

본태성 고혈압쥐에서 Myogenic Tone의 변화 : RhoA 단백질 및 Protein Kinase C의 역할

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Changes in Myogenic Tone in Spontaneously Hypertensive Rat : Role of RhoA and Protein Kinase C

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ABSTRACT

Background and Objectives : The myogenic response was originally described as a contraction of a blood vessel that occurred following an increase in intravascular distending pressure. Conversely, a reduction in intravascular pressure produces myogenic vascular relaxation. Recent attention has focused on the potential role of this myogenic mechanism in the control of tone in the resistance vasculature, and in particular on how this mechanism may contribute to the increased vascular resistance seen in hypertension. Therefore, in the present study, we investigated the role of myogenic tone in the generation and/or maintenance of hypertension. **Materials and Methods :** Myogenic tone was developed by stretching of the basilar arteries of WKY (Wistar Kyoto rat) and SHR (spontaneously hypertensive rats). Contractile responses, PKC (protein kinase C) immunoblots and translocation of PKC and RhoA were measured. **Results :** In the presence of extracellular Ca^{2+} , the stretching of the resting vessel evoked a myogenic contraction in the basilar arteries of SHR and WKY. Myogenic tone was significantly greater in SHR than in WKY. However, in the absence of extracellular Ca^{2+} , stretching evoked a myogenic contraction in SHR, but not in WKY. The stretch-induced myogenic tone was inhibited by nifedipine. The effect of nifedipine was similar in both SHR and WKY rats. H-7, calphostin C and Y-27632, also inhibited stretch-induced myogenic tone in both SHR and WKY. The inhibitory effects of these drugs were greater in SHR than in WKY. Immunoblotting showed rho A and PKC were translocated from the cytosol to the cell membrane with stretching in both SHR and WKY. PKC, however, was translocated to the cell membrane with stretching in SHR, but not in WKY. **Conclusion :** These results suggest that stretch-induced myogenic tone is significantly greater in SHR than in WKY. Furthermore, the increase in amount and/or activity of PKC and ROK (rhoA-associated kinase) may be a key mechanism accounting for the enhanced myogenic tone in SHR. (**Korean Circulation J 2002;32(3):257-267**)

KEY WORDS : Myogenic tone ; Protein kinase C ; RhoA GTP-binding protein ; Rats, inbreds SHR.

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서 론
 (perfusion pressure)
 (blood flow)
 가
 (autoregulation)¹⁾
 (myogenic), (neurogenic) (metab-
 olic)²⁾
 (myogenic response)
 가
 ,
³⁾ in vivo
 in vitro
 (stretch)
 가
 (myogenic tone)
 ,
 Ca^{2+} 가 가
⁴⁾ Ca^{2+} 가 1)
 stretch activated cation
 Ca^{2+} ⁵⁾ 2) Ca^{2+}
 Ca^{2+} ⁶⁾ 3)
 Ca^{2+} 2
 Ca^{2+} 6)
 Ca^{2+} 가
 ,
 Ca^{2+} PKC(protein kinase C)가
 Ca^{2+} (Ca^{2+} sensit-
 ization) 가 ⁷⁾
 small G - protein rhoA가 Ca^{2+}
⁸⁾
 Ca^{2+}
 ,
 small G - protein rhoA
 ROK(rho - kinase)가 가
 MLCP(myosin light chain phosphatase) $M_{110-130}$
 regulatory subunit
 activity
 rhoA/ROK
 Ca^{2+}
 (basal vascular resi-
 stance)¹¹⁾
 가
 가
 가
 가
 (ca-
 pillary pressure)
 ,
 (SHR, spontaneously
 hypertensive rat)¹³⁾
 가
¹⁴⁾
 가
¹⁵⁾
 (myogenic constriction)¹⁾
 가
¹⁶⁾
 가
 aortic -
 coarctation - induced hypertension 가
 3 4
 PKC 가
¹⁴⁾
 SHR 가
 SHR
 1) SHR WKY(Wistar Kyoto rat)
 , 2) SHR WKY

densitometry . homogenization 4 10 in -
cubation , 100,000 g
(4) 60
PKC isoform rhoA translocation PKC PKC rhoA
translocation SHR WKY protein - matched sample western blot
western blot immunoblot .
8 basilar 통계처리
artery . 50.5 mM K⁺
가 mean ± SE
incubation strip , “ n ”
liquid N₂ - cooled liquid strip
chlorodifluoromethane homog - Student t - test
enization {200 mM Tris - HCl(pH 7.4), 0.3 M
sucrose, 5 mM EDTA, 5 mM DTT, 10 mM EGTA,
0.3 mM phenylmethylsulfonyl fluoride, 0.3% 2 -
mercaptoethanol} homogenization . Ho -
mogenates hom -
ogenates 100,000 g(4) 60
0.1% Triton X - 100 가 SHR WKY

결 과

SHR과 WKY의 basilar artery에서 신장이 장력에 미치는 영향
SHR WKY

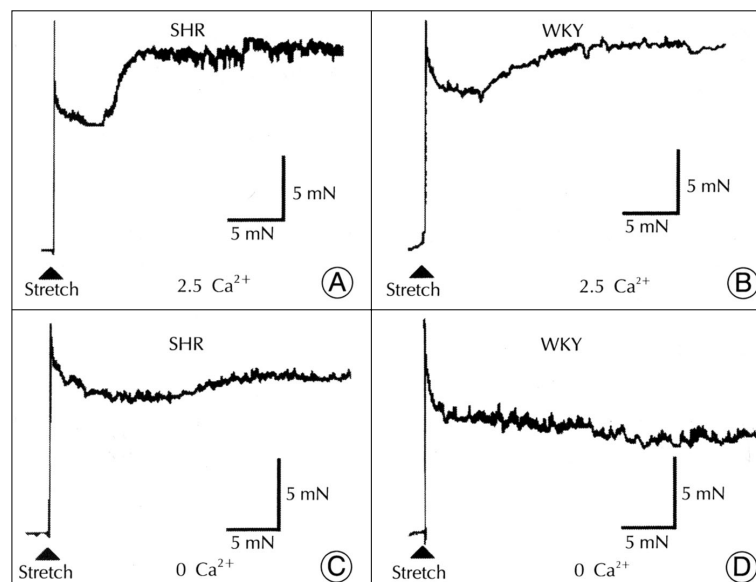


Fig. 1. Typical records of myogenic tone induced by stretch in basilar artery of SHR and WKY. A and B : typical record of changes in tension by stretch in the presence of extracellular Ca²⁺ (2.5 mM Ca²⁺ ; 2.5Ca²⁺). C and D : typical record of changes in tension by stretch in the absence of extracellular Ca²⁺ (0Ca²⁺). Helical strips were stretched passively to the optimal length by imposing a stretch of 50% of resting length. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

basilar artery

. Fig. 1

가

SHR WKY

2.5 mM Ca^{2+}

(passive tension)

SHR WKY

5 mM K^+

38.2 ± 6%

62.1 ± 12.2%

WKY

SHR WKY

(Fig. 1).

15 ± 1.2% (Fig. 2 ; n=14)

Ca^{2+}

Fig. 2 SHR 50.

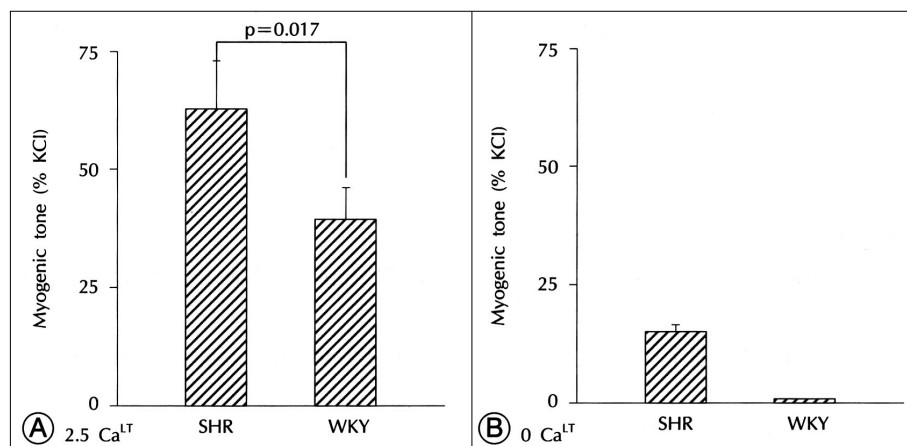


Fig. 2. Statistical analysis of the amplitude of myogenic tone induced by stretch between SHR and WKY. A : comparison of amplitudes of myogenic tone between SHR and WKY in the presence extracellular Ca^{2+} (2.5 mM Ca^{2+} ; 2.5 Ca^{2+}). B : comparison of amplitudes of myo-genic tone between SHR and WKY in the absence of extracellular Ca^{2+} (0 Ca^{2+}). Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=14). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

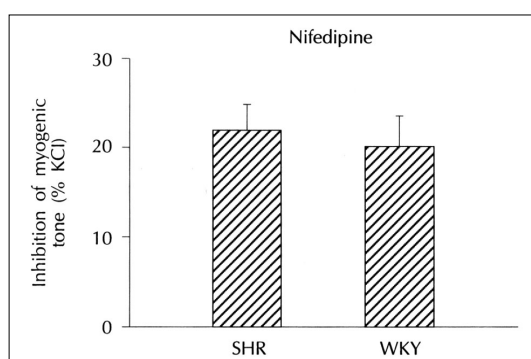


Fig. 3. Effect of nifedipine on the stretch-induced myogenic tone. Nifedipine (10^{-7} M) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=8). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

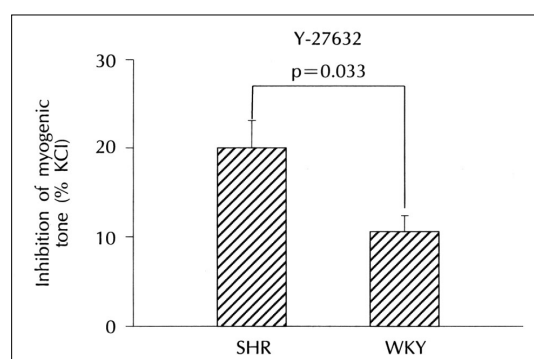


Fig. 4. Effect of Y-27632 on the stretch-induced myogenic tone. Y-27632 (1 μ M) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=9). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

Nifedipine이 신장에 의한 장력 변화에 미치는 영향

10⁻⁶
M Y - 27632 Y - 27632 SHR
WKY
Ca²⁺ nife -
dipine (Fig. 3). SHR WKY
가가
10⁻⁷ M nifedipine
nifedipine
SHR 23.2 ± 2.3%(n=8), WKY 20.2 ± 3.6%
(n=8) SHR WKY 가
5 × 10⁻⁷ M calphostin - C
SHR WKY

Rho-kinase와 PKC 억제제가 신장에 의한 장력 변화에 미치는 영향

rhoA
PKC가 rho - kinase
Y - 27632(Fig. 4) PKC H - 7 ca -
lphostin C(Fig. 5) , SHR
WKY small G - protein rhoA
rho - kinase Y - 27632
(Fig. 4). SHR WKY
SHR (H - 7 ; p=0.0335, calphostin
C ; p=0.0344)
H - 7 calphostin - C
가 12.2 ± 2.2% 12.3 ± 3.1%(n=8)
, WKY 5.3 ± 2.1% 6.3 ± 1.7%(n=8)

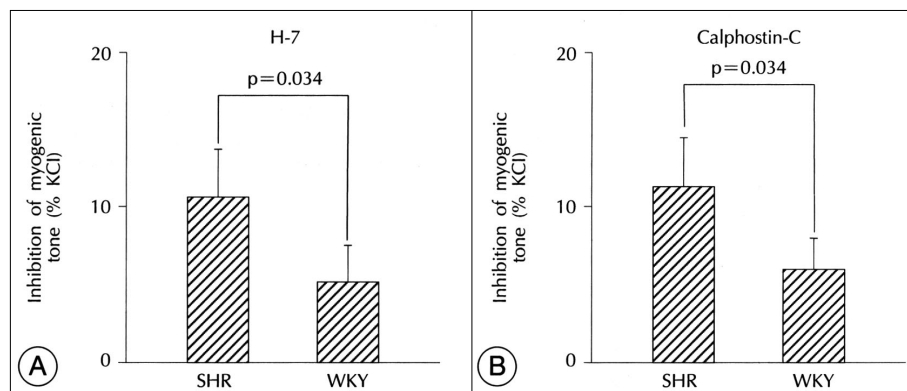


Fig. 5. Effect of H-7 and calphostin-C on the stretch-induced myogenic tone. H-7 (10⁻⁵ M) and calphostin-C (5 × 10⁻⁷ M) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K⁺ response. Results are expressed as mean ± SE (n=8). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

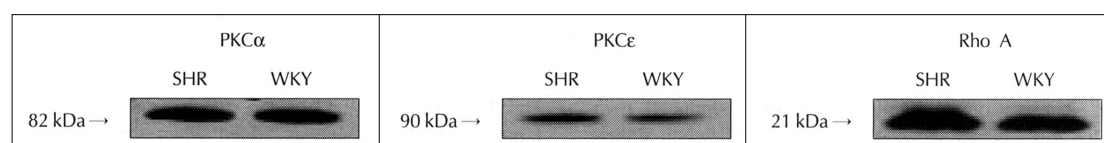


Fig. 6. Immunoblots of PKC- α , - ϵ , and rhoA in isolated basilar artery. Immunoblots are representative of five independent preparations. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat, PKC : protein kinase C alpha, PKC : protein kinase C epsilon.

신장이 rhoA 단백질과 PKC의 translocation에 미치는 영향

SHR WKY basilar artery rhoA
PKC isoforms rhoA
PKC isoform specific antibody western blot

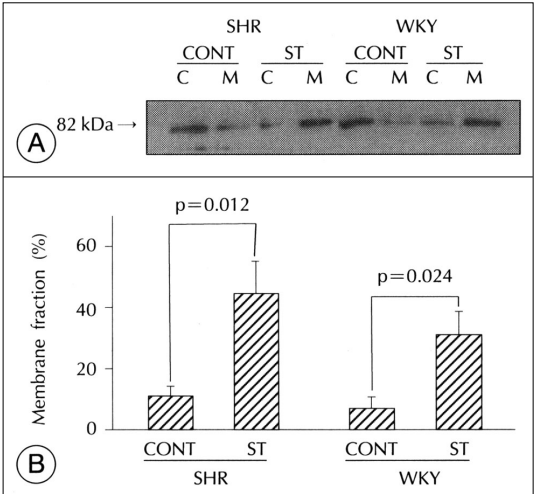


Fig. 7. Stretch-induced translocation of rhoA. A : typical data for stretch-induced translocation of rhoA. Results are representative of five experiments showing that rhoA is translocated from the cytosol (C) to the membrane (M) fraction by stretch (ST) in both SHR and WKY. B : statistical analysis for changes in membrane fraction by stretch in both SHR and WKY. Results are expressed as mean \pm SE. CONT : non-stretched tissues. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

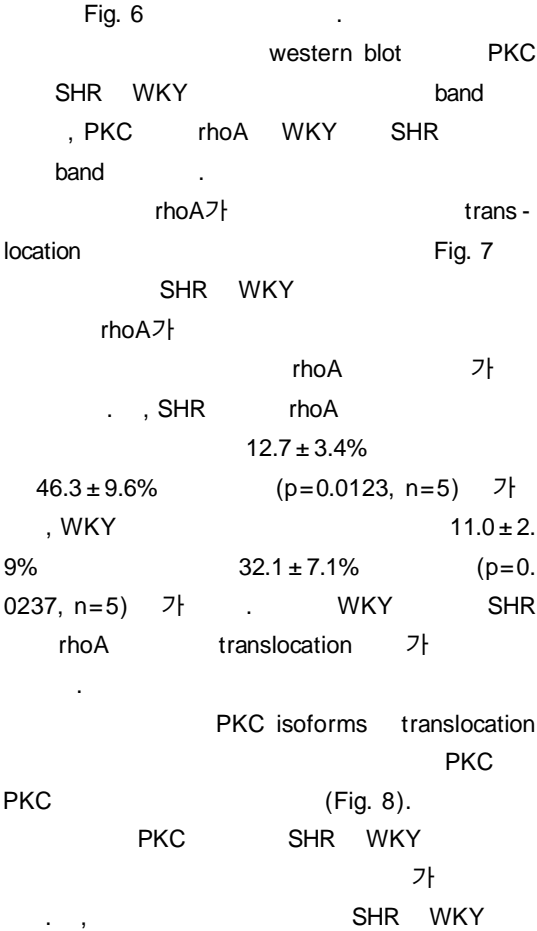


Fig. 8. Stretch-induced translocation of PKC (A) and PKC (C). Results are representative of five experiments. B and D : statistical analysis for changes in membrane fraction by stretch in both SHR (B) and WKY (D). Results are expressed as mean \pm SE. ST : stretched tissues, CONT : non-stretched tissues. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat. C : cytosol, M : membrane.

SHR 가

Ca²⁺ WKY , PKC SHR

SHR 가 SHR 가

Ca²⁺ WKY

Ca²⁺ nifedipine isoform classical PKC(, 1, 2) Ca²⁺, diacylglycerol phosphatidylserine novel PKC(, ,) Ca²⁺ - independent isoform dia - cylglycerol phosphatidylserine

Ca²⁺ rhoA . Atypical PKC(,) phosphatidylser - ine

Ca²⁺ rhoA PKC SHR

Ca²⁺ SHR rhoA WKY , PKC

SHR 가 SHR 가

rho - kinase Y - 27632가 rhoA PKC translocation

WKY SHR

rhoA DNA western blot rhoA

rhoA band가 WKY SHR PKC가 myosin light chain

SHR WKY 가 myosin light chain phosphatase my - osin light chain 가 my - osin light chain phosphatase

rhoA PKC

Ca²⁺ 가 Ca²⁺ 가가

SHR 가

Ca²⁺ rhoA/rho - kinase PKC가

PKC H - 7 calphostin - 가

WKY SHR

SHR WKY

western blot PKC SHR WKY

band PKC band

WKY SHR

PKC가

요 약

SHR WKY PKC western blot PKC

translocation PKC SHR WKY transloca - tion PKC SHR

translocation PKC

배경 및 목적 : (myogenic response)

가

가

가

SHR WKY (str - , 2) SHR

etch)

WKY

방 법 :

basilar artery (stretch)

basilar artery

, rhoA PKC immun -

oblot translocation

결 과 :

Ca²⁺ basilar ar -

tery

SHR WKY

WKY SHR

Ca²⁺

SHR

WKY

Ca²⁺ nifedipine SHR WKY

kinase Y - 27632 SHR WKY

WKY SHR

PKC H - 7 calphostin - C

WKY SHR

PKC isoforms - specific antibody im -

munoblotting PKC SHR WKY

band rhoA PKC WKY

SHR band RhoA

SHR WKY 가

translocation translocation

WKY SHR

PKC SHR WKY

translocation

PKC SHR translocation

WKY

결 론 :

SHR WKY

가 rhoA

가

가

중심 단어 : ; Protein kinase C ; RhoA

;

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