Intracellular acidosis induced myogenic tone change in rabbit basilar artery

- Role of extracellular Ca^{2+}, SR Ca^{2+} release and Ca^{2+} sensitization

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Directed by Associate professor Young-Ho Lee

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Abstract

Intracellular acidosis induced myogenic tone change in rabbit basilar artery - Role of Ca^{2+} influx, SR Ca^{2+} release and Ca^{2+}sensitization

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(Directed by Associate Professor Young-Ho Lee)

Myogenic tone is a constriction response of arteries to the increase in intraluminal pressure and it is known as an important mechanism of blood flow regulation. Changes in intracellular pH may also have important effects on the contractility of vascular smooth muscle cell by altering the regulation of $[Ca^{2+}]_i$ or by changing the Ca^{2+} sensitivity. Although there are many reports concerned with effect of changes of pH_i on vascular tone, most investigations of the relationship between pH_i and tone have been carried out in conduit or pre-contracted arteries. Thus, this study investigates the effect of pH_i on myogenic tone in rabbit basilar artery. Myogenic tone was developed by stretch and measured with isometric myograph and intracellular acidification was induced by the bath application of sodium acetate.

1. Sodium acetate increased myogenic tone transiently, followed by a slowly decreased and sustained tone over the resting level.
2. Elimination of extracellular Ca^{2+} and pretreatment of 10^{-6}M nifedipine, VOCC blocker, blocked sodium acetate-induced increase of myogenic

tone.
3. 2×10^{-5}M cyclopiazonic acid, SERCA inhibitor had no effect on the sodium acetate-induced change of myogenic tone.
4. Several inhibitors involved in Ca^{2+} sensitization pathways, 10^{-6}M Y-27632(ROCK inhibitor), 5×10^{-7}M calphostin C(PKC inhibitor) and 10^{-5}M PD 98059(MAPK inhibitor) had no effect on the sodium acetate-induced change of myogenic tone.
5. 10^{-5}M gadolinium(stretch-activated cation channel inhibitor) and 5-Nitro-2-(3-phynylpropylamino)benzoic acid, Cl^- channel blocker, had no effect on the sodium acetate-induced change of myogenic tone. On the other hand, 5mM TEA(nonselective K^+ channel blocker) inhibited sodium acetate-induced increase of myogenic tone.

These results suggest that intracellular acidosis increases stretch-induced myogenic tone in rabbit basilar artery. Furthermore, Ca^{2+} influx via VOCC may play an important role in intracellular acidosis-induced increase of myogenic tone and inhibition of K^+ channels by intracellular acidosis may be partly involved in activation of VOCC.

Key Words : intracellular acidosis, myogenic tone, rabbit basilar artery, voltage-operated Ca^{2+} channel, K^+ channel

Intracellular acidosis induced myogenic tone change in rabbit basilar artery - Role of Ca^{2+} influx, SR Ca^{2+} release and Ca^{2+} sensitization

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Ⅰ. INTRODUCTION

Myogenic tone is defined as the constrictor response of arteries to an increase in the intraluminal pressure.[1] An increase in pressure leads to vasoconstriction, whereas a decrease in pressure leads to vasodilatation.[2] This response is inherent to smooth muscle and independent of neuronal, metabolic, and hormonal influences.[3] In the vascular system, the myogenic response has been proposed to participate in a number of physiologically important functions. The two importances of these are 1) establishment of basal vascular tone and 2) autoregulation of blood flow and capillary hydrostatic pressure.[3]

The mechanism of the myogenic tone development is thought to be highly dependent on an extracellular Ca^{2+} influx through voltage-operated calcium channels(VOCC).[2] Nonselective cation channels, Cl^- channels and K^+ channels can modulate the VOCC activities. SR calcium release through phospholipase C(PLC) activation and Ca^{2+}

sensitization mechanisms are also known to be involved in the development of myogenic tone.[3]

Changes in intracellular pH(pH_i) may also have an important effect on the vascular smooth muscle contraction by altering the regulation of intracellular Ca^{2+} concentration($[Ca^{2+}]_i$) and/or by changing the sensitivity of myofibrils to Ca^{2+}.[4] And it is considered as an important factor of a metabolic theory of a local blood flow control having a potent effect on a diameter of blood vessels.[5,6]

It has been known that changes in extracellular pH(pH_o) and pH_i have a profound effect on vascular tone and play a major role in the local control of blood flow. Their importance and mechanism of action are still unclear, but changes in pH_i induced by pH_o may be an important role in vascular tone.[7,8] The effects of altered pH_i on vascular tone are various. It has been reported that intracellular alkalinization by treatment with NH_4Cl increased in force of canine pulmonary arteries[9] or elicited a rapid and transient decrease in force followed by a sustained increase.[5,10,11] And it has been reported that intracellular acidification produced a transient increase in force[10,11,12] or sustained increase in force.[13] On the contrary, marked dilatation was also shown in rat basilar artery in sodium propionate-induced intracellular acidosis.[14]

Although there are many reports concerned with effects of pH_i changes on vascular tone, most investigations of the relationship between pH_i and tone have been carried out in conduit or pre-contracted arteries. No previous study appears to have examined the effects of alteration of pH_i on myogenic tone which may play an important role in blood flow control in small arteries. Therefore, this study is the first to investigate the changes of myogenic tone to intracellular acidosis and its mechanisms in rabbit basilar arteries.

Ⅱ. MATERIALS AND METHODS

1. Tissue preparation

New Zealand White Rabbits weighing 1.7～2.2kg were anesthetized with Ketamine(11mg/kg) and heparin(2,000IU/kg). All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use committee. The brain was excised and placed in a Krebs-Henseleit solution(KH solution). The basilar arteries were dissected out from the brain and prepared ring segments(about 1㎜ length and 300～500㎛ outside diameter). To avoid the possible influences of endothelium-derived factors, the endothelium of the strip was removed by gentle rubbing the endothelial surface.

2. Measurements of myogenic tone

Arterial rings were mounted isometrically on two stainless-steel wires, which were fastened to a strain gauge transducer and a L-shape rod in a chamber, respectively. And then, a period of 30min was used to equilibrate the rings in a 15ml organ bath at 37℃ containing KH solution which is aerated with a gas mixture of 95% O_2 and 5% CO_2 to maintain pH at 7.4.

After equilibration in normal KH solution, the ring segments were stretched passively to a resting tension of 700mg. When stretch-induced myogenic tone was developed and maintained, buffer solution was changed to Ca^{2+} free(0-Ca^{2+}) KH solution to confirm the magnitude of myogenic tone(Fig. 1).

3. Solutions and chemicals

KH solution contained(mM): 119 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.5 $MgSO_4$, 25 $NaHCO_3$ and 11 glucose. The 0-Ca^{2+} KH solution was prepared by omission of Ca^{2+} from the KH solution. Intracellular acidosis

was produced by replacing 20mM NaCl in KH solution with equimolar sodium acetate. Nifedipine, gadolinium, tetraethylammonium chloride(TEA), ruthenium red, 5-Nitro-2-(3-phenylprepylamino)benzoic Acid(NPPB) were obtained from Sigma Chemical Co.(St Lousi, MO, USA). PD98059 was obtained from Calbiochem(Darmstadt, Germany). Cyclopiazonic acid was obtained from TOCRIS(Bristol, UK). Y-27632 was obtained from Biomol(Plymouth, PA).

4. Statistics

Data are expressed as means±S.E. and *n* indicates the number of strips. Force was expressed as a relative percentage of myogenic tone. Differences between means tested using Student's *t*-tests. Significant differences were taken at the $p<0.05$ level.

Ⅲ. RESULTS

1. Effect of decreased pH_i on myogenic tone

To investigate the effects of intracellular acidosis on myogenic tone in basilar artery, 20mM sodium acetate was applied when the myogenic tone was stable. As shown in Fig. 1, application of sodium acetate increased myogenic tone transiently(transient tone: 60.12±2.72%) followed by a slowly decreased and sustained tone over the resting level(sustained tone: 21.41±2.50%) Removal of sodium acetate caused a transient decrease of myogenic tone and then slow return to the resting level(data were not shown).

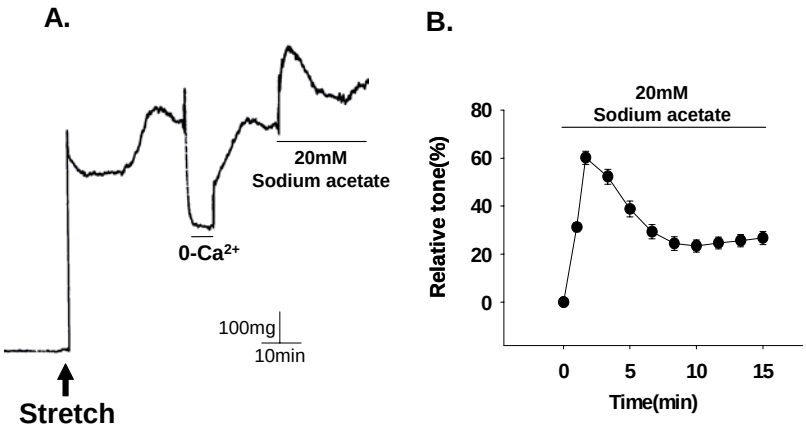

Fig. 1. Effect of sodium acetate on myogenic tone in rabbit basilar artery. A: Typical record for response of myogenic tone to 20mM sodium acetate. When stretch-induced tone was maintained, extracellular Ca^{2+} was eliminated to measure amplitude of myogenic tone. After tension returned to the original level by treatment of external Ca^{2+}, 20mM sodium acetate was treated. B: Statistical evaluation for sodium acetate-induced change of myogenic tone. Data are expressed as the mean±S.E. of 67 independent experiments.

2. Mechanisms of intracellular acidosis-induced increase of myogenic tone

A. Role of extracellular Ca^{2+} influx

To determine the mechanisms involved in intracellular acidosis-induced increase of myogenic tone, role of extracellular Ca^{2+} influx was examined. As shown in Fig. 2, in the presence of extracellular Ca^{2+}, 20mM sodium acetate induced transient and sustained increase of myogenic tone. However, in the absence of extracellular Ca^{2+}, 20mM sodium acetate did not induce any changes of myogenic tone.

To further investigate the role of Ca^{2+} influx, the effect of nifedipine(10^{-6}M), VOCC blocker, was examined. As shown in Fig. 2B, pretreatment of nifedipine blocked the sodium acetate-induced transient and sustained tone.

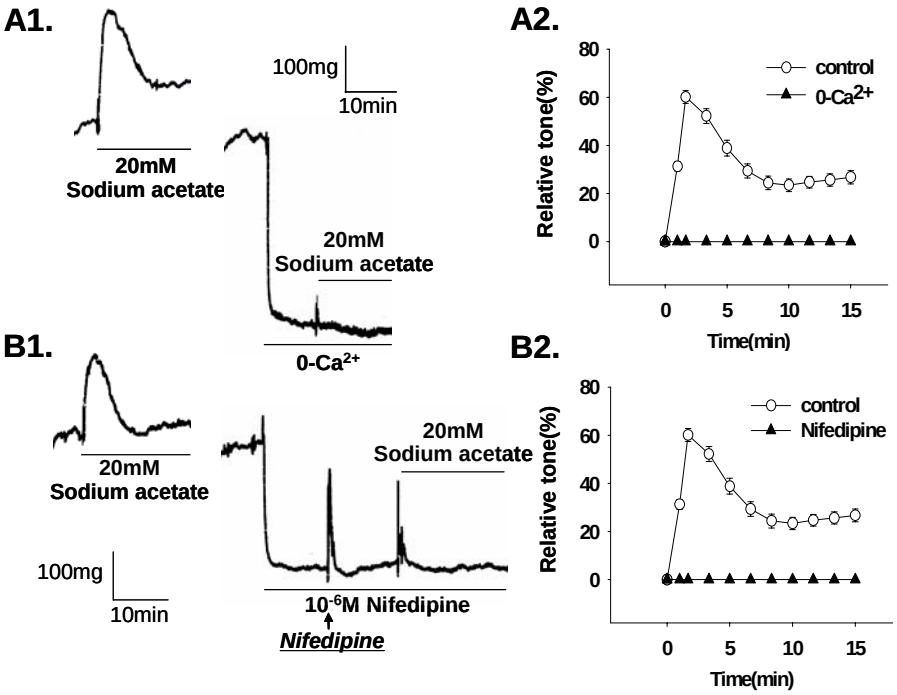

Fig. 2. Role of extracellular Ca^{2+} influx in the change of myogenic tone induced by sodium acetate. A1: Typical record for response of myogenic tone to 20mM sodium acetate in the presence or absence of extracellular Ca^{2+}. B1: Typical record for response of myogenic tone to 20mM sodium acetate in the presence or absence of 10^{-6}M nifedipine, voltage-operated Ca^{2+} channel blocker. A2 & B2: Statistical evaluation of effects of 0-Ca^{2+}(n=4) and nifedipine(n=8), repectively, on sodium acetate-induced increase of myogenic tone. Data are expressed as the mean±S.E.

B. Role of Ca^{2+} release from sarcoplasmic reticulum

To investigate the role of Ca^{2+} release from sarcoplasmic reticulum(SR), effect of 2×10^{-5}M cyclopiazonic acid, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase(SERCA) inhibitor, on the 20mM sodium acetate-induced change of myogenic tone was examined. As shown in Fig. 3, pretreatment of cyclopiazonic acid increased resting myogenic tone(60.15±12.46%), but had no effect on the sodium acetate-induced change of myogenic tone. The mean peak amplitudes of tone in response to sodium acetate without and with cyclopiazonic acid were 60.12±2.72% and 52.3±3.59%(n=10), respectively.

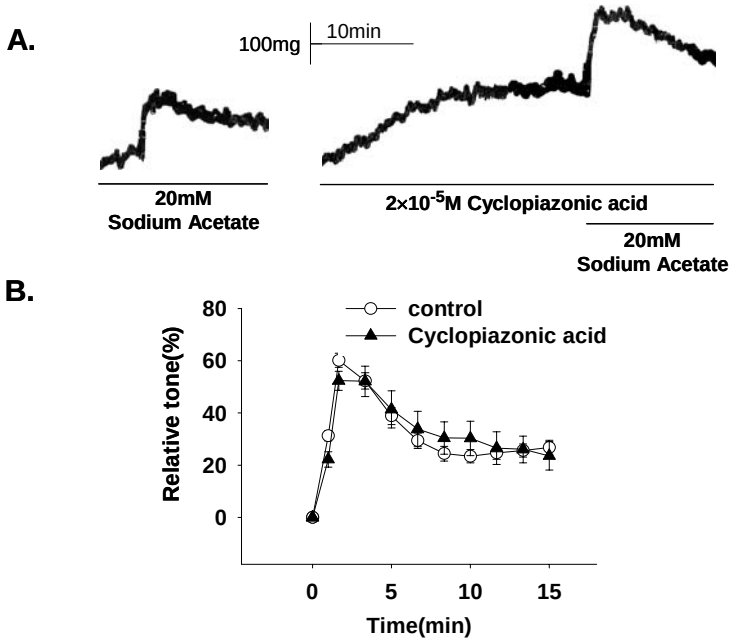

Fig. 3. Role of sarcoplasmic reticulum Ca^{2+} release in the change of myogenic tone induced by sodium acetate. A: Typical record for the effect of 2×10^{-5}M cyclopiazonic acid, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase(SERCA) inhibitor, on the sodium acetate-induced change of myogenic tone. B: Statistical evaluation of the effect of cyclopiazonic acid on sodium acetate-induced increase of myogenic tone. Data are expressed as the mean±S.E.(n=10).

C. Role of Ca^{2+} sensitization mechanisms

To determine if Ca^{2+} sensitization mechanisms may play a role in the sodium acetate-induced change of myogenic tone, role of rho A kinase(ROK), protein kinase C(PKC) and mitogen-activated protein kinase(MAPK) was examined. As shown in Fig. 4, application of 10^{-6}M Y-27362, ROK inhibitor, reduced resting myogenic tone(-102.24±18.83%), but did not affect 20mM sodium acetate-induced increase of myogenic tone. The mean peak amplitude of tone without and with Y-27632 were 60.12±2.72% and 69.08±5.16%(n=4), respectively. Pretreatment of 5×10^{-7}M calphostin C, PKC inhibitor and 10^{-5}M PD98059, MAPK inhibitor, also had no effect on sodium acetate-induced increase of myogenic tone. The peak amplitudes of tone in response to sodium acetate without and with inhibitors, respectively, were 60.12±2.72% and 64.98±16.43(n=6) in calphostin C and 60.12±2.72% and 75.69±21.73%(n=5) in PD98059.

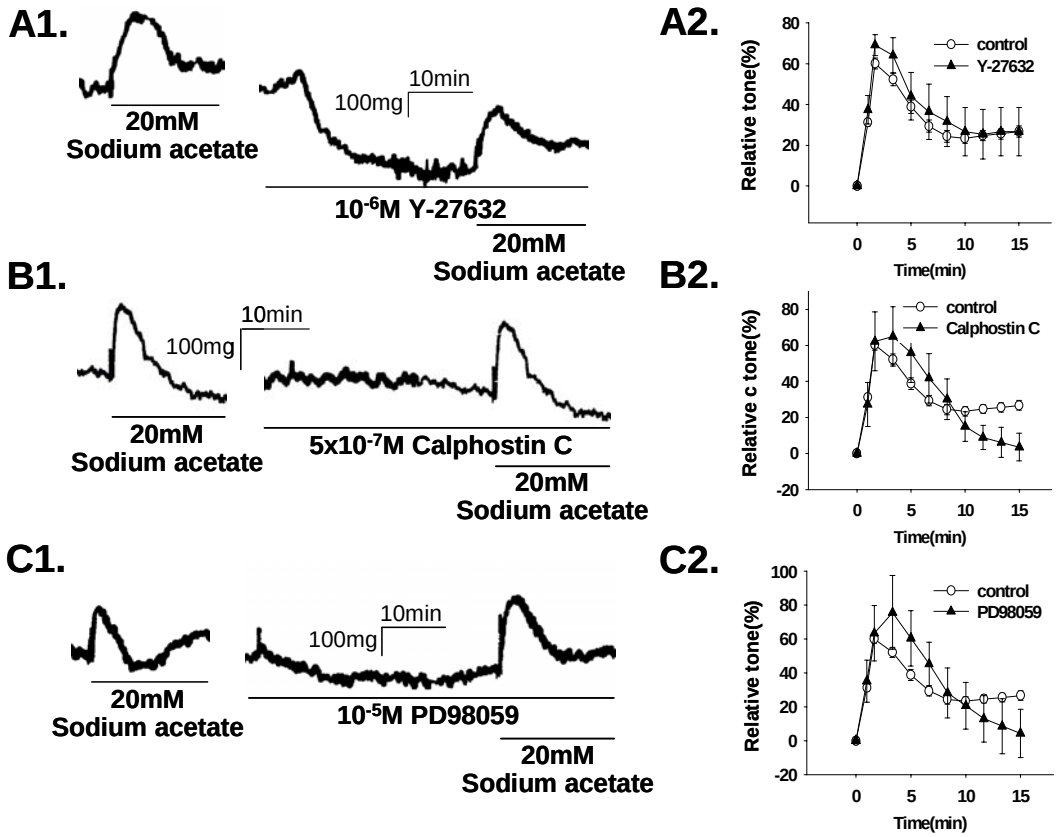

Fig. 4. Effect of several inhibitors involved in Ca^{2+} sensitization pathways on the sodium acetate-induced change of myogenic tone. A1 & A2: Effect of Rho-kinase inhibitor, 10^{-6}M Y-27632 on the 20mM sodium acetate-induced change of myogenic tone(n=4). B1 & B2: Effect of protein kinase C inhibitor, 5×10^{-7}M calphostin C on the sodium acetate-induced change of myogenic tone(n=6). C1 & C2: Effect of mitogen-activated protein kinase inhibitor, 10^{-5}M PD98059 on the sodium acetate-induced change of myogenic tone(n=9). Data are expressed as means±S.E.

D. Role of stretch-activated cation channels

To determine if stretch-activated cation channels may play a role in sodium acetate-induced increase of myogenic tone, the effect of two stretch-activated cation channel blockers was tested. As shown in Fig. 5, 10^{-5}M gadolinium, stretch-activated cation channel blocker, had no effect on resting myogenic tone and the sodium acetate-induced increase of myogenic tone. The peak amplitudes of tone in response to sodium acetate without and with gadolinium were 60.12±2.72% and 73.92±9.59%(n=5), respectively. Effect of ruthenium red known as nonselective cation channel blocker was also tested. When 3×10^{-5}M ruthenium red was pretreated, resting myogenic tone decreased to almost 0-Ca^{2+} level(-91.49±6.83%), and sodium acetate-induced increase of myogenic tone was completely abolished by pretreatment of ruthenium red.

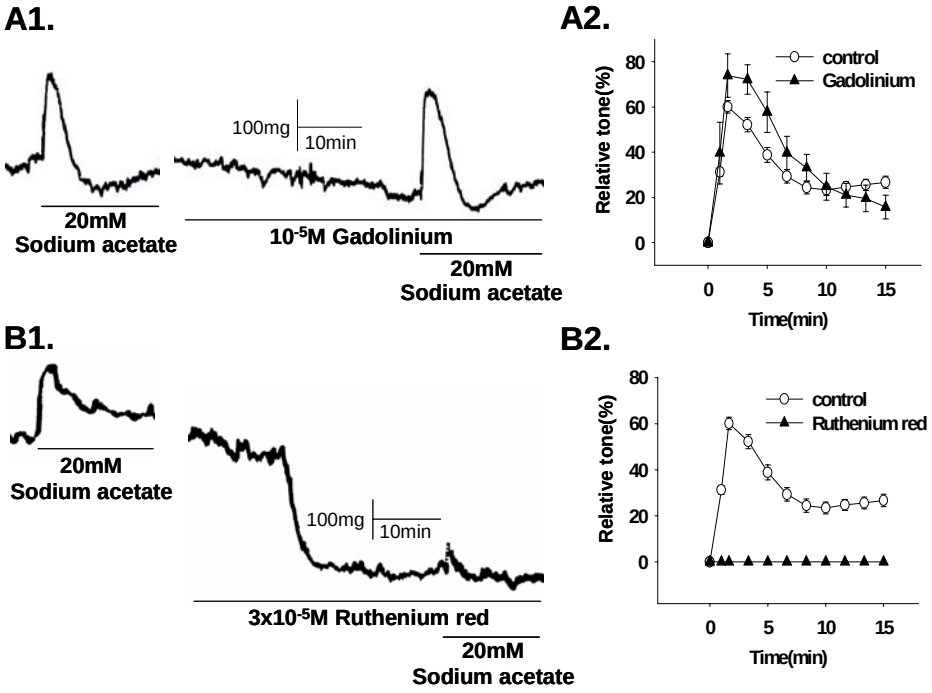

Fig. 5. Effects of stretch-activated cation channel on the sodium acetate-induced change of myogenic tone. A1 & A2: Effect of 10^{-5}M gadolinium, stretch-activated cation channel blocker, on the sodium acetate-induced change of myogenic tone(n=5). B1 & B2: Effect of 3×10^{-5}M ruthenium red, nonselective cation channel inhibitor, on the sodium acetate-induced change of myogenic tone(n=7). Data are expressed as means±S.E.

E. Role of Cl^- channels

To determine if Cl^- channels may play a role in sodium acetate-induced increase of tone, the effect of 5-Nitro-2-(3-phynylpropylamino)benzoic acid(NPPB), Cl^- channel inhibitor, was tested. As shown in Fig. 6, pretreatment NPPB changed neither myogenic tone nor sodium acetate-induced myogenic tone. The peak amplitudes of tone in response to sodium acetate without and with NPPB were 60.12±2.72% and 63.91±12.62%(n=7), respectively.

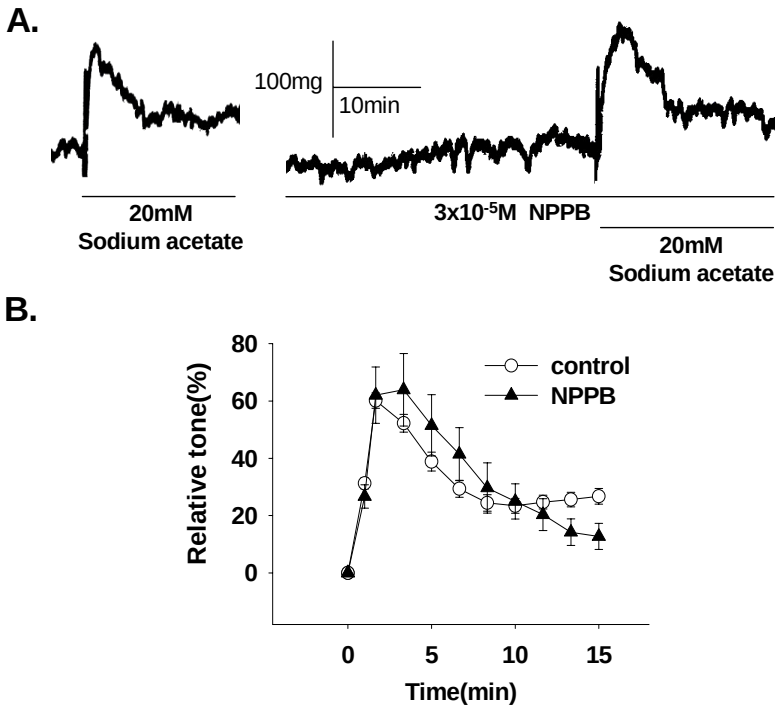

Fig. 6. Effects of Cl^- channel on the sodium acetate-induced change of myogenic tone. A: Effect of 3×10^{-5}M 5-Nitro-2-(3-phynylpropylamino)benzoic acid(NPPB), Cl^- channel inhibitor, on the 20mM sodium acetate-induced change of myogenic tone. B: Statistical analysis of the effect of NPPB on the sodium acetate-induced change of myogenic tone. Data are expressed as the mean±S.E.(n=7).

F. Role of K^+ channels

To determine the role of K^+ channels on sodium acetate-induced increase of myogenic tone, the effect of tetraethylammonium chlorid(TEA), nonselective K^+ channel blocker, was examined. As shown in Fig. 7, pretreatment of 5mM TEA produced increase of resting myogenic tone(151.93±18.84%) and inhibited the sodium acetate-induced increase of myogenic tone. The peak amplitudes of tone in response to sodium acetate without and with TEA were 60.12±2.72% and 34.35±6.64%(n=12), respectively.

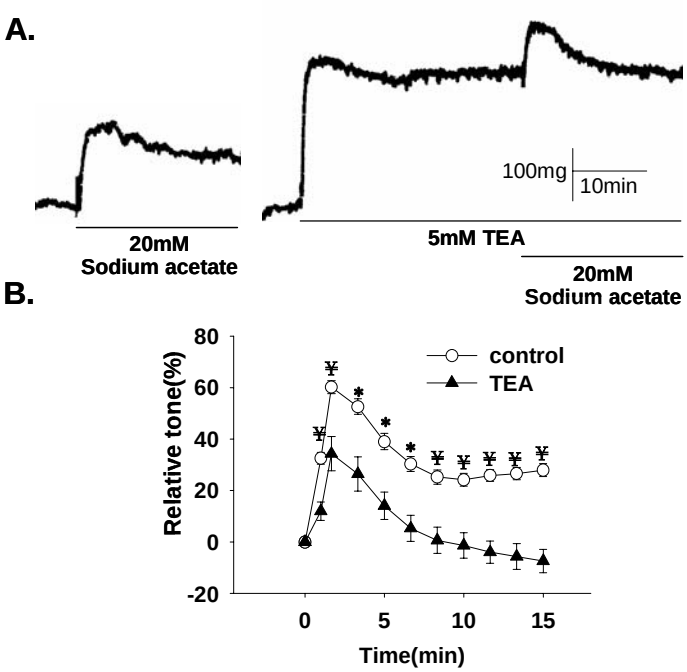

Fig. 7. Effects of K^+ channel on the sodium acetate-induced change of myogenic tone. A: Effect of 5mM tetraethylammonium chloride(TEA), nonselective K^+ channel blocker, on the 20mM sodium acetate-induced change of myogenic tone. B: Statistical analysis of the effect of TEA on the sodium acetate-induced change of myogenic tone. Data are expressed as the mean±S.E.(n=12). ¥ : $p<0.01$, * : $p<0.05$

Ⅳ. DISCUSSION

In the present study, it has been shown that intracellular acidosis induces increase of stretch-induced myogenic tone in rabbit basilar artery, and acidosis-induced increase of myogenic tone may be due to Ca^{2+} influx via nifedipine sensitive VOCC. Furthermore, it has been also shown that activation of VOCC by intracellular acidosis may be due to direct and indirect effects via membrane depolarization by inhibition of K^+ channels when hydrogen ion increases.

It has been known that acute lowering of pH_i in vascular smooth muscle was generally associated with transient tone development[15,16], but prolonged intracellular acidosis induced dilatation.[4,17] In the present study, intracellular acidosis induced small transient increase of tone followed by sustained tone. These results are consistent with a number of earlier reports in that the small transient tension developed in small resistant arteries in response to acute acidosis.[7,8,11] In earlier reports, intracellular acidification has been induced by raising pCO_2 or NH_4Cl pre-pulse, in which washout of NH_4Cl is associated with immediate and profound intracellular acidification. In the present study, we used treatment of sodium acetate to induce intracellular acidosis. Although we did not measure changes of pH_i by treatment of sodium acetate, it has been reported that sodium acetate permeates the cell and releases protons, which produce intracellular acidification.[11]

1. Extracellular Ca^{2+} influx

To determine the mechanism of sodium acetate-induced increase of myogenic tone, we measured the effect of extracellular Ca^{2+} removal and the pretreatment of nifedipine on intracellular acidosis-induced increase of tone. We showed that extracellular Ca^{2+} elimination and pretreatment

of nifedipine abolished sodium acetate-induced increase of myogenic tone. These results suggest that increase of stretch-induced myogenic tone by intracellular acidosis may be due to Ca^{2+} influx via nifedipine sensitive L-type VOCC. These results are consistent with previous results that intracellular acidosis-induced increase of tone is inhibited by treatment of Ca^{2+} channel antagonist.[5,9,18] Matthews *et al* have shown that transient tension development induced by NH_4Cl pre-pulse was inhibited by removal of extracellular Ca^{2+} and diltiazem in mesenteric resistant artery.[5] It was also shown that inhibition of Ca^{2+} entry through VOCC with nifedipine attenuated transient contraction induced by NH_4Cl pre-pulse.[9] These results are consistent with our results.

However, effects of intracellular acidosis on vascular tone are variable. Klöckner and Isenberg have shown that elevation of pCO_2 in bath solution reduced Ca^{2+} current in porcine coronary artery.[19] These differences between experiments may be depend on the vessel diameter, methods evoked intracellular acidosis and the state of vascular tone such as its resting state or pre-contracted state.

To activate VOCC, elevation of hydrogen ion has direct effects on VOCC or indirect effects via membrane depolarization by modulation of several channels. To determine the mechanisms of depolarization induced by intracellular acidosis, we investigated the role of stretch-activated cation channels, Cl^- channels and K^+ channels in sodium acetate-induced increase of myogenic tone.

In isolated vascular smooth muscle cells, longitudinal stretch activates a nonselective inward cation conductance.[18] Although Ca^{2+} influx would be relatively small, it is generally believed that stretch mainly contributes to a membrane depolarization with subsequent opening of VOCCs.[2] However, in the present study, gadolinium, stretch-activated cation channel blocker, had no effect on resting and the intracellular acidosis-induced change of myogenic tone. These results are consistent with previous results that gadolinium did not block changes in myogenic

tone.[20] We also tested the effect of ruthenium red, nonselective cation channel inhibitor, on the sodium acetate-induced change of myogenic tone. Ruthenium red completely blocked the sodium acetate-induced increase of myogenic tone. These results are questionable. But it has been reported that ruthenium red has various effects such as inhibition of RyR-3, caffeine-insensitive Ca^{2+} release, mitochondrial Ca^{2+} accumulation, Ca^{2+}-ATPase in the plasma membrane and VOCC.[21] Therefore, the inhibitory effect of ruthenium red may be due to its non-specific effects.

To determine role of Cl^- channel in sodium acetate-induced increase of myogenic tone, the effect of 5-Nitro-2-(3-phynylpropylamino)benzoic acid(NPPB), Cl^- channel blocker, was tested. It has been proposed that activation of Cl^- channels may explain the stretch-induced depolarization of vascular smooth muscle.[22] It has been reported that DIDS, Cl^- channels inhibitor, hyperpolarized rat cerebral arteries and inhibited myogenic tone of pressurized cerebral arteries.[23] However, in the present study, NPPB had no effect on resting myogenic tone and intracellular acidosis-induced increase of tone.

It has been known that stretch-induced depolarization could result from inhibition of any or various K^+ currents identified in smooth muscle.[24] These results indicate that K^+ channels were activated when the vessels have a basal myogenic tone, even though there is no evidence of the direct role of K^+ channels in initiating myogenic tone. And it has been shown that K^+ currents can and do counteract myogenic tone development, and voltage-dependent and Ca^{2+}-activated K^+ channels are involved in theses phenomena.[25,26] In the present study, we showed that 5mM TEA, nonselective K^+ channel blocker, increased resting myogenic tone.

Intracellular acidification has been previously shown different effects on K^+ channels associated with circulation-specific, and sometimes opposing. In the present study, TEA inhibited intracellular

acidosis-induced increase of myogenic tone. This result indicates that intracellular acidosis blocks K^+ channels activated by stretch and induces further activation of VOCCs via membrane depolarization. These results are consistent with previous results that intracellular acidosis inhibited voltage-dependent K^+ currents in pulmonary arteries,[27] and activation of G protein coupled inward rectifying K^+ channels.[28]

In the present study, we did not elucidate which types of K^+ channels are involved in intracellular acidosis. Although it has been reported that Ca^{2+}-activated and/or voltage-dependent K^+ channels are involved in stretch-induced myogenic tone, additional studies are required to elucidate which types of K^+ channels are contribute to these conditions.

2. Ca^{2+} release from sarcoplasmic reticulum

In vascular smooth muscle, Ca^{2+} can be released from the SR by two different mechanisms: Ca^{2+}-induced Ca^{2+} release(CICR) and IP_3-induced Ca^{2+} release(IICR). It is known that these mechanisms play a role in myogenic tone development.[3] There are many studies investigated the role of SR in changes of vascular contractilities by pH_i changes and these studies elucidated that SR Ca^{2+} release is modified by $[H^+]_i$ changes. Batlle *et al* have shown that increase in $[Ca^{2+}]_i$ produced by cell acidification in rat thoracic aorta may be partly due to SR Ca^{2+} release.[29] It has been also reported that alteration of spontaneous force with intracellular pH changes has a requirement of Ca^{2+} release from caffeine-sensitive intracellular store in rat portal vein[18], and acidic pH-induced contraction is caused by SR Ca^{2+} release and VOCC activation in SHR and WKY aorta.[13] However, in the present study, cyclopiazonic acid, SERCA inhibitor, had no effect on the acidosis-induced increase of myogenic tone. These results suggest that SR may not be involved in the acidosis-induced change of myogenic tone in rabbit basilar artery. These results are consistent with the

results that SR Ca^{2+} release has less important role in development of myogenic tone in basilar artery because stretch-induced myogenic tone is highly dependent on a Ca^{2+} influx via cell membrane.[20]

3. Ca^{2+} sensitization mechanism

A major determinant of smooth muscle cell contractility is the phosphorylation state of the 20kDa myosin light chain(MLC_{20}). The phosphorylation of MLC_{20} is dually regulated through MLCK which enhances phophorylation and MLCP which decreases phosphorylation. It has been well known that regulation of Ca^{2+} sensitization in smooth muscle cells occurs through the inhibition of MLCP, and PKC, rhoA/ROCK and MAPK are involves in this pathway.[20,30] It has been also reported that rhoA/ROCK and PKC are involved in stretch-induced myogenic tone.[20] In the present study, we tested effects of these kinases on the intracellular acidosis-induced change of myogenic tone. However, these inhibitors concerned with PKC, rhoA/ROCK and MAPK had no effects on the intracellular acidosis-induced change of myogenic tone, although Y-27632, ROCK inhibitor, inhibited resting myogenic tone. These results are consistent with many previous results in that extracellular Ca^{2+} and/or SR Ca^{2+} release play a role in pH_i-induced changes of vascular contractilities not Ca^{2+} sensitization mechanism.[5,6,31]

In summary, present study shows that intracellular acidosis increases stretch-induced myogenic tone in rabbit basilar artery. Furthermore, Ca^{2+} influx via VOCCs may be play an important role in intracellular acidosis-induced increase of myogenic tone and inhibition of K^+ channels by intracellular acidosis may be partly involved in activation of VOCCs.

Ⅴ. CONCLUSION

Myogenic tone is a constriction response of arteries to the increase in intraluminal pressure and it is known as an important mechanism of blood flow regulation. Changes in intracellular pH may also have important effects on the contractility of vascular smooth muscle cell by altering the regulation of $[Ca^{2+}]_i$ or by changing the Ca^{2+} sensitivity. Although there are many reports concerned with effect of changes of pH_i on vascular tone, most investigations of the relationship between pH_i and tone have been carried out in conduit or pre-contracted arteries. Thus, this study investigates the effect of pH_i on myogenic tone in rabbit basilar artery. Myogenic tone was developed by stretch and measured with isometric myograph and intracellular acidification was induced by the bath application of sodium acetate.

1. Sodium acetate increased myogenic tone transiently, followed by a slowly decreased and sustained tone over the resting level.
2. Elimination of extracellular Ca^{2+} and pretreatment of 10^{-6}M nifedipine, VOCC blocker, blocked sodium acetate-induced increase of myogenic tone.
3. 2×10^{-5}M cyclopiazonic acid, SERCA inhibitor had no effect on the sodium acetate-induced change of myogenic tone.
4. Several inhibitors involved in Ca^{2+} sensitization pathways, 10^{-6}M Y-27632(ROCK inhibitor), 5×10^{-7}M calphostin C(PKC inhibitor) and 10^{-5}M PD 98059(MAPK inhibitor) had no effect on the sodium acetate-induced change of myogenic tone.
5. 10^{-5}M gadolinium(stretch-activated cation channel inhibitor) and 5-Nitro-2-(3-phynylpropylamino)benzoic acid, Cl^- channel blocker, had no effect on the sodium acetate-induced change of myogenic tone. On the other hand, 5mM TEA(nonselective K^+ channel blocker) inhibited

sodium acetate-induced increase of myogenic tone.

These results suggest that intracellular acidosis increases stretch-induced myogenic tone in rabbit basilar artery. Furthermore, Ca^{2+} influx via VOCC may play an important role in intracellular acidosis-induced increase of myogenic tone and inhibition of K^{+} channels by intracellular acidosis may be partly involved in activation of VOCC.

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Abstract(in Korean)

토끼 기저동맥에서 세포 내 산성화에 따른 myogenic tone의 변화 - 칼슘 유입, 소포체로부터 칼슘 유리와 칼슘 민감도의 역할

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근원성 장력(myogenic tone)은 혈관 내압이 증가하는 경우 혈관 평활근이 수축하는 반응으로서 혈류 조절의 중요한 기전으로 알려져 있다. 한편, 근원성 장력 이외에 세포 내 pH의 변화 또한 세포 내 Ca^{2+} 농도의 변화 및 Ca^{2+} sensitization 기전을 통해 혈관의 수축정도를 조절한다고 보고되어 있다. 비록 지금까지 혈관 장력에 미치는 세포 내 pH의 영향에 대한 연구는 많이 이루어져 있지만, 직경이 큰 혈관이나 미리 수축시킨 혈관에서 세포 내 pH와 혈관 장력과의 관계를 연구한 것이 대부분이었다. 그러므로 본 연구에서는 토끼 기저동맥에서의 근원성 장력에 미치는 세포 내 pH의 영향에 대하여 알아보고자 하였다. 신장(stretch)에 의한 근원성 장력의 크기 변화를 근원성 장력 변환기로 측정하였으며, sodium acetate를 이용하여 세포 내 산성화를 유도하였다.

1. 근원성 장력은 20mM sodium acetate로 유도한 세포 내 산성화에 의해 일시적으로 증가 된 후 서서히 저하되어 근원성 장력 이상으로 유지되었다.
2. Sodium acetate로 인한 장력의 증가는 외부 칼슘이 제거된 상태나 막전압 의존성 칼슘 통로 차단제인 10^{-6}M nifedipine을 전처치한 경우에서 소실되었다.
3. SERCA 억제제인 2×10^{-5}M cyclopiazonic acid는 세포 내 산성화로 인한

장력의 변화에 영향을 주지 않았다.
4. 10^{-6}M Y-27632(ROCK 억제제), 5×10^{-7}M calphostin C(PKC 억제제) 그리고 10^{-5}M PD98059(MAPK 억제제)는 세포 내 산성화로 인한 장력의 변화에 영향을 주지 않았다.
5. 10^{-5}M gadolinium(stretch-activated cation channel 억제제), 3×10^{-5}M 5-Nitro-2-(3-phynylpropylamino)benzoic acid(Cl^- 통로 억제제)는 세포 내 산성화로 인한 장력의 변화에 영향을 주지 않았으나 5mM TEA(비선택적 K^+ 통로 억제제)는 세포 내 산성화로 인한 장력의 증가를 현저하게 억제하였다.

이상의 실험을 종합하여 볼 때, 세포 내 산성화는 신장으로 인해 나타나는 근원성 장력을 증가시키며, 막전압 의존성 칼슘 통로를 통한 칼슘의 유입이 세포 내 산성화로 인한 근원성 장력의 증가에 중요한 역할을 할 것으로 생각된다. 세포 내 산성화에 의한 K^+ 통로의 억제 또한 막전압 의존성 칼슘 통로를 활성화하는 데 부분적으로 관여할 것으로 생각된다.

핵심되는 말 : 세포 내 산성화, 근원성 장력, 토끼 기저동맥, 막전압 의존성 칼슘 통로, K^+ 통로