Effect of Protein Kinase C and Protein Kinase A Inhibitors on Contraction of Isolated Femoral Arteries of SHR and Wistar Rats

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Running title: The role of PKA and PKC in vasoconstriction of SHR

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SUMMARY

Alterations of calcium handling and other second messenger cascades including protein kinase C (PKC) and A (PKA) were suggested to be responsible for abnormal vascular function in spontaneously hypertensive rats (SHR). However, the relative contribution of these pathways to vasoconstriction is still not completely understood. We investigated the effect of Ro 31-8220 (PKC inhibitor) and H89 (PKA inhibitor) on vasoconstriction induced by 120 mM KCl or by addition of 10 µM noradrenaline (NA) in isolated femoral arteries of control Wistar rats and SHR. Moreover, we investigated these responses in the presence and absence of Ca^{2+} ions in the incubation medium in order to assess the role of calcium influx in these contractions. We observed that while the vasoconstriction in the presence of calcium was not different between Wistar and SHR, the difference between constriction elicited by NA addition in the absence and presence of external calcium was larger in SHR. The inhibition of PKC had no effect on constrictions in SHR, but diminished constrictions in Wistar rats. PKA inhibition slightly enhanced constrictions in Wistar rats, but reduced them in the presence of calcium in SHR. We conclude that vasoconstriction elicited by adrenergic stimulation is more dependent on extracellular calcium influx in SHR compared to Wistar rats. Moreover, the activation of PKA contributes to this calcium-dependent vasoconstriction in SHR but not in Wistar. On the other hand, PKC activation seems to play a less important role in vasoconstriction in SHR than in Wistar rats.

Keywords: Protein kinase C, Protein kinase A, Femoral artery, Calcium, Myograph

Introduction

Spontaneously hypertensive rats (SHR) represent an attractive model of essential hypertension. Augmented vasoconstrictor ability of arteries isolated from SHR was proposed to be involved in this type of hypertension. Several studies have reported abnormalities of vascular smooth muscle in SHR. These include disturbed cell Ca^{2+} handling (Cox 2002, Hermsmeyer *et al.* 1989, Lompre 1999, Martens *et al.* 1998) probably linked to abnormal properties of L-type voltage-dependent Ca^{2+} channels (VDCC) in SHR (Hermsmeyer *et al.* 1989, Matsuda *et al.* 1997), which display higher L-type Ca^{2+} current density due to a more frequent opening of the channels (Ohya *et al.* 1998). Available studies also suggest that there may be increased number of L-type Ca^{2+} channel pores (Pratt *et al.* 2002) or increased Ca^{2+} stores (Goldberg *et al.* 1977, Miquel *et al.* 2005). Furthermore the Ca^{2+}-sensitivity of vascular smooth muscle could be modulated by protein kinase C (Kitazawa *et al.* 2000) or protein kinase A (Wooldridge *et al.* 2004). PKC was proposed to be up-regulated (Kanashiro *et al.* 2001) and PKA less active in SHR (Alemany *et al.* 2006).

In vascular smooth muscle, PKC and PKA are activated by noradrenaline through stimulation of G_q and G_s proteins, respectively (Nishizuka 1988, Somlyo *et al.* 1988) or to lesser extent by membrane depolarization (Takuwa and Rasmussen 1987, Ko *et al.* 2008). The role of PKC in the regulation of smooth muscle was suggested by the finding that tumor-promoting phorbol esters, which specifically activate PKC, induce sustained contractions in smooth muscles (Castagna *et al.* 1982, Rasmussen *et al.* 1987).

The second messenger adenosine 3', 5'-cyclic monophosphate (cAMP) was shown to mediate its effects through PKA activation leading to relaxation of smooth muscle

(Somlyo *et al.* 1994) mainly by Ca^{2+} -dependent or -independent effects (Somlyo *et al.* 2003, Nishimura *et al.* 1989).

The aim of our study was to evaluate the relative contribution of calcium influx to vascular constriction induced by KCl or adrenergic stimulation in SHR. Furthermore, we intended to elucidate the role of PKC and PKA recruitment in this vasoconstriction.

Methods

Male 3-month-old SHR and Wistar rats were sacrificed by ether overdosage. Femoral arteries were isolated, cleaned, and cut into segments of equal length. Attention was paid to preserve the endothelium of the arteries. The segments were subsequently mounted on standard Mulvany-wire myograph (610M; Danish Myo Technology, Aarhus, Denmark) filled with modified Krebs-Henseleit solution (KHS, p*H* 7.4) of the following composition (in mM): NaCl 120, $NaHCO_3$ 25, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, and oxygenated with 95% O_2: 5% CO_2. The rings were first equilibrated for 1 h at 37°C and then constricted with 120 mM KCl followed by addition of 10 µM noradrenaline (NA). After reaching a steady-state, the rings were washed with KHS containing nominally 0 mM $CaC1_2$, (KHS 0 Ca^{2+}) and once the resting tension was restored, the protocol was repeated in KHS 0 Ca^{2+} solution (Fig. 1). In fresh arteries, the same protocol was implemented after a 30 min preincubation with PKC (Ro 31-8220) or PKA (H-89) inhibitor (both Biaffin, Germany). H-89 was dissolved as a stock solution of 1 mM in 100% dimethyl sulfoxide, the final concentration of both inhibitors used was 1 µM (Budzyn *et al.* 2006). All other drugs were dissolved and diluted in deionized water. During the experiment, the generated force was recorded and wall tension was

automatically calculated by the myograph system. Data are expressed as mean ± SEM, $P<0.05$ (one-way, two-tailed, unpaired Student's t-test) was considered significant. All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Physiology AS CR, and conform to the *European Convention on Animal Protection and Guidelines on Research Animal Use*.

Results

Basic parameters of the isolated arteries

The normalized inner diameter under tension corresponding to pressure of 100 mm Hg was 953.4 ± 24.3 µm in Wistar controls *vs.* 909.5 ± 21.5 µm in SHR (non-significant). The segment length was not different between Wistar and SHR vessels (2.16 ± 0.07 mm in Wistar *vs*. 2.19 ± 0.06 mm in SHR).

Constriction induced by KCl and KCl + noradrenaline

The vasoconstriction elicited by KCl or KCl + NA in KHS 1.6 Ca^{2+} or by KCl in KHS 0 Ca^{2+} was not different between control rats and SHR. The vasoconstriction elicited by KCl + NA in KHS 0 Ca^{2+} was lower in SHR compared to controls ($P<0.05$) (Fig 2).

Effect of PKC inhibition on constriction induced by KCl or KCl + noradrenaline

When PKC inhibitor was present in KHS 1.6 Ca^{2+} medium, the constrictions of arteries isolated from control Wistar rats were decreased compared to constrictions seen in its absence ($P<0.05$). The incubation with PKC inhibitor did not affect constrictions of

arteries from SHR and therefore the constriction elicited by KCl + NA in SHR became higher compared to Wistar controls ($P<0.05$) (Fig. 3A).

The presence of PKC inhibitor in KHS 0 Ca^{2+} medium decreased the constrictions induced by KCl + NA in arteries isolated from Wistar rats compared to constrictions observed in its absence ($P<0.05$). The incubation with PKC inhibitor did not affect constrictions of arteries from SHR and therefore constriction elicited by KCl + NA in SHR was not different from respective controls (Fig. 3B).

Effect of PKA inhibition on constriction induced by KCl or KCl + noradrenaline

In the presence of PKA inhibitor in KHS 1.6 Ca^{2+} medium the constrictions of arteries isolated from control Wistar rats were non-significantly higher compared to those recorded in its absence. The incubation with PKA inhibitor reduced constrictions of arteries from SHR ($P<0.05$) and therefore constriction in SHR became lower compared to controls ($P<0.05$) (Fig. 4A).

In the presence of PKA inhibitor in KHS 0 Ca^{2+} medium the constrictions of arteries isolated from control rats were slightly but non-significantly higher compared to the conditions when PKA inhibitor was absent. The incubation with PKA inhibitor tended to reduce the constrictions of arteries from SHR, but the constriction in SHR was lower compared to Wistar controls ($P<0.05$) (Fig. 4A).

Discussion

In our *in vitro* experiments on isolated femoral arteries we observed that the constriction elicited by KCl or KCl + noradrenaline (NA) in Krebs-Henseleit solution

containing 1.6 mM Ca^{2+} (KHS 1.6 Ca^{2+}) or by KCl in the nominally calcium-free KHS (KHS 0 Ca^{2+}) was not different between control Wistar rats and SHR. However, the constriction elicited by KCl + NA in KHS 0 Ca^{2+} was less pronounced in SHR. A larger inhibition of vasoconstriction of isolated mesenteric arteries after calcium chelation has already been reported by Kahonen *et al.* (1994) and is in agreement with our previous data reporting greater relaxation after nifedipine in SHR than in controls (Paulis *et al.* 2007). These results suggest that the activation of intracellular pathways and/or intracellular calcium stores is reduced in SHR. The relative contribution of extracelullar calcium influx to vascular constriction is therefore increased in SHR, which have enhanced activity (Ohya *et al.* 1998) or higher number (Pratt *et al.* 2002) of voltage-dependent calcium channels (VDCC). Thus SHR seem to be more dependent on extracellular calcium influx and may require higher level of adrenergic stimulation.

The stimulation by noradrenaline involving the recruitment of G_q and G_s proteins leads to activation of protein kinase C (PKC) and protein kinase A (PKA), respectively (Nishizuka 1988, Somlyo *et al.* 1988). In our experiments we aimed to investigate the significance of PKC and PKA in the recruitment of extracellular calcium influx and mobilization of intracellular calcium stores in SHR. Recent studies using inhibitors with improved selectivity for PKC have yielded conflicting results regarding its physiological importance in the vasculature (Chrissobolis *et al.* 2002, McNair *et al.* 2004, Shirao *et al.* 2002). In our experiment, the inhibition of PKC with Ro 31-8220 had no effect on constrictions in SHR, but diminished constrictions in control Wistar rats. Moreover, the constrictions in controls were attenuated in KHS 1.6 Ca^{2+} as well as in KHS 0 Ca^{2+} to similar extent. This fact indicates that in control rats PKC recruited either by membrane depolarization or by adrenergic stimulation enhances the smooth muscle tone

independently on extracellular calcium influx either by modulation of sarcoplasmatic reticulum calcium release (Bonev *et al.* 1997) or by direct action on myosine (Lamounier-Zepter *et al.* 2003). These pathways seems to be of less importance in SHR, despite the previously reported higher PKC expression in SHR (Kanashiro *et al.* 2001).

In contrast to the effect of PKC inhibition, the inhibition of PKA with H-89, a selective and potent inhibitor (Lochner *et al.*, 2006), slightly enhanced constrictions in control rats in both KHS 1.6 Ca^{2+} and KHS 0 Ca^{2+} but reduced them in SHR in KHS 1.6 Ca^{2+}. Apparently in control rats the PKA is involved in relaxation. The relaxing effect of PKA in Ca^{2+}-free conditions is supposed to be mediated mainly by inhibition of myosin light chain kinase (Conti and Adelstein, 1981). On the other hand, the reduced contraction after PKA inhibition in SHR, observed in our experiment, is more difficult to explain. The mild activation of PKA by lower cAMP concentrations was reported to increase VDCC current in smooth muscle cells and may lead to vasoconstriction (Taguchi *et al.*, 1997, Ruiz-Velasco *et al.*, 1998). In our experiments the effect of PKA on vasoconstriction in SHR was dependent on the presence of extracellular calcium supporting this hypothesis. Moreover, in rat aortic rings pre-incubated with the PKA inhibitor H-89 the albuterol-induced relaxation was attenuated (Ferro *et al.*, 2004).

We conclude that vasoconstriction elicited by adrenergic stimulation is more dependent on extracellular calcium influx in SHR compared to controls. Moreover, the activation of PKA contributes to the vasoconstriction in SHR but not in Wistar. On the other hand, PKC activation seems to play a less important role in vasoconstriction in SHR than in Wistar.

References

ALLEMANY R, VOGLER O, TERES S, EGEA C, BAAMONDE C, BARCELO F, DELGADO C, JAKOBS KH, ESCRIBA PV: Antihypertensive action of 2-hydroxyoleic acid in SHRs via modulation of the protein kinase A pathway and Rho kinase. *J Lipid Res* **47:** 1762-1770, 2006.

BONEV AD, JAGGAR JH, RUBART M, NELSON MT: Activators of protein kinase C decrease Ca^{2+} spark frequency in smooth muscle cells from cerebral arteries. *Am J Physiol* **273:** C2090-C2095, 1997.

BUDZYN K, PAULL M, MARLEY PD, SOBEY CG: Segmental differences in the roles of rho-kinase and protein kinase C in mediating vasoconstriction. *J Pharmacol Exp Ther* **317:** 791-796, 2006.

CASTAGNA M, TAKAI Y, KAIBUCHI K, SANO K, KIKKAWA U, NISHIZUKA Y: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257:** 7847-7851, 1982.

CHRISSOBOLIS S, SOBEY CG: Inhibitory effects of protein kinase C on inwardly rectifying K^+ and ATP-sensitive K^+ channel-mediated responses of the basilar artery. *Stroke* **33:** 1692-1697, 2002.

CONTI MA, ADELSTEIN RS: The relationship between calmodulin binding and phosphorylation of smooth muscle myosin kinase by the catalytic subunit of 3':5' cAMP-dependent protein kinase. *J Biol Chem* **256:** 3178-3181, 1981.

COX RH: Changes in the expression and function of arterial potassium channels during hypertension. *Vascul Pharmacol* **38:** 13-23, 2002.

FERRO A, COASH M, YAMAMOTO T, ROB J, JI Y, QUEEN L: Nitric oxide-dependent beta2-adrenergic dilatation of rat aorta is mediated through activation of both protein kinase A and Akt. *Br J Pharmacol* **143:** 397-403, 2004.

GOLDBERG MT, TRIGGLE CR: An analysis of the action of lanthanum on aortic tissue from normotensive and spontaneously hypertensive rats. *Can J Physiol Pharmacol* **55:** 1084-1090, 1977.

HERMSMEYER K, ERNE P: Cellular calcium regulation in hypertension. *Am J Hypertens*, **2:** 655-658, 1989.

KAHONEN M, ARVOLA P, WU X, PORSTI I: Arterial contractions induced by cumulative addition of calcium in hypertensive and normotensive rats: influence of endothelium. *Naunyn-Schmiedeberg´s Arch Pharmacol* **349:** 627-636, 1994.

KANASHIRO CA, KHALIL RA: Gender-related distinctions in protein kinase C activity in rat vascular smooth muscle. *Am J Physiol* **280:** C34-C45, 2001.

KITAZAWA T, ETO M, WOODSOME TP, BRAUTIGAN DL: Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. *J Biol Chem* **275:** 9897-9900, 2000.

KO EA, HAN J, JUNG ID, PARK WS: Physiological roles of K^+ channels in vascular smooth muscle cells. *J Smooth Muscle Res* **44:** 65-81, 2008.

LAMOUNIER-ZEPTER V, EHRHART-BORNSTEIN M, KARCZEWSKI P, HAASE H, BORNSTEIN SR, MORANO I: Human adipocytes attenuate cardiomyocyte contraction: characterization of an adipocyte-derived negative inotropic activity. *FASEB J* **20:** 1653-1659, 2006.

LOCHNER A, MOOLMAN JA: The many faces of H89: a review. *Cardiovasc Drug Rev* **24:** 261-74, 2006.

LOMPRE AM: Sarcoplasmic reticulum in vascular cells in hypertension and during proliferation. *Clin Exp Pharmacol Physiol* **26:** 553-557, 1999.

MARTENS JR, GELBAND CH: Ion channels in vascular smooth muscle: alterations in essential hypertension. *Proc Soc Exp Biol Med* **218:** 192-203, 1998.

MATSUDA K, LOZINSKAYA I, COX RH: Augmented contributions of voltage-gated Ca^{2+} channels to contractile responses in spontaneously hypertensive rat mesenteric arteries. *Am J Hypertens* **10:** 1231-1239, 1997.

MCNAIR LL, SALAMANCA DA, KHALIL RA: Endothelin-1 promotes Ca^{2+} antagonist-insensitive coronary smooth muscle contraction via activation of epsilon-protein kinase C. *Hypertension* **43:** 897-904, 2004.

MIQUEL R, GISBERT R, SERNA E, PEREZ-VIZCAINO F, ANSELMI E, NOGUERA MA, IVORRA MD, D'OCON MP: Acute and chronic captopril, but not prazosin or nifedipine, normalize alterations in adrenergic intracellular Ca^{2+} handling observed in the mesenteric arterial tree of spontaneously hypertensive rats. *J Pharmacol Exp Ther* **313:** 359-367, 2005.

NISHIMURA J, VAN BREEMEN C: Direct regulation of smooth muscle contractile elements by second messengers. *Biochem Biophys Res Commun* **163:** 929-935, 1989.

NISHIZUKA Y: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* **334:** 661-665, 1988.

OHYA Y, TSUCHIHASHI T, KAGIYAMA S, ABE I, FUJISHIMA M: Single L-type calcium channels in smooth muscle cells from resistance arteries of spontaneously hypertensive rats. *Hypertension* **31:** 1125-1129, 1998.

PAULIS Ľ, LÍŠKOVÁ S, PINTÉROVÁ M, DOBEŠOVÁ Z, KUNEŠ J, ZICHA J: Nifedipine-sensitive noradrenergic vasoconstriction is enhanced in spontaneously hypertensive rats: the influence of chronic captopril treatment. *Acta Physiol (Oxf)* **191:** 255-266, 2007.

PRATT JH, AMBROSIUS WT, AGARWAL R, ECKERT GJ, NEWMAN S: Racial difference in the activity of the amiloride-sensitive epithelial sodium channel. *Hypertension*, **40,** 903-908, 2002.

RASMUSSEN H, TAKUWA Y, PARK S: Protein kinase C in the regulation of smooth muscle contraction. *FASEB J* **1:** 177-85, 1987.

RUIZ-VELASCO V, ZHONG J, HUME JR, KEEF KD: Modulation of Ca^{2+} channels by cyclic nucleotide cross activation of opposing protein kinases in rabbit portal vein. *Circ Res*, **82:** 557-565, 1998.

SALAMANCA DA, KHALIL RA: Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. *Biochem Pharmacol* **70:** 1537-1547, 2005.

SHIRAO S, KASHIWAGI S, SATO M, MIWA S, NAKAO F, KUROKAWA T, TODOROKI-IKEDA N, MOGAMI K, MIZUKAMI Y, KURIYAMA S, HAZE K, SUZUKI M, KOBAYASHI S: Sphingosylphosphorylcholine is a novel messenger for Rho-kinase-mediated Ca^{2+} sensitization in the bovine cerebral artery: unimportant role for protein kinase C. *Circ Res*, **91:** 112-119, 2002.

SOMLYO AP, SOMLYO AV: Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* **83:** 1325-1358, 2003.

SOMLYO AP, SOMLYO AV: Signal transduction and regulation in smooth muscle. *Nature* **372:** 231-236, 1994.

SOMLYO AP, WALKER JW, GOLDMAN YE, TRENTHAM DR, KOBAYASHI S, KITAZAWA T, SOMLYO AV: Inositol trisphosphate, calcium and muscle contraction. *Philos Trans R Soc Lond B Biol Sci* **320:** 399-414, 1988.

TAGUCHI K, UEDA M, KUBO T: Effects of cAMP and cGMP on L-type calcium channel currents in rat mesenteric artery cells. *Jpn J Pharmacol* **74:** 179-186, 1997.

TAKUWA Y, RASMUSSEN H: Measurement of cytoplasmic free Ca^{2+} concentration in rabbit aorta using the photoprotein, aequorin. Effect of atrial natriuretic peptide on agonist-induced Ca^{2+} signal generation. *J Clin Invest* **80:** 248-257, 1987.

WOOLDRIDGE AA, MACDONALD JA, ERDODI F, MA C, BORMAN MA, HARTSHORNE DJ, HAYSTEAD TA: Smooth muscle phosphatase is regulated in vivo by exclusion of phosphorylation of threonine 696 of MYPT1 by phosphorylation of Serine 695 in response to cyclic nucleotides. *J Biol Chem* **279:** 34496-34504, 2004.

FIGURE LEGENDS

Fig. 1. Original records of force generated by femoral artery of control (Wistar, upper panel) and spontaneously hypertensive rats (SHR, lower panel) stimulated with 120 mM KCl (6 min) followed by addition of 10 µM noradrenaline (NA) in Krebs-Henseleit solution (KHS) containing 1.6 mM Ca^{2+} and in KHS nominally free of Ca^{2+} Horizontal bar represents 5 min.

Fig. 2. Vasoconstriction obtained after stimulation of femoral artery of control (Wistar) and spontaneously hypertensive rats (SHR) stimulated with 120 mM KCl and KCl + 10 µM noradrenaline (NA) in Krebs-Henseleit solution (KHS) containing 1.6 mM Ca^{2+} (A) and in KHS nominally free of Ca^{2+} (B). **P*<0.05 *vs.* controls.

Fig. 3. Vasoconstriction in the presence of protein kinase C inhibitor Ro-31-8220 obtained after stimulation of femoral artery of control (Wistar) and spontaneously hypertensive rats (SHR) stimulated with 120 mM KCl and KCl + 10 µM noradrenaline

(NA) in Krebs-Henseleit solution (KHS) containing 1.6 mM Ca^{2+} (A) and in KHS nominally free of Ca^{2+} (B). *P<0.05 *vs.* controls, ^{+}P<0.05 *vs.* respective group in the absence of the inhibitor.

Fig. 4. Vasoconstriction in the presence of protein kinase A inhibitor H-89 obtained after stimulation of femoral artery of control (Wistar) and spontaneously hypertensive rats (SHR) stimulated with 120 mM KCl and KCl + 10 μM noradrenaline (NA) in Krebs-Henseleit solution (KHS) containing 1.6 mM Ca^{2+} (A) and in KHS nominally free of Ca^{2+} (B). *P<0.05 *vs.* controls, ^{+}P<0.05 *vs.* respective group in the absence of the inhibitor.

Figure 1

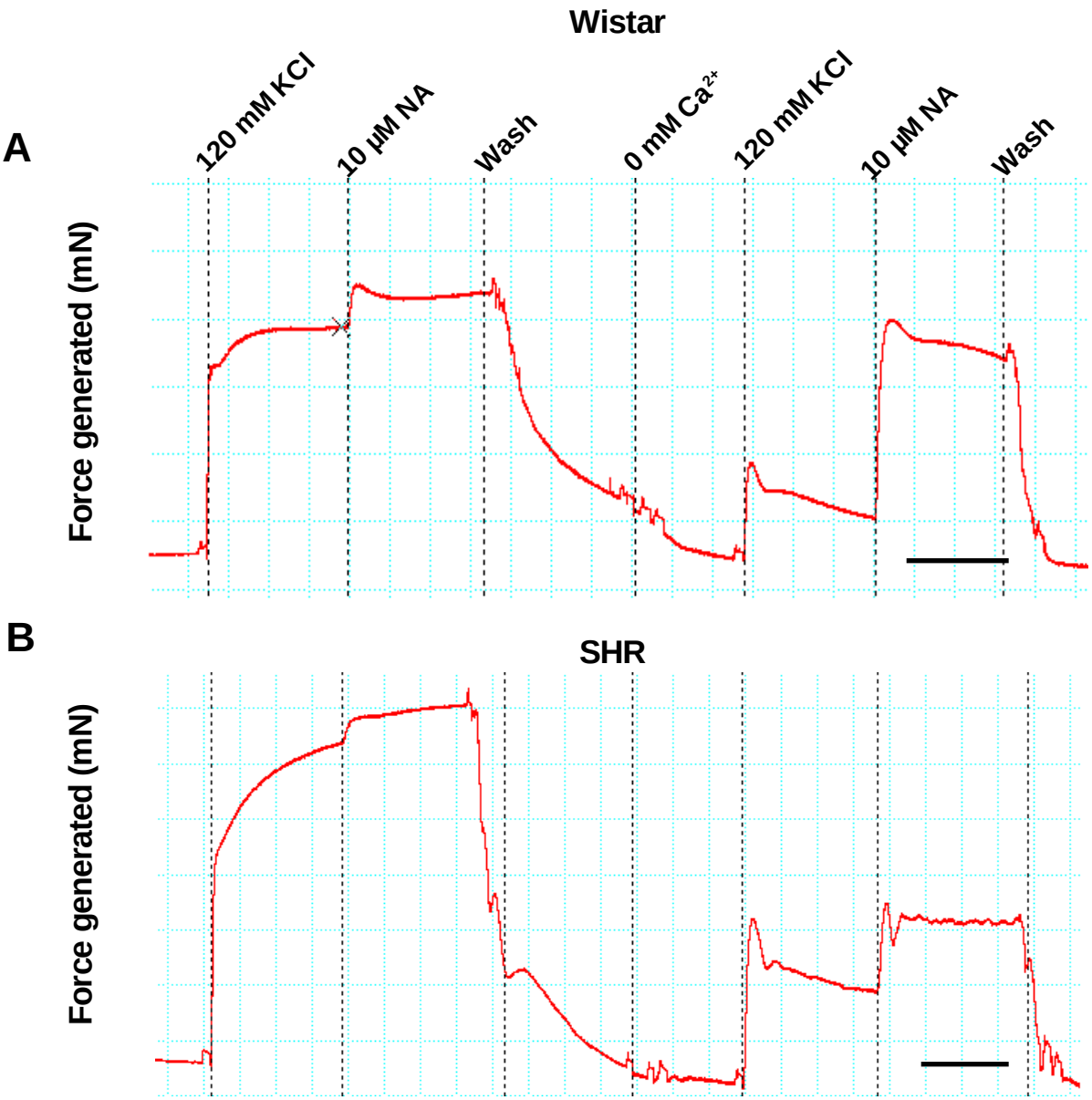

A
Wistar
120 mM KCl
10 μM NA
Wash
0 mM Ca2+
120 mM KCl
10 μM NA
Wash
Force generated (mN)
B
SHR
Force generated (mN)

Figure 2

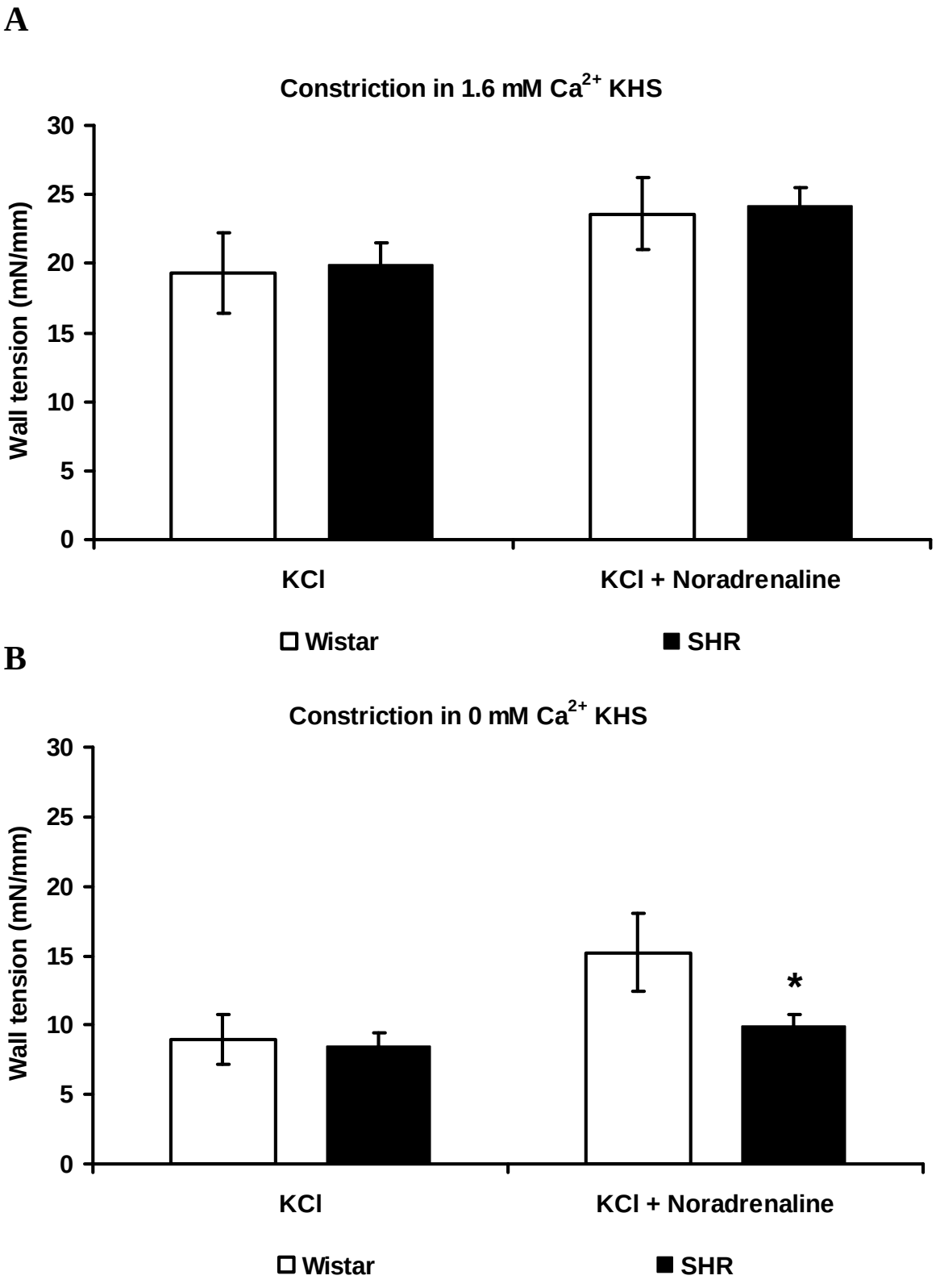

A
Constriction in 1.6 mM Ca2+ KHS
Wall tension (mN/mm)
30
25
20
15
10
5
0
KCl
KCl + Noradrenaline
Wistar
SHR
B
Constriction in 0 mM Ca2+ KHS
Wall tension (mN/mm)
30
25
20
15
10
5
0
*
KCl
KCl + Noradrenaline
Wistar
SHR

Figure 3

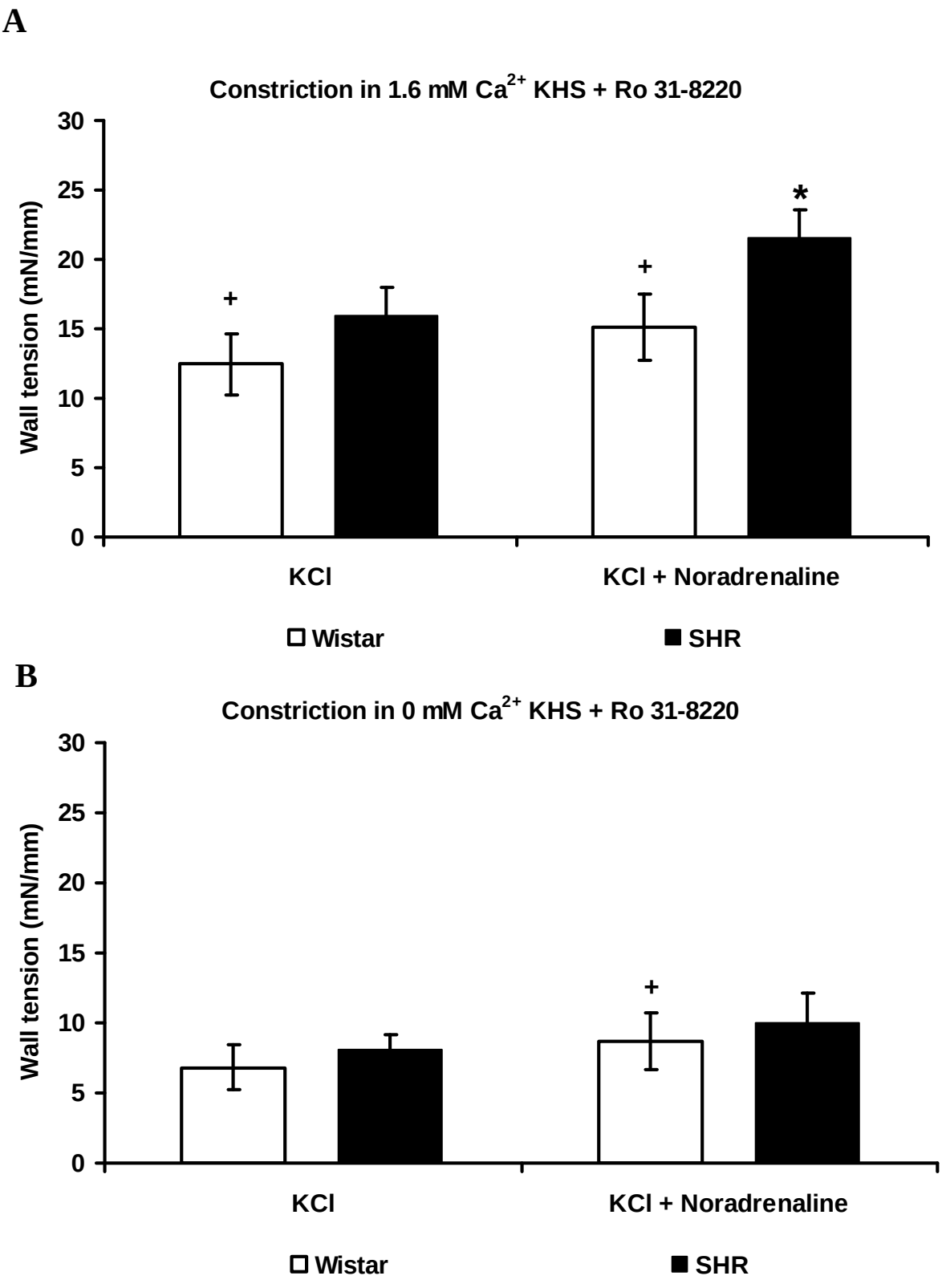
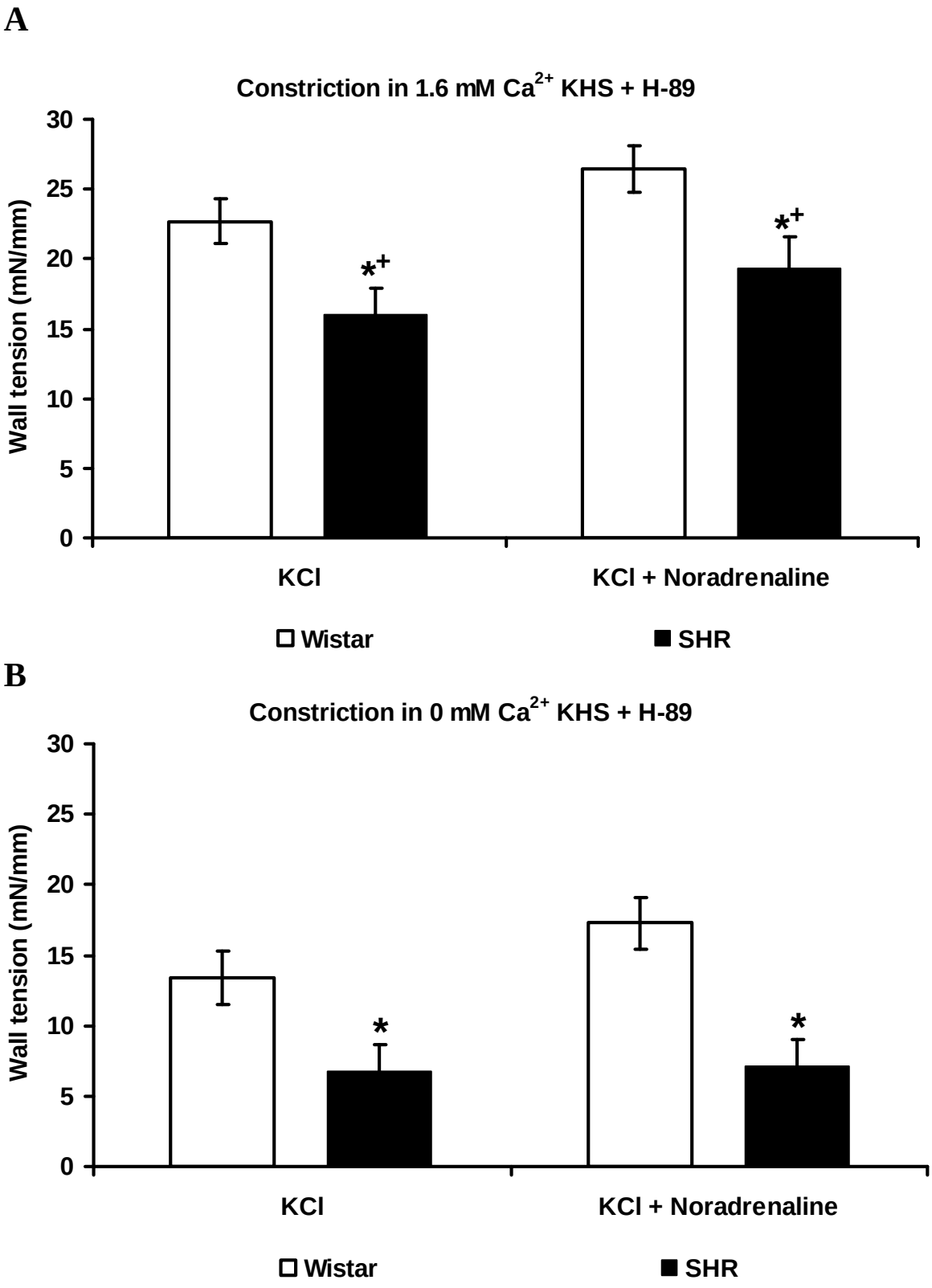

A
Constriction in 1.6 mM Ca2+ KHS + Ro 31-8220
Wall tension (mN/mm)
30
25
20
15
10
5
0
+
+
*
KCl
KCl + Noradrenaline
Wistar
SHR
B
Constriction in 0 mM Ca2+ KHS + Ro 31-8220
Wall tension (mN/mm)
30
25
20
15
10
5
0
+
KCl
KCl + Noradrenaline
Wistar
SHR

Figure 4


A
Constriction in 1.6 mM Ca2+ KHS + H-89
Wall tension (mN/mm)
30
25
20
15
10
5
0
*+
*+
KCl
KCl + Noradrenaline
Wistar
SHR
B
Constriction in 0 mM Ca2+ KHS + H-89
Wall tension (mN/mm)
30
25
20
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10
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0
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*
KCl
KCl + Noradrenaline
Wistar
SHR