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Enhanced Ca^{2+}-Dependent Activation of PI3KC2α–Rho Axis in Blood Vessels of Spontaneously Hypertensive Rats

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Abstract

Rho-mediated inhibition of myosin light chain (MLC) phosphatase (MLCP), together with Ca^{2+}-dependent MLC kinase activation, constitutes the major signaling mechanisms for vascular smooth muscle (VSM) contraction. We recently unveiled the involvement of Ca^{2+}-induced, phosphoinositide 3-kinase (PI3K) class IIα-isoform (PI3K-C2α)-dependent Rho activation and resultant Rho kinase-dependent MLCP suppression in membrane depolarization- and receptor agonists-induced contraction. It is unknown whether Ca^{2+}- and PI3K-C2α-dependent regulation of MLCP is altered in VSM of hypertensive animals and is involved in hypertension. Therefore, we studied the role of the Ca^{2+}−PI3K-C2α−Rho−MLCP pathway in spontaneously hypertensive rats (SHRs). PI3K-C2α was readily detected in various vascular beds of Wistar-Kyoto rats and activated by high KCl. High KCl also stimulated vascular Rho activity and phosphorylation of the MLCP regulatory subunit MYPT1 at Thr^{853} in a PI3K inhibitor wortmannin-sensitive manner. In mesenteric and other vessels of SHRs at the hypertensive but not prehypertensive stage, the activity of PI3K-C2α but not class I PI3K p110α was elevated with concomitant rises of Rho activity and Thr^{853}-phosphorylation of MYPT1, as compared with normotensive controls. Infusion of the Ca^{2+} channel antagonist nicardipine reduced blood pressure with suppression of vascular activity of PI3K-C2α−Rho and phosphorylation of MYPT1 in hypertensive SHRs. Infusion of wortmannin lowered blood pressure with inhibition of PI3K-C2α−Rho activities and MYPT1 phosphorylation in hypertensive SHRs. These observations suggest that an increased

activity of the Ca^{2+}−PI3K-C2α−Rho signaling pathway with resultant augmented MLCP suppression contributes to hypertension in SHRs. The Ca^{2+}− and PI3K-C2α−dependent Rho stimulation in VSM may be a novel, promising target for treating hypertension.

Key words: hypertension, L-type Ca^{2+} channel, PI3K-C2α, Rho, myosin light chain phosphatase

Introduction

Accumulated evidence shows that enhanced peripheral vascular constriction is an important component in human essential hypertension and other animal models of hypertension.[1] Various contractile receptor agonists and mechanical strain induce vascular smooth muscle (VSM) contraction, contributing to vascular tone. Contractile stimuli induce an increase in the intracellular free Ca^{2+} concentration, which mediates myosin light chain kinase (MLCK) activation to result in phosphorylation of the 20 kDa myosin light chain (MLC) and the initiation of actin-myosin interaction.[2,3] Various receptor agonists also activate the distinct signaling pathway comprising Rho and its effector Rho kinase via $G_{12/13}$, which leads to inhibition of myosin light chain phosphatase (MLCP) through mechanisms involving Rho kinase-dependent phosphorylation of the MLCP-regulatory subunit MYPT1 at the inhibitory phosphorylation sites Thr^{853} and/or Thr^{696} (numbering of human MYPT1).[2-9] Phosphorylation of MYPT1 at Thr^{853} is to the larger extent stimulated by receptor agonists in a Rho kinase-dependent manner compared with phosphorylation at Thr^{696}.[10,11] We and others previously demonstrated in VSM that membrane depolarization-induced Ca^{2+} influx elicits Rho activation and consequent Rho kinase-dependent phosphorylation of MYPT1 at Thr^{853} and to the lesser extent at Thr^{696} (Ca^{2+}-induced Ca^{2+}-sensitization).[11-14] The Ca^{2+}-induced Rho activation and Rho kinase-dependent MLCP inhibition are dependent on phosphoinositide 3-kinase (PI3K) class II α-isoform (PI3K-C2α).[11,15] This Ca^{2+}-induced, PI3K-C2α-dependent Rho activation mechanism

is also involved in receptor agonist-induced Rho stimulation.[11, 15] Thus, in VSM stimulated by either receptor agonists or membrane depolarization, MLCK activation and MLCP inhibition synergistically act to induce effective MLC phosphorylation and contractile responses.

In our previous study[11], we observed that administration of the PI3K inhibitor, wortmannin, into normal rats induced dose-dependent reductions in arterial blood pressure with suppression of vascular activities of PI3K-C2α and Rho and phosphorylation level of MYPT1, suggesting that PI3K-C2α plays a substantial role *in vivo* in maintaining vascular tone and blood pressure through regulating Rho and MLCP. Previous studies suggested that hyperactivity of Rho-Rho kinase pathway augments Ca^{2+}-sensitivity of myofilaments in VSM of rat hypertensive models including spontaneously hypertensive rats (SHRs) and human hypertensive patients.[9, 16-20] However, it is totally unknown whether Ca^{2+}-induced, PI3K-C2α-dependent regulation of Rho and MLCP is altered in VSM of hypertensive animals and is involved in hypertension.

In the present study, we studied the role of the Ca^{2+}−PI3K-C2α−Rho−MLCP pathway in hypertension by comparing vascular activities of PI3K-C2α, Rho and MLCP between SHRs and normotensive Wistar-Kyoto (WKY) rats and examining the effects of the Ca^{2+}-channel antagonist nicardipine and WMN on blood pressure and vascular activities of PI3K-C2α, Rho and MLCP in SHRs. Our results show that vascular PI3K-C2α activity, Rho activity, and MYPT1 phosphorylation at Thr^{853} in blood vessels are all elevated in SHRs. Either nicardipine or wortmannin effectively reversed hyperactivities of PI3K-C2α and Rho and

hyperphosphorylation of MYPT1 in blood vessels with correction of hypertension. The observations suggest the critical role of the Ca^{2+}−PI3K-C2α−Rho−MLCP pathway in hypertension of SHRs.

Materials and Methods

The extended materials and methods used are described in the online Data Supplement.

Results

The expression of PI3K-C2α, membrane depolarization-induced stimulation of PI3K-C2α, Rho and MYPT1 phosphorylation, and their inhibition by wortmannin in various vascular beds of WKY rats

We studied the expression of PI3K-C2α in various vascular beds of normotensive WKY rats. Western analysis readily detected PI3K-C2α protein in all endothelium-denuded blood vessels examined, which included aorta, carotid artery, pulmonary artery, mesenteric artery, and femoral artery and vein (Figure 1A). These vascular beds also expressed class I PI3K p110α. The PI3K inhibitor wortmannin inhibited high KCl membrane depolarization-induced contraction of these blood vessels with the similar dose-response relationships (Figure 1 B). The structurally different

PI3K inhibitor LY294002 also inhibited contraction in a dose-dependent manner (Fig. S1). Wortmannin suppressed PI3K-C2α activity in aortae with the similar dose-response relationship as that for inhibition of the contractile response (Fig. S2). In the employed *in vitro* assay condition for immunoprecipitated PI3K-C2α activity, we confirmed a linear time-dependent increase in the PI3K-C2α product (Fig. S3). High KCl stimulation of aortae and mesenteric arteries induced 1.8-2.0 fold increases in the activity of PI3K-C2α immunoprecipitated from the blood vessels, which were abolished by pretreatment of vessels with wortmannin (Figure 2 A). High KCl also increased the amounts of GTP-bound, active RhoA (GTP-Rho) (Figure 2 B). Consistent with this, high KCl stimulated phosphorylation at Thr^{853} of MYPT1 in aortae and mesenteric arteries (Figure 2 C). Wortmannin inhibited KCl-induced increases in both RhoA activity and MYPT1 phosphorylation at Thr^{853}. High KCl did not change phosphorylation at Thr^{696} of MYPT1 in aortae (Figure S4 A). Wortmannin, which also inhibits MLCK in a ATP-sensitive manner[21], did not inhibit MLCK in permeabilized VSM cells or isolated MLCK at a physiological mM order of ATP concentration (Fig. S5).

Increases in the activities of PI3K-C2α and Rho and phosphorylation of MYPT1 in aortae of hypertensive SHRs but not prehypertensive young SHRs

Twelve-week-old SHRs exhibited a marked increase in systolic blood pressure compared with age-matched WKY rats (180±2 (n=13) in SHRs vs. 113±2 mm Hg (n=13) in WKY rats, $p<0.01$).

PI3K-C2α activity in aortae of SHRs was 2.7-fold higher than in WKY rats (Figure 3A). The expression level of PI3K-C2α protein in the vessels was similar between SHRs and WKY rats when the same amounts of the tissue protein were analyzed (Figure 3 A, *middle panel of Western blots*). In contrast, the activity and protein expression level of class I PI3K p110α in blood vessels were similar between SHRs and WKY rats (Figure 3 B). We compared vascular PI3K-C2α activity in young 4-week-old SHRs at the prehypertensive stage with that of 12-week-old hypertensive SHRs. PI3K-C2α activity in 4-week-old SHRs (systolic blood pressure 117±2 mm Hg (n=4)) was similar to that in normotensive 12-week-old WKY rats (110±4 mm Hg (n=4)), and lower than in 12-week-old SHRs (176±2 mm Hg, n=4) whereas the expression level of PI3K-C2α protein in vessels was not different among the three rat groups (Figure 3 C). In isolated 12-week-SHR aortae, both the basal and KCl-stimulated PI3K-C2α activities were higher compared with WKY vessels (Figure S6). The amount of GTP-Rho in 12-week-old SHRs was 2.2-fold higher than in the age-matched WKY rats (Figure 3 D), which is consistent with the report by Seko et al.[18] Consistently, the extent of phosphorylation at Thr^{853} of MYPT1 were elevated in aortae of 12-week-old SHRs than in age-matched WKY rats (Figure 3 E) whereas phosphorylation at Thr^{696} of MYPT1 was not different between WKY rats and SHRs (Figure S4 B). These observations suggest that MLCP in VSM is suppressed in hypertensive SHRs compared with normotensive WKY rats.

Ca^{2+} channel blocker nicardipine lowers systolic blood pressure, the activities of PI3K-C2α and Rho, and MYPT1 phosphorylation in aortae of hypertensive SHRs

We previously showed that PI3K-C2α is stimulated in a Ca^{2+}-dependent manner in isolated VSM contracted by either membrane depolarization or receptor agonists.[11, 15] Therefore, we tested the *in vivo* effects of the Ca^{2+}-channel antagonist nicardipine on the activities of PI3K-C2α, Rho, and MLCP in vessels of SHRs. Infusion of either nicardipine or hydralazine, the latter of which was used as a control anti-hypertensive, induced similar magnitudes of decreases in systolic blood pressure with the maximal declines of about 85 mm Hg and 80 mm Hg, respectively, at 10 min after infusion in 12-week-old SHRs (Figure 4 A). Nicardipine but not hydralazine reduced vascular PI3K-C2α activity in aortae of SHRs to the level in age-matched WKY control rats (Figure 4 B). Nicardipine also lowered Rho activity and Thr^{853} phosphorylation levels of MYPT1 in aortae of SHR nearly to the levels in WKY rats (Figure 4 C and 4 D). Nicardipine did not change Thr^{696} phosphorylation of MYPT1 (Figure S4 B). Hydralazine did not significantly inhibit Rho activity or phosphorylation of MYPT1 in SHRs.

Wortmannin lowers systolic blood pressure, the activities of PI3K-C2α and Rho, and MYPT1 phosphorylation in aortae of hypertensive SHRs

In normotensive rats, we previously showed that the PI3K inhibitor wortmannin reduced blood pressure due to its direct vasodilator effect.[11, 22] We tested the effects of wortmannin infusion on

blood pressure and vascular activities of PI3K-C2α, Rho and MLCP in SHRs. Wortmannin substantially lowered systolic blood pressure with the maximal decline of about 70 mm Hg at 10 min after infusion in 12-week-old SHRs (Fig. 5 A). Wortmannin infusion reduced enhanced activities of PI3K-C2α activity and Rho activity and Thr^{853} phosphorylation of MYPT1 in aortae of SHRs (Figure 5 B-D). Wortmannin did not affect Thr^{696} phosphorylation of MYPT1 (Figure S4 B).

Nicardipine and wortmannin inhibit the activities of PI3K-C2α and Rho and phosphorylation of MYPT1 in mesenteric arteries of hypertensive SHRs

We studied the effects of infusions of nicardipine and wortmannin on the activity of PI3K-C2α−Rho−MLCP pathway in the resistance artery, mesenteric artery. The infusions of the same doses of either nicardipine or wortmannin as in Figures 4 and 5 suppressed PI3K-C2α activity in mesenteric artery with the declines of blood pressure (Figure 6 A and 6 B). Nicardipine and wortmannin also decreased the amounts of GTP-Rho and MYPT1 phosphorylation at Thr^{853} (Figure 6 C and 6 D). Similarly as in aortae, phosphorylation at Thr^{696} of MYPT1 in mesenteric arteries was not different between WKY rats and SHRs (Figure S4 C).

These observations collectively suggest that Ca^{2+}-dependent PI3K-C2α stimulation in VSM contributes to hypertension in SHRs through the mechanism involving the regulation of the Rho-MLCP pathway.

Discussion

High blood pressure is a disease affecting 20-30% of the world's adult population.[23] Although pathogenic mechanisms of hypertension are not completely elucidated, it is widely accepted that increased peripheral VSM contraction is an important mechanism underlying the development of hypertension.[1] VSM tone is regulated by multitudes of contractile and relaxing stimuli including neurotransmitters, circulating vasoactive hormones, locally produced vasoactive substances, and mechanical forces.[1, 3, 4] The involvement of hyperactivities of these contractile stimuli in hypertension was intensively investigated previously. However, possible alterations of the intracellular contractile signaling in VSM of hypertensive animals are only poorly understood. Ca^{2+} is the primary intracellular messenger to determine contractile state of VSM through regulating MLCK-catalyzed phosphorylation of MLC.[2, 4] Recently, we found that Ca^{2+} also activates the small GTPase Rho via PI3K-C2α to result in MLCP suppression.[11-12, 15, 22, 24] The identification of this second Ca^{2+}-activated contractile signaling pathway comprising PI3K-C2α−Rho−MLCP implies that Ca^{2+} induces stimulation of MLC phosphorylation and contraction through both MLCK activation and MLCP inhibition.[13, 14, 23]

In the present study, we showed that PI3K-C2α is widely expressed in various vascular beds including aorta, the muscular and smaller resistance arteries, and vein (Fig. 1 A). In vessels isolated from these vascular beds, wortmannin inhibited membrane depolarization-induced contraction with the similar dose-response relationships among these vessels (Fig. 1 B). These

observations suggest that a PI3K-dependent contractile mechanism is operating in various vascular beds of rats. The observed dose-response relationship is similar to that observed in rabbit blood vessels of our previous study[11], suggesting that a responsible PI3K is relatively less sensitive to wortmannin, which is a characteristic of PI3K-C2α but not any other PI3K member.[25] Consistent with this, wortmannin inhibited KCl-induced PI3K-C2α activation in the blood vessels with the similar dose-response relationship to that for contraction (Figure S2). A previous study[21] demonstrated a MLCK inhibitor activity of wortmannin. Our previous observations[11, 15] and present results together suggest that the major *in vivo* target of wortmannin in inhibition of VSM contraction is PI3K-C2α but not MLCK in VSM although it is not totally precluded that wortmannin inhibition of MLCK to some extent contributes to inhibition of contraction.

SHRs are the hypertension model that at least in part involves blood vessel hypercontractility and increased vascular Rho activity through mechanisms including endothelial dysfunction, increased sympathetic tone, and/or abnormalities inherent in VSM.[1, 16-18, 23] The present observations suggest the link of PI3K-C2α, which we previously showed to be involved in Ca^{2+}-induced stimulation of Rho- and Rho kinase-dependent MLCP inhibition,[11, 15] to an increased Rho activity in vessels of hypertensive SHRs: first, the activity of PI3K-C2α but not class I PI3K p110α in mesenteric arteries and aortae of hypertensive SHRs is elevated with concomitant increased Rho activity and MYPT1 phosphorylation as compared with

normotensive, age-matched WKY rats (Figs. 3 and 6). Secondly, PI3K-C2α is activated by membrane depolarization-induced Ca^{2+} entry in blood vessels with the larger extent of activation in SHRs (Fig. 2 and Fig. S6). Thirdly, blockade of Ca^{2+} entry by nicardipine infusion suppressed vascular activities of PI3K-C2α and Rho with a decline of blood pressure in hypertensive SHRs (Figs. 3 and 6). Finally, infusion of the PI3K inhibitor wortmannin into hypertensive SHRs suppressed vascular Rho activity with a decrease in blood pressure. Moreover, our data in nicardipine- or wortmannin-administered hypertensive SHRs show that inhibition of vascular Rho activity was accompanied by reductions in phosphorylation of MYPT1 at the inhibitory site Thr^{853} in hypertensive SHRs. Thus, these findings suggest that elevated vascular Rho activity results in augmented suppression of MLCP and consequently contributes to increased vascular contraction and hypertension in SHRs. This is the first evidence for an altered activity of vascular PI3K-C2α–Rho-MLCP axis in hypertension and the involvement of this axis in hypertension. We previously showed that wortmannin infusion into normotensive rats resulted in inhibition of vascular PI3K-C2α with reductions in Rho activity, MYPT1 phosphorylation, and blood pressure.[12] These observations together suggest that PI3K-C2α in VSM is involved in the blood pressure regulation under both the physiological and pathological conditions through its effects on the Rho-MLCP pathway. It is unknown at present how PI3K-C2α is involved in Rho activation in VSM.

In isolated VSM, PI3K-C2α is activated by Ca^{2+} entry across the plasma membrane

particularly through L-type voltage-dependent Ca^{2+} channels (Fig. 2 A).[12] The inhibitory effects of nicardipine infusion on not only PI3K-C2α activity but also Rho and MYPT1 phosphorylation indicated that Ca^{2+} influx is a major regulator of the PI3K-C2α−Rho−MLCP pathway in blood vessels of hypertensive SHRs *in vivo*. Several lines of evidence indicates that Ca^{2+} entry through L-type of Ca^{2+} channels in VSM of hypertensive SHRs is elevated, which plays a critical role in elevation of vascular tone. For example, Ca^{2+} channel blockers at doses that had little hypotensive effect in normotensive WKY rats profoundly reduced blood pressure in hypertensive SHRs.[26] VSM isolated from hypertensive SHRs shows augmented contraction in responses to stimulation of voltage-dependent Ca^{2+} channels by Bay k 8644.[27] In addition, blood vessels from SHRs exhibit upregulation of L-type of Ca^{2+} channel expression.[28] Differently from adult hypertensive SHR, vascular PI3K-C2α activity in young prehypertensive SHRs, in which alterations in Ca^{2+} channel activity are not detected,[26] was not elevated compared with normotensive WKY rats (Fig. 3). Hence, increased vascular PI3K-C2α activity in hypertensive SHRs may be secondary to increased Ca^{2+} entry through L-type of Ca^{2+} channels.

Unlike the Ca^{2+} channel antagonist nicardipine, the vasodilator hydralazine was nearly ineffective in inhibiting PI3K-C2α−MLCP pathway but with a marginal effect on Rho (Fig. 4). Although the molecular mechanism of the vasodilator effect of hydralazine is not yet completely defined, available evidence suggests that the major site of hydralazine action is inhibition of inositol-1,4,5-trisphosphate-induced Ca^{2+} release from the sarcoplasmic reticulum.[29] Relative

ineffectiveness of hydralazine on the PI3K-C2α−Rho pathway may suggest that PI3K-C2α in VSM is more sensitive to Ca^{2+} entry across the plasma membrane than Ca^{2+} mobilized from the intracellular Ca^{2+} store *in vivo*. Because upregulation of PI3K-C2α activity in blood vessels from hypertensive rats is still detectable in the immunoprecipitation-lipid kinase assay after tissue homogenization in the presence of a Ca^{2+} chelator, the stimulatory effect of Ca^{2+} on the PI3K-C2α−Rho pathway is likely indirect. It remains to be clarified how increased Ca^{2+} entry brings about stimulation of PI3K-C2α activity in VSM. The previous investigation[17] showed that the Ca^{2+}-sensitivity of myofilaments is increased in β-escin-permeabilized artery from SHRs. It is interesting to examine possible alterations of the PI3K-C2α−Rho−MLCP pathway in β-escin-permeabilized artery from SHRs.

Perspectives

The present study demonstrated a rise in the activity of PI3K-C2α in mesenteric arteries and other vessels of hypertensive SHRs compared with normotensive control rats and pre-hypertensive SHRs. Increased PI3K-C2α activity brought about stimulation of Rho and consequently augmented MLCP suppression in blood vessels. Ca^{2+} entry plays a critical role in the altered activity of PI3K-C2α−Rho−MLCP pathway *in vivo* in hypertensive SHRs. The correction of the altered activity of Ca^{2+}−PI3K-C2α−Rho−MLCP leads to lowering of blood pressure in SHRs, suggesting a causal link of this pathway to development of hypertension in

SHRs and the possibility that the pathway may be a promising molecular target for novel, anti-hypertensive drugs. The present study also provided novel mechanistic insights into the role of Ca^{2+} in contraction of VSM and the action of a Ca^{2+} channel antagonist.

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Disclosures

None

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Figure Legends

Figure 1. Expression of PI3K-C2α protein in various vascular beds of rats and wortmannin sensitivity of KCl membrane depolarization-induced contraction. A, Six different segments of endothelium-denuded blood vessels isolated from 10-week-old WKY rats were analyzed for the expression of PI3K-C2α, class I PI3K p110α, and α-smooth muscle actin (α-SMA) as internal control by Western blot analysis. A; artery, V; vein. B, Isometric tension of various vascular segments stimulated with 60 mM KCl in the presence of indicated concentractions of wortmannin. Wortmannin (WMN) was added 60 min prior to KCl stimulation. The data are expressed as means±SE of 4 values.

Figure 2. KCl-induced stimulation of activities of PI3K-C2α and Rho and phosphorylation of MYPT1 and their inhibition by wortmannin in aortae and mesenteric arteries of WKY rats. Denuded aortae or mesentery arteries were stimulated with 60 mM KCl for 5 min (A, B) or 10 min (C) after pretreatment with 1 μM WMN for 1 h. Vessels were quickly isolated from WKY rats, rapidly frozen as described in "Materials and Methods", and analyzed for PI3K-C2α activity (A), the amounts of GTP-bound RhoA (GTP-Rho) (B), and phosphorylation of MYPT1 (C) by the immunoprecipitation kinase assay for PI3K assay, the pull-down assay for GTP-Rho, and Western blotting using anti-phospho (Thr^{853})-MYPT1. Portions of tissue homogenates were also analyzed for protein amounts of PI3K-C2α and α-SMA (A), RhoA (B), and total

MYPT1(C), and shown in the lower panels of Western blots. Values are means±SE. The numbers in parentheses on the top of bars indicate the numbers of analyzed rats.

Figure 3. Stimulation of activities of PI3K-C2α and Rho and phosphorylation of MYPT1 in aortae of SHRs. Aortae were quickly isolated from SHRs and WKY rats of indicated ages, rapidly frozen as described in "Materials and Methods", and analyzed for PI3K-C2α activity (A and C), PI3K-p110α activity (B), and the amounts of GTP-bound RhoA (GTP-Rho) (D), and phosphorylation of MYPT1 (E) by the immunoprecipitation kinase assay for PI3K assay, the pull-down assay for GTP-Rho, and Western blotting using anti-phospho (Thr^{853})-MYPT1. Portions of tissue homogenates were also analyzed for protein amounts of PI3K-C2α (A and C), PI3K-p110α (B), α-SMA (A-C), RhoA (D), and total MYPT1(E), and shown in the lower panels of Western blots. In (C), 4-week (wk)-old SHRs at the prehypertensive stage, 12-wk-old hypertensive SHRs and 12-wk-old normotensibe WKY rats were analyzed. Values are means±SE. The numbers in parentheses on the top of bars indicate the numbers of analyzed rats. The symbols * and ** denote statistical significance at the level of $p<0.05$ and $p<0.01$, respectively, compared with "WKY" and 4-week SHRs in C.

Figure 4. Ca^{2+} channel blocker nicardipine lowers the activities of PI3K-C2α and Rho, and phosphorylation of MYPT1 in aortae of SHRs. Nicardipine (NIC) (1 mg/kg BW), hydralazine

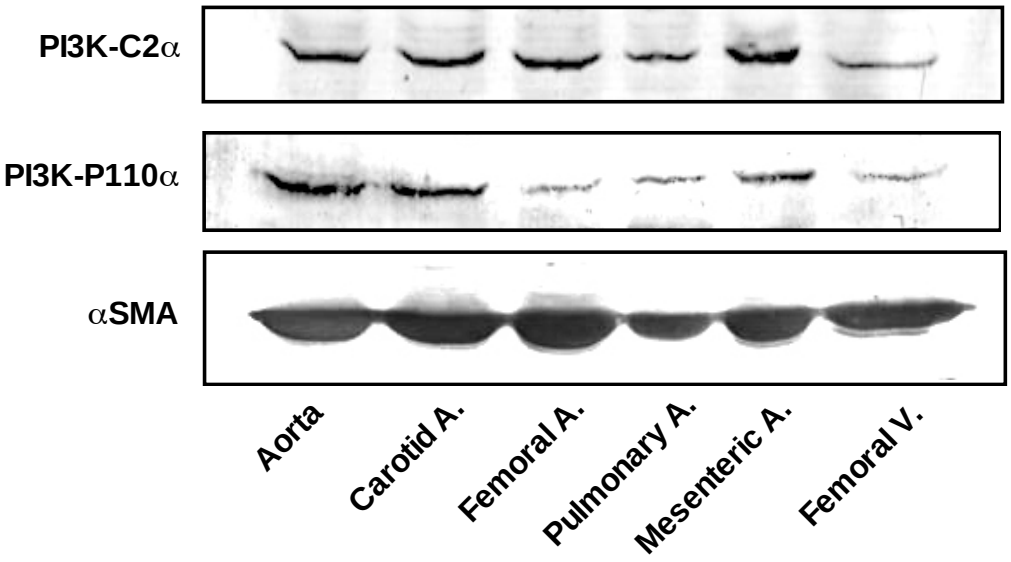
(HDZ) (10 mg/kg BW) or vehicle solution were infused through the tail vein of conscious 12 week-old SHR rats. 10 min later, rats were sacrificed and the blood vessels were isolated and analyzed as in Fig. 1. (A) systolic blood pressure (BP) at 10 min after infusion. (B) PI3K-C2α activity. (C) the amounts of GTP-Rho. (D) phosphorylation of MYPT1 at Thr^{853}. Values are means±SE. The symbols * and ** denote statistical significance at the levels of $p<0.05$ and $p<0.01$, respectively, compared with WKY rats. The symbol †† denotes statistical significance at the level of $p<0.01$ compared with SHRs receiving vehicle.

Figure 5. Wortmannin lowers the activities of PI3K-C2α and Rho, and phosphorylation of MYPT1 in aortae of SHRs. Wortmannin (WMN) (5 mg/kg BW) or vehicle solution was infused through the tail vein of conscious 12 week-old SHRs. (A) systolic blood pressure (BP) at 10 min after infusion. (B) PI3K-C2α activity. (C) the amounts of GTP-Rho. (D) phosphorylation of MYPT1 at Thr^{853}. Values are means±SE. The symbol † denotes statistical significance at the level of $p<0.05$ compared with SHRs receiving vehicle.

Figure 6. Infusion of nicardipine and wortmannin lower the activities of PI3K-C2α and Rho, and phosphorylation of MYPT1 in mesenteric arteries of SHRs. Nicardipine, wortmannin or vehicle solution were infused through the tail vein of conscious 12-week-old SHRs and age-matched WKY rats and analyzed as described in Figures 4 and 5. (A) systolic blood pressure

(BP) at 10 min after infusion. (B) PI3K-C2α activity. (C) the amounts of GTP-Rho. (D) phosphorylation of MYPT1 at Thr^{853}. Values are means±SE. The symbols * and †denote statistical significance at the level of $p<0.05$ compared with WKY rats and SHRs receiving vehicle, respectively.

A


PI3K-C2α
PI3K-P110α
αSMA
Aorta
Carotid A.
Femoral A.
Pulmonary A.
Mesenteric A.
Femoral V.

B

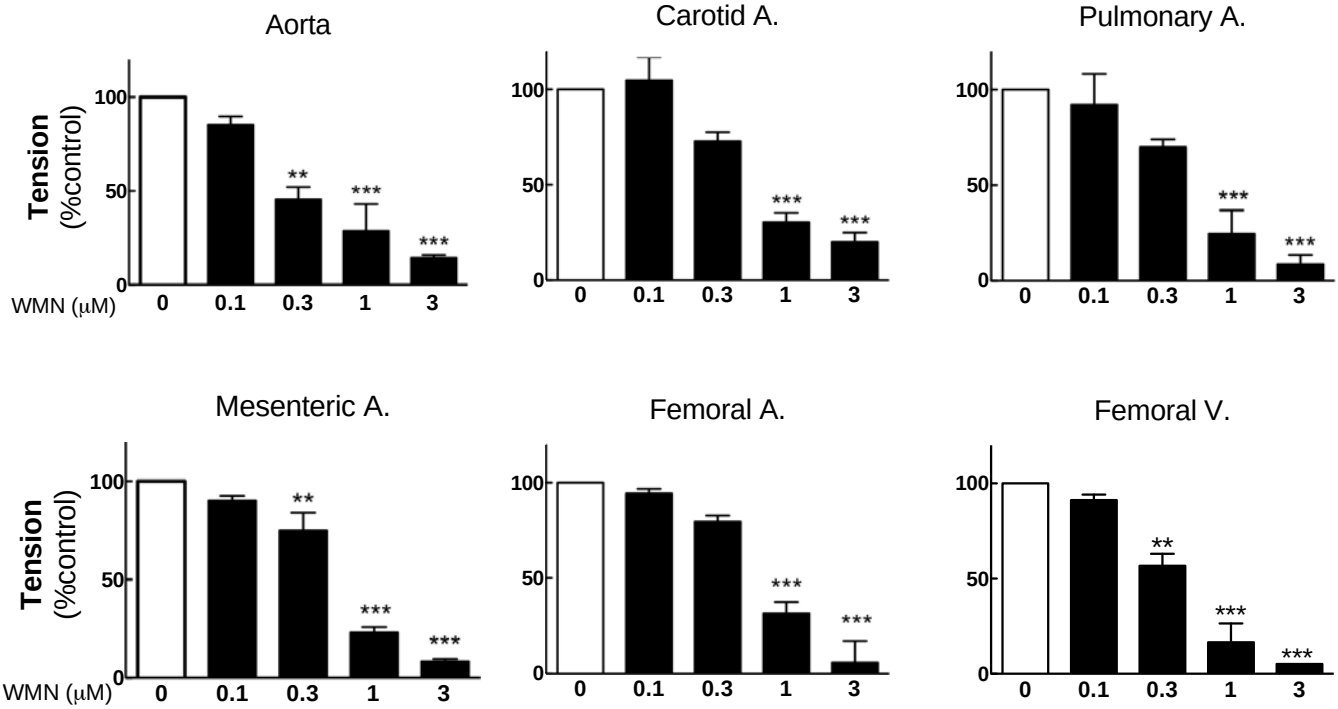

Aorta
Carotid A.
Pulmonary A.
Mesenteric A.
Femoral A.
Femoral V.
Tension (%control)
WMN (μM)
0
0.1
0.3
1
3
50
100
**
***

Figure 1

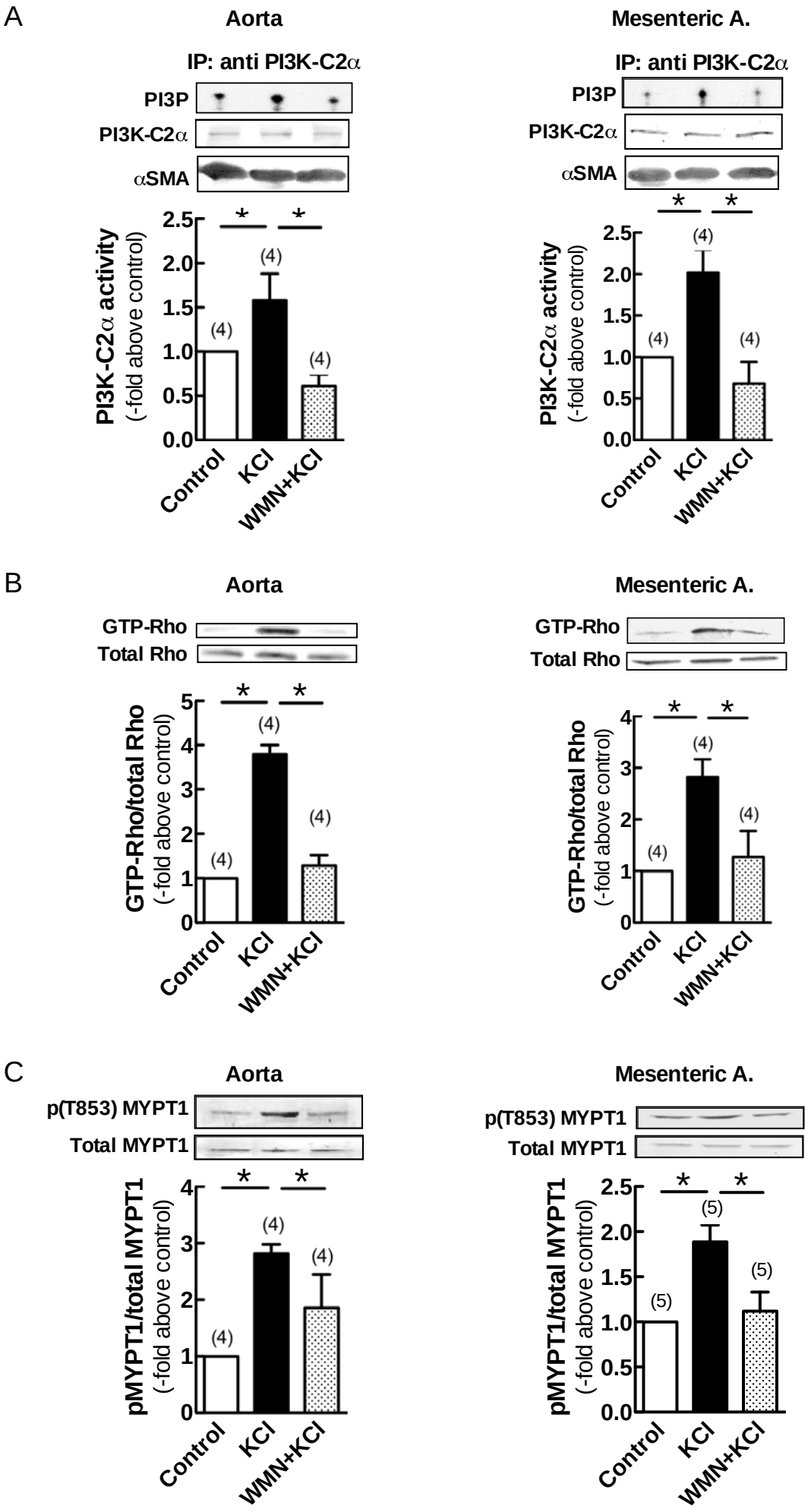

A
Aorta
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
PI3K-C2α activity
(-fold above control)
Control
KCl
WMN+KCl
Mesenteric A.
B
GTP-Rho
Total Rho
GTP-Rho/total Rho
C
p(T853) MYPT1
Total MYPT1
pMYPT1/total MYPT1

Figure 2

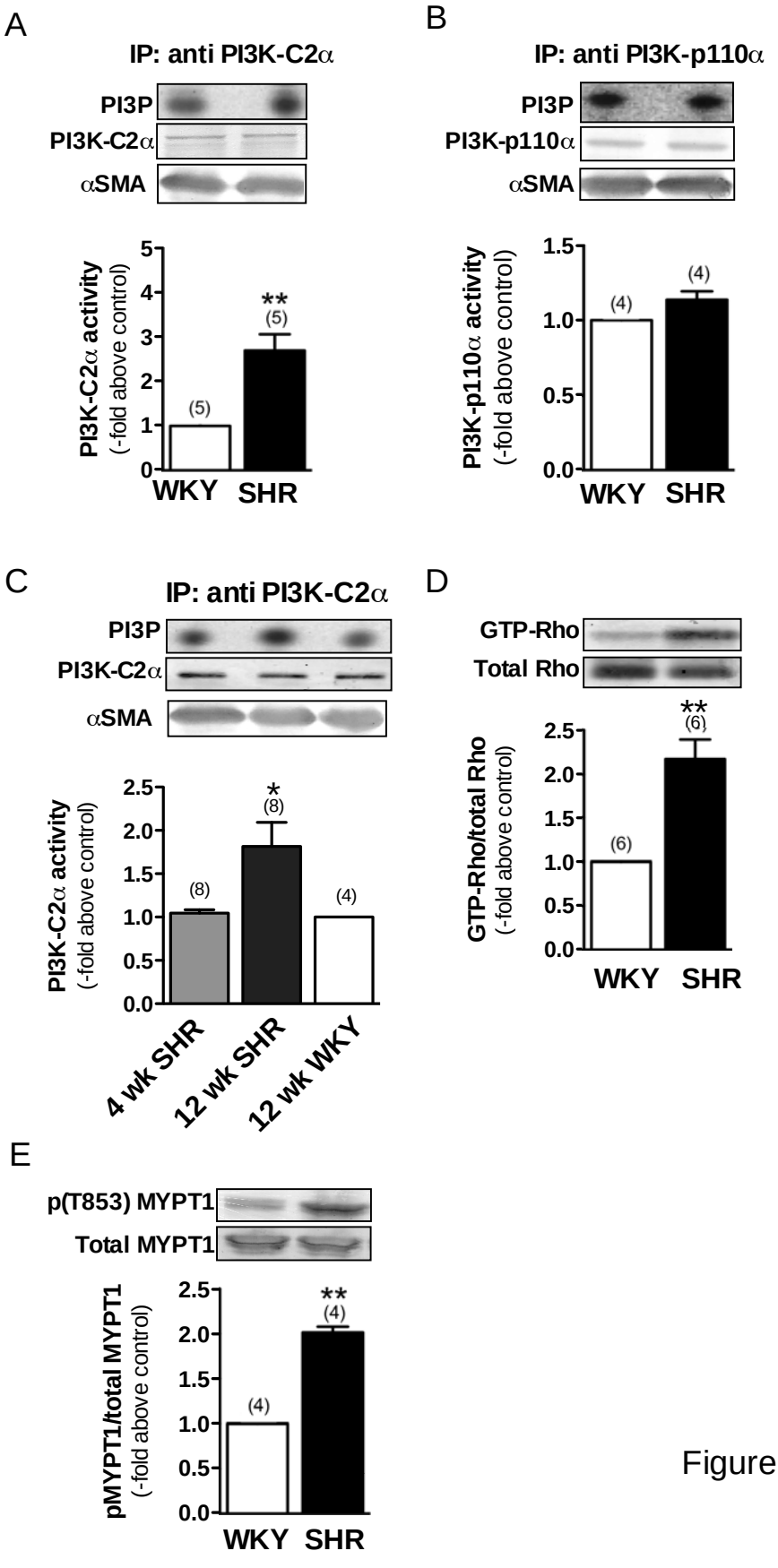

A
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
PI3K-C2α activity
(-fold above control)
5
4
3
2
1
0
**
(5)
(5)
WKY
SHR
B
IP: anti PI3K-p110α
PI3P
PI3K-p110α
αSMA
PI3K-p110α activity
(-fold above control)
1.5
1.0
0.5
0.0
(4)
(4)
WKY
SHR
C
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
PI3K-C2α activity
(-fold above control)
2.5
2.0
1.5
1.0
0.5
0.0
*
(8)
(8)
(4)
4 wk SHR
12 wk SHR
12 wk WKY
D
GTP-Rho
Total Rho
GTP-Rho/total Rho
(-fold above control)
**
(6)
(6)
WKY
SHR
E
p(T853) MYPT1
Total MYPT1
pMYPT1/total MYPT1
(-fold above control)
**
(4)
(4)
WKY
SHR

Figure 3

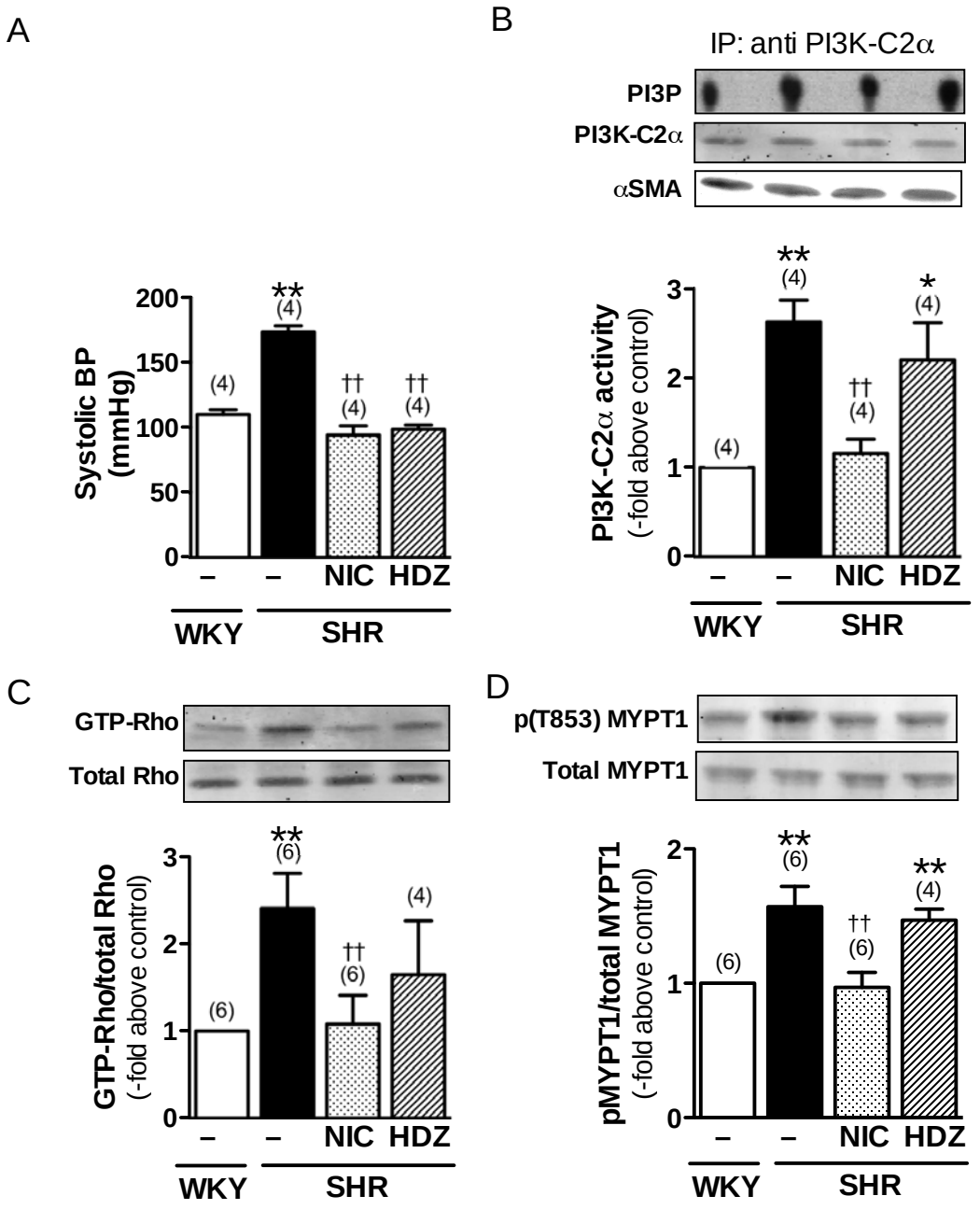

A
B
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
Systolic BP (mmHg)
200
150
100
50
0
**
(4)
(4)
††
(4)
††
(4)
–
–
NIC
HDZ
WKY
SHR
PI3K-C2α activity
(-fold above control)
3
2
1
0
**
(4)
(4)
††
(4)
*
(4)
C
D
GTP-Rho
Total Rho
GTP-Rho/total Rho
(-fold above control)
**
(6)
(6)
††
(6)
(4)
p(T853) MYPT1
Total MYPT1
pMYPT1/total MYPT1
(-fold above control)
**
(6)
(6)
††
(6)
**
(4)

Figure 4

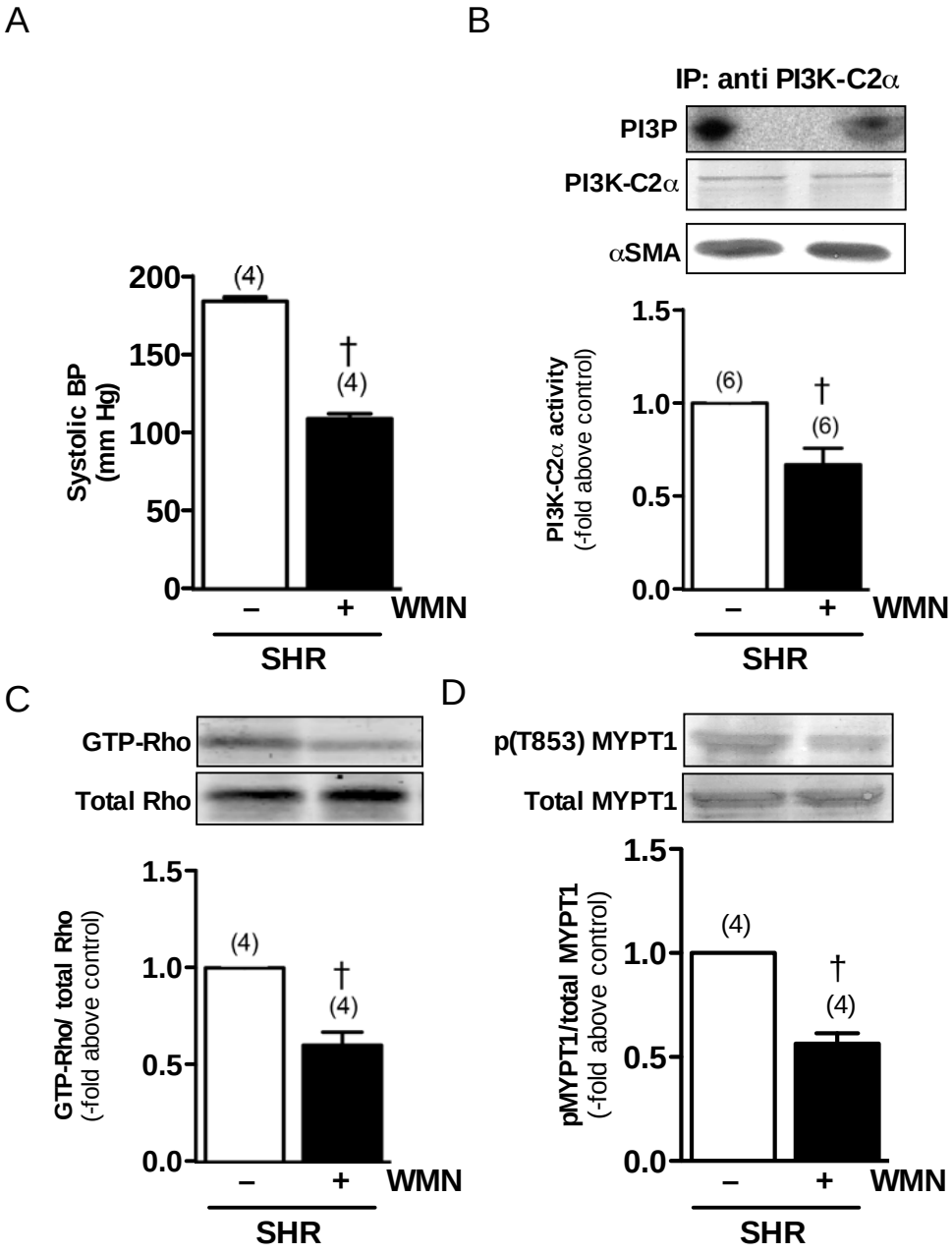

A
B
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
Systolic BP (mm Hg)
200
150
100
50
0
(4)
†
(4)
– + WMN
SHR
PI3K-C2α activity (-fold above control)
1.5
1.0
0.5
0.0
(6)
†
(6)
– + WMN
SHR
C
D
GTP-Rho
Total Rho
GTP-Rho/ total Rho (-fold above control)
1.5
1.0
0.5
0.0
(4)
†
(4)
– + WMN
SHR
p(T853) MYPT1
Total MYPT1
pMYPT1/total MYPT1 (-fold above control)
1.5
1.0
0.5
0.0
(4)
†
(4)
– + WMN
SHR

Figure 5

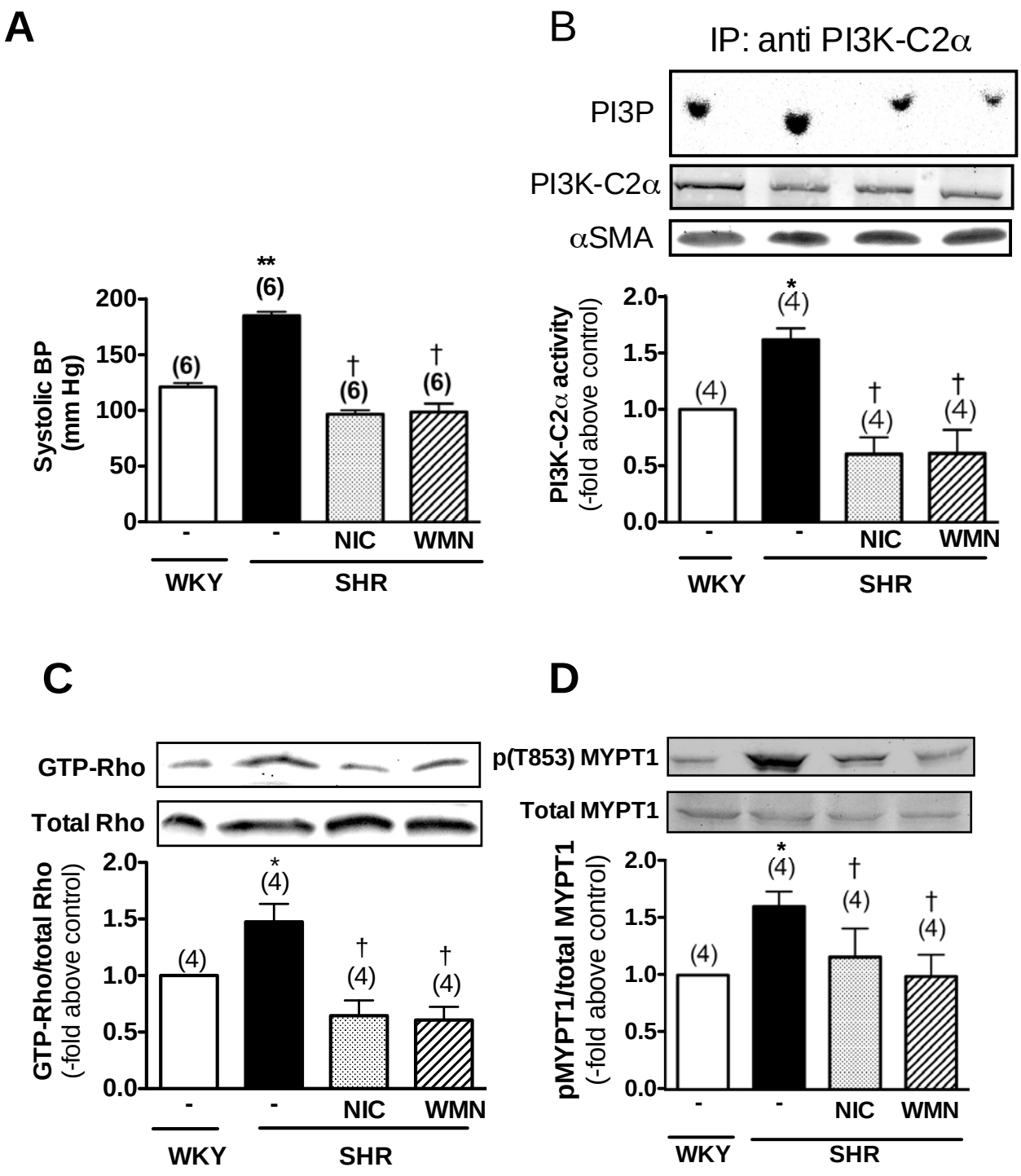

A
Systolic BP (mm Hg)
200
150
100
50
0
**
(6)
(6)
†
(6)
†
(6)
-
-
NIC
WMN
WKY
SHR
B
IP: anti PI3K-C2α
PI3P
PI3K-C2α
αSMA
PI3K-C2α activity (-fold above control)
2.0
1.5
1.0
0.5
0.0
*
(4)
(4)
†
(4)
†
(4)
-
-
NIC
WMN
WKY
SHR
C
GTP-Rho
Total Rho
GTP-Rho/total Rho (-fold above control)
2.0
1.5
1.0
0.5
0.0
*
(4)
(4)
†
(4)
†
(4)
-
-
NIC
WMN
WKY
SHR
D
p(T853) MYPT1
Total MYPT1
pMYPT1/total MYPT1 (-fold above control)
2.0
1.5
1.0
0.5
0.0
*
(4)
(4)
†
(4)
†
(4)
-
-
NIC
WMN
WKY
SHR

Figure 6