusion is supported by data indicating that Pyr3 and CIRBP-TAT essentially abolished IP_3-induced I_{Cat} in both WKY and SHR myocytes. These data also indicate that hypertension is not associated with the induction of promiscuous coupling of IP_3Rs to TRP channels other than C3, a potential mechanism that may have also elevated I_{Cat}.

Small arteries from hypertensive humans and rats generate higher spontaneous tone and constrict more in response to receptor agonists (1;2;24;35–39). Here, although ET-1 and membrane depolarization stimulated larger vasoconstriction in SHR arteries, ET-1 sensitivity was similar, consistent with previous studies in perfused mesenteric arteries (40;41). When combined with other findings in this study, these data suggest that ET-1 stimulates larger vasoconstriction, in part, through stimulating more effective IP_3R1-TRPC3 physical coupling in myocytes of hypertensive rats. This leads to larger I_{Cat}, membrane depolarization, voltage-dependent Ca^{2+} channel activation, and vasoconstriction. Our data also indicate that SR Ca^{2+} release does not contribute to ET-1-induced vasoconstriction in either SHR or WKY mesenteric arteries, supporting the concept that physical coupling to TRPC3 channels is a primary mechanism by which IP_3Rs stimulate vasoconstriction in mesenteric arteries. Data also show that IP_3Rs and TRPC3 channels do not contribute to depolarization-induced vasoconstriction and that 2-APB, Pyr3 and CIRBP-TAT do not block voltage-dependent Ca^{2+} channels which elicit this response. Many vasoconstrictors, including angiotensin II and epinephrine, bind to phospholipase C-coupled receptors, leading to an elevation in intracellular IP_3 in smooth muscle cells. Therefore, our study also raises the possibility that other agonists may induce vasoconstriction by stimulating IP_3R1 to TRPC3 coupling and that this mechanism may contribute to elevated contractility during hypertension.

In summary, data indicate that IP_3R1 stimulates vasoconstriction primarily through physical coupling to TRPC3 channels in mesenteric artery myocytes. We also demonstrate that genetic hypertension is associated with an increase in TRPC3 protein, TRPC3 channel cell surface expression and enhanced physical coupling of TRPC3 to IP_3R1 channels. This pathological alteration elevates ET-1-induced vasoconstriction in hypertension.

Perspective

An elevation in vascular resistance is a hallmark of hypertension (1;2). Identifying mechanisms that underlie vasoconstriction in hypertension is essential to develop novel therapies to induce vasodilation. We show that IP_3R1 physically and functionally couples to

TRPC3 channels in mesenteric artery myocytes, similarly to previous data in cerebral artery myocytes (11). We also identify a novel mechanism by which enhanced molecular and functional coupling of TRPC3 channels to IP_3R1 elevates arterial contractility in hypertension. An increase in TRPC3 channel protein, TRPC3 channel surface expression, and molecular localization of these channels nearby IP_3R1 enhances physical and functional coupling of IP_3R1 to TRPC3 in SHR mesenteric artery myocytes. This pathological alteration in IP_3R1 to TRPC3 coupling augments agonist and IP_3-induced I_{Cat} in arterial myocytes and vasoconstriction in hypertension. Our study also provides evidence that interfering with physical and functional coupling between IP_3R1 and TRPC3 channels may be a novel therapeutic strategy to induce vasodilation in hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

1. What is new?

- IP_3R1 is located in close proximity to TRPC3 channels in mesenteric artery myocytes.
- Hypertension is associated with an increase in molecular localization of IP_3R1 to TRPC3 channels.
- Enhanced physical and functional interaction between IP_3R1 and TRPC3 channels induces vasoconstriction in hypertension

2. What is relevant?

- Smooth muscle cells regulate arterial diameter and thus, control blood flow.
- Vascular diseases, including hypertension, are associated with changes in vascular smooth muscle cell contractility that modifies blood flow and can lead to organ damage.
- Identifying mechanisms that elevate myocyte contractility in hypertension is important to develop new therapies for vascular diseases.

Summary

Enhanced physical coupling between type 1 IP_3 receptors and TRPC3 channels in smooth muscle cells stimulates vasoconstriction during hypertension.

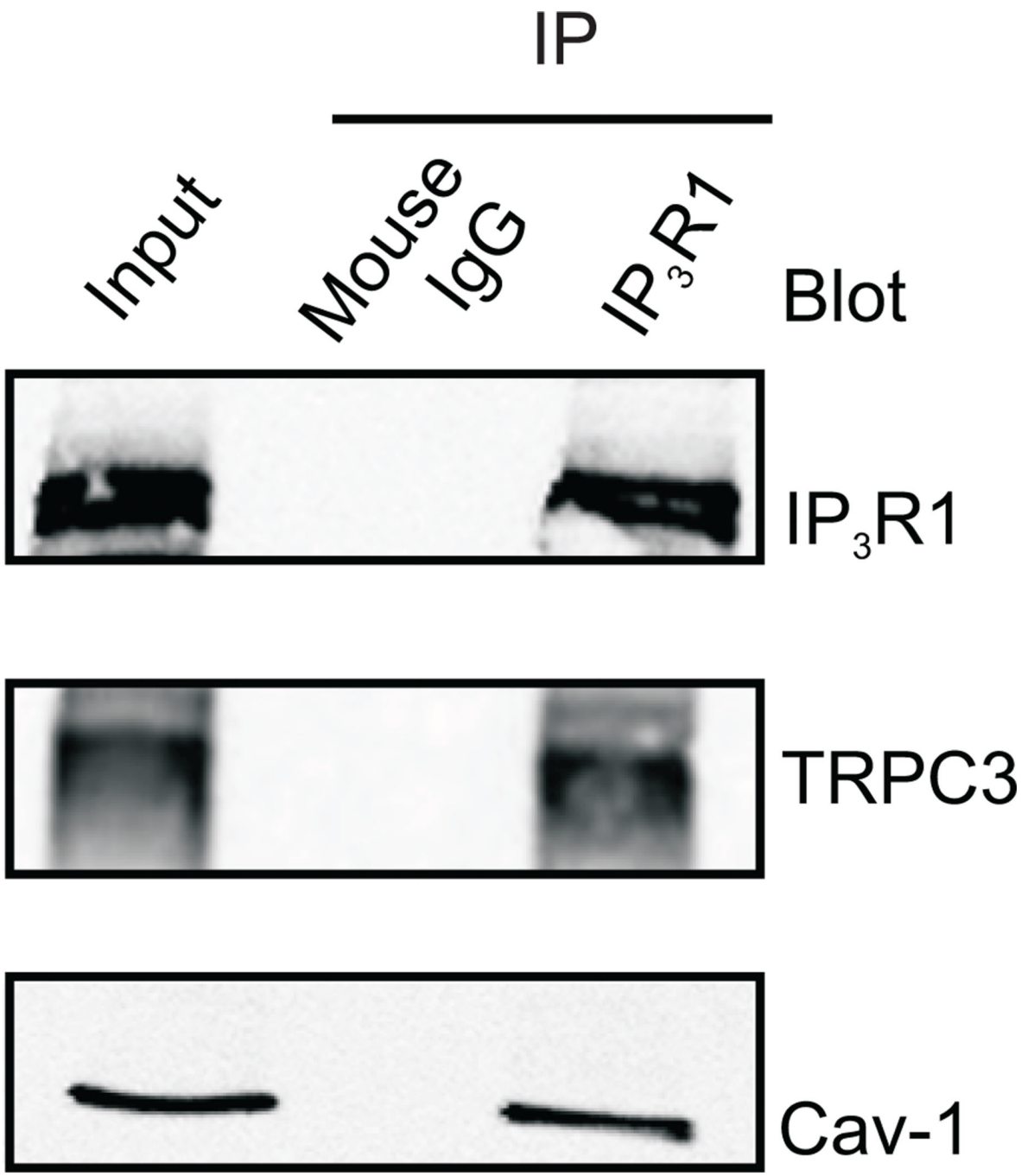

Figure 1. IP_3R1, TRPC3 and cav-1 are located in a macromolecular complex in rat mesenteric arteries

Monoclonal mouse anti-IP_3R1 antibody co-immunoprecipitated IP_3R1 (~270 kDa), TRPC3 (~90 kDa), and cav-1 (~ 22 kDa). Lysate supernatant (~40 μg protein) was used as the input control and mouse IgG as the negative control. Arteries pooled from ~ 8 rats were used in this experiment.

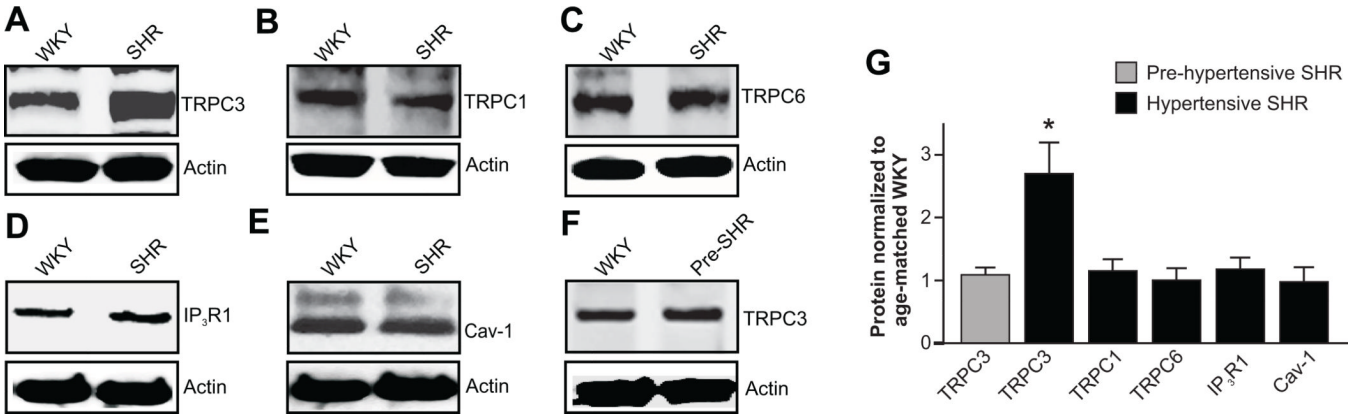

Figure 2. Hypertension is associated with an elevation in TRPC3, but not TRPC1, TRPC6, IP_3R1 or cav-1, protein in arteries

A–E, Western blots illustrating that TRPC3, but not TRPC1, TRPC6, IP_3R1 or cav-1 protein, is higher in mesenteric arteries of hypertensive (13 week old) SHR than age-matched WKY rats. **F**, Western blot of TRPC3 in 6 week old pre-hypertensive SHR and age-matched WKY rat arteries. **G**, mean data. N=4–5; $*P<0.05$.

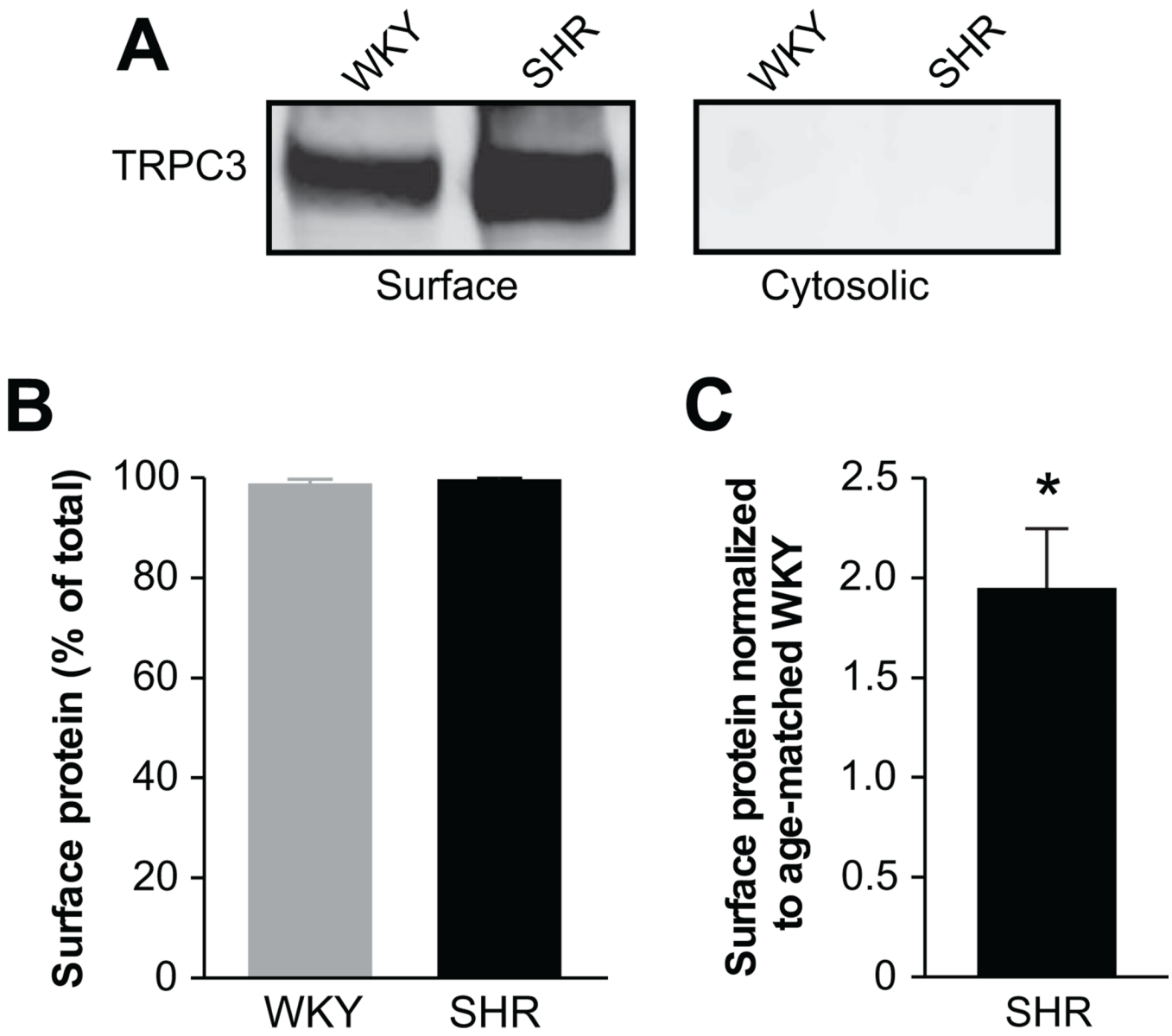

Figure 3. Plasma membrane-localized TRPC3 channel protein is higher in hypertensive SHR than WKY rat arteries

A, Western blot and **B**, mean data (n=4) illustrating cellular distribution of TRPC3 in SHR and WKY rat arteries. **C**, mean data indicating that surface TRPC3 channel protein is higher in hypertensive SHR than WKY arteries (n=4). Protein samples were run on the same gel, but lanes were not contiguous. *$P<0.05$

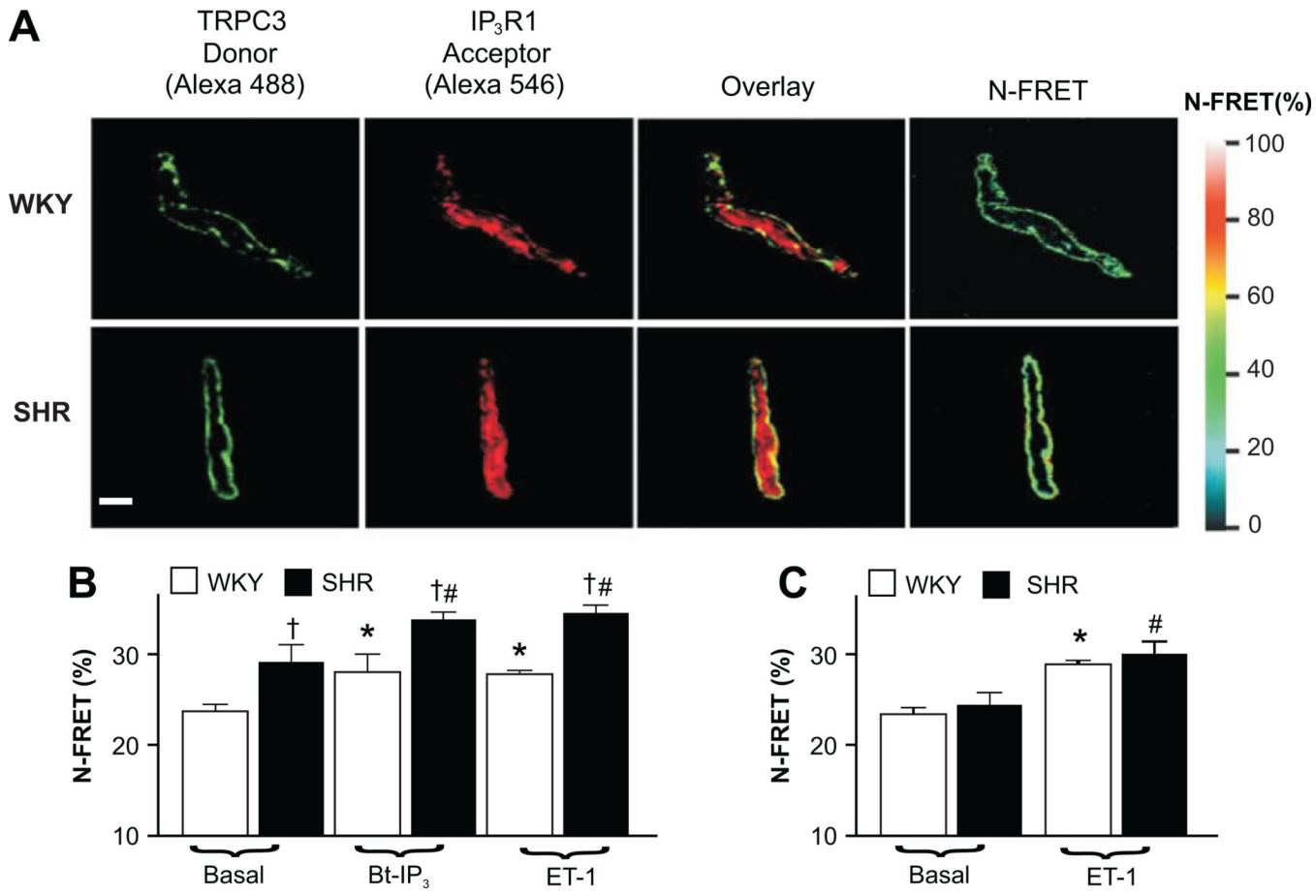

Figure 4. Hypertension is associated with an elevation in spatial localization of IP_3R1 and TRPC3 channels in mesenteric artery myocytes

A, Exemplar images illustrating imunofluorescence and N-FRET for IP_3R1 and TRPC3 channels in hypertensive SHR and WKY rat isolated myocytes. Shown are fluorescent images generated by Alexa 488- and 546-conjugated antibodies, pixel overlay, and N-FRET for the same cells. **B**, mean N-FRET data for IP_3R1 and TRPC3 channels in 13 week old rat myocytes (untreated control: WKY, n=9; SHR, n=6, Bt-IP_3, 10 nM; WKY, n=7; SHR, n=11, and ET-1, 10 nM; WKY, n=6; SHR, n=6). **C**, mean N-FRET data for IP_3R1 and TRPC3 channels in 6 week old rat myocytes (untreated control: WKY, n=6; SHR, n=6) and ET-1 (10 nM; WKY, n=6; SHR, n=7). $^{*}P<0.05$ versus WKY basal N-FRET; $^{\#}P<0.05$ versus SHR basal N-FRET; $^{\dagger}P<0.05$ versus WKY in the same condition. Scale bar, 10 μm.

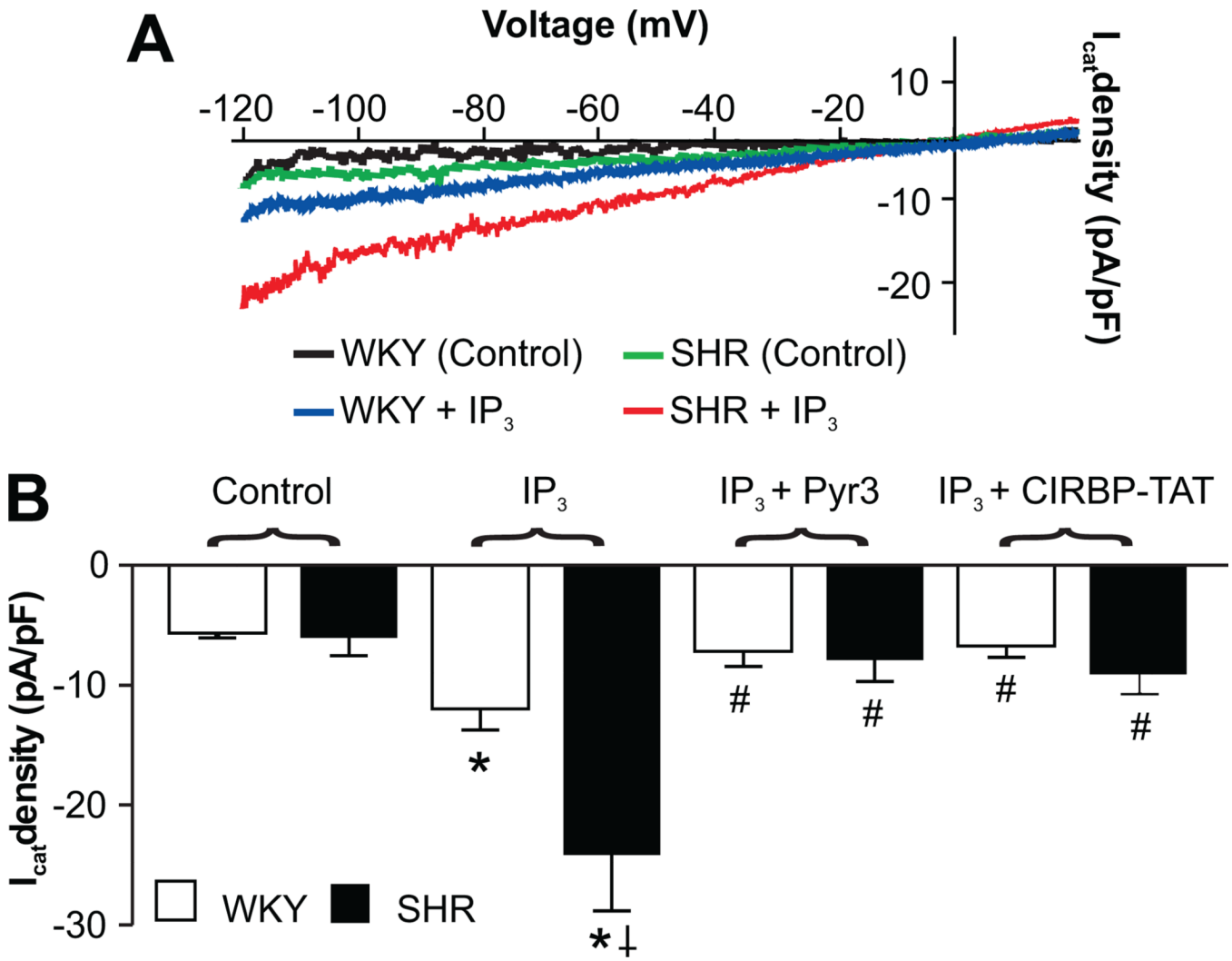

Figure 5. IP_3-induced I_{Cat} is larger in hypertensive SHR than WKY rat arterial myocytes

A, Exemplar traces and **B**, mean data indicating that control I_{Cat} density is similar and IP_3 (10 μM)-induced $I_{Ca}t$ is larger in hypertensive SHR than WKY rat myocytes. Pyr3 (1 μM) and CIRBP-TAT (10 μM) inhibited IP_3-induced I_{Cat} in both WKY and hypertensive SHR myocytes. Control WKY, n=4; control SHR, n=5; IP_3 WKY, n=6; IP_3 SHR, n=6; IP_3+Pyr3 WKY, n=7; IP_3+Pyr3 SHR, n=6; IP_3+CIRBP-TAT WKY, n=5; IP_3+CIRBP-TAT SHR, n=5. *P<0.05, versus control; $^{\dagger}P$<0.05 versus WKY; $^{\#}P$<0.05 versus IP_3.

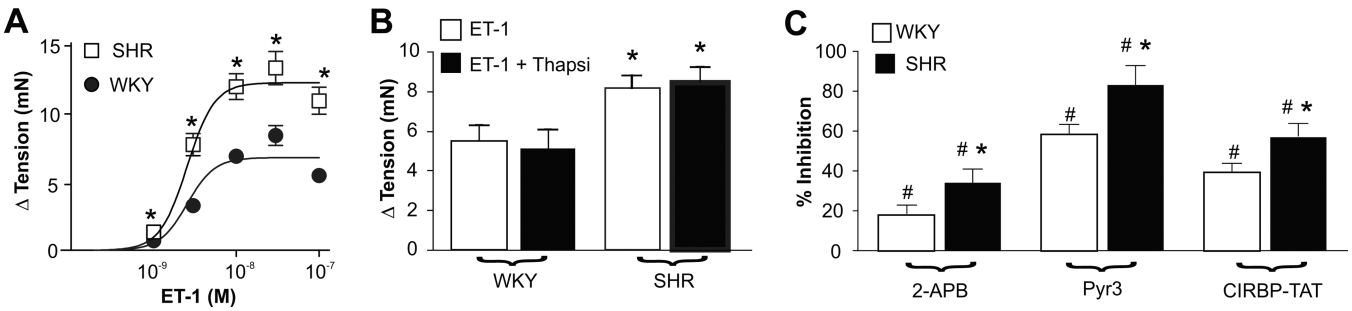

Figure 6. ET-1-induced vasoconstriction is larger in hypertensive SHR arteries, independent of SR Ca^{2+} release, and attenuated by 2-APB, Pyr3 and CIRB-TAT

A, ET-1 concentration-response in SHR (EC_{50}, 3.05 nM, n=8) and WKY (EC_{50}, 2.4 nM, n=6) arterial rings. **B**, mean data (SHR, n=6 for each; WKY, n=8 for each) in the absence and presence of thapsigargin (Thapsi; 100 nM). **C**, mean data illustrating that 2-APB (50 μM), Pyr3 (1 μM) and CIRBP-TAT (3 μM) more effectively inhibit ET-1 (10 nM)-induced constriction in SHR arteries (n=6–9) **P*<0.05, versus WKY; #*P*<0.05 versus control.