

Calcium Sensitization Induced by Sodium Fluoride in Permeabilized Rat Mesenteric Arteries

Enyue Yang^{1,*}, Joon Yong Cho^{2,*}, Uy Dong Sohn³, and In Kyeom Kim^{1,4}

Departments of ¹Pharmacology, ²Thoracic and Cardiovascular Surgery, ⁴Cardiovascular Research Institute, Kyungpook National University School of Medicine, Daegu 700-422, ³Department of Pharmacology, College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea

It was hypothesized that NaF induces calcium sensitization in Ca²⁺-controlled solution in permeabilized rat mesenteric arteries. Rat mesenteric arteries were permeabilized with β -escin and subjected to tension measurement. NaF potentiated the concentration-response curves to Ca²⁺ (decreased EC₅₀ and increased E_{max}). Cumulative addition of NaF (4.0, 8.0 and 16 mM) also increased vascular tension in Ca²⁺-controlled solution at pCa 7.0 or pCa 6.5, but not at pCa 8.0. NaF-induced vasoconstriction and GTP γ S-induced vasoconstriction were not additive. NaF-induced vasoconstriction at pCa 7.0 was inhibited by pretreatment with Rho kinase inhibitors H1152 or Y27632 but not with a MLCK inhibitor ML-7 or a PKC inhibitor Ro31-8220. NaF induces calcium sensitization in a Ca²⁺-dependent manner in β -escin-permeabilized rat mesenteric arteries. These results suggest that NaF is an activator of the Rho kinase signaling pathway during vascular contraction.

Key Words: Calcium sensitization, Permeabilization, Mesenteric artery, Sodium fluoride, Rho kinase

INTRODUCTION

Smooth muscle contraction is activated by an increase in cytosolic calcium provided with calcium entry through voltage-sensitive calcium channels or with calcium release from the sarcoplasmic reticulum (SR). However, some agonists activating GPCRs can also lead to further contraction at constant level of intracellular calcium, so called calcium sensitization. Accumulating evidence indicates that RhoA/Rho kinase and protein kinase C (PKC) are the major pathways for calcium sensitization, which involve inhibition of myosin phosphatase activity [1,2]. Several cardiovascular diseases such as coronary vasospasm, cerebral vasospasm, hypertension, atherogenesis and endothelial dysfunction are associated with activation of RhoA-Rho kinase signaling pathway [3].

Sodium fluoride (NaF), an acknowledged Ser/Thr phosphatase inhibitor and G protein activator, has a wide range of effects on cellular metabolism and physiology. Both G protein-dependent and -independent pathways are likely to be involved in the physiological response to fluoride [4]. Fluoride has been reported to activate the stress-response signaling cascade involving MAP kinase [5]. Addition of aluminum fluoride (AlF₄⁻) induces calcium sensitization in various smooth muscles such as guinea pig vas deferens and airway smooth muscle [6,7]. However, the mechanism

by which NaF induces calcium sensitization is poorly understood. In a previous study, NaF-induced contraction was inhibited by a Rho kinase inhibitor, but not by inhibitors of MLCK or PKC, suggesting that vascular tension induced by NaF is, at least in part, mediated by Rho kinase [8,9].

β -Escin is reported to permeabilize the plasma membrane of smooth muscle without disrupting receptor function. Smooth muscle preparations treated with β -escin have been shown to respond to excitatory agonists through IP₃-mediated release of Ca²⁺ and to exert calcium sensitization [10].

This study investigated whether NaF induces calcium sensitization in Ca²⁺-controlled solution by using β -escin-permeabilized rat mesenteric arteries, and which mechanisms are involved in calcium sensitization.

METHODS

Ethical approval

The investigation is in accordance with Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH 1996). Nineteen male Sprague-Dawley rats, weighing 350~400 g, were used. Animals were anesthetized by sodium pentobarbital (50 mg kg⁻¹ i.p.).

ABBREVIATIONS: ANOVA, analysis of variance; CPI17, PKC-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa; MLC20, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; MYPT1, myosin phosphatase-targeting subunit 1; PDBu, phorbol 12, 13-dibutyrate.

Received January 15, 2010, Revised February 10, 2010,
Accepted February 22, 2010

Corresponding to: In Kyeom Kim, Department of Pharmacology, Kyungpook National University School of Medicine, 101, Donggin-2-ga, Daegu 700-422, Korea. (Tel) 82-53-420-4833, (Fax) 82-53-426-7345, (E-mail) inkim@knu.ac.kr

*These authors contributed equally to this paper.

Tissue preparation

Under anesthesia, the mesentery bed was removed and placed in HEPES buffered modified Tyrode's solution composed of (in mM) NaCl, 134; KCl, 5.6; CaCl₂, 2.5; MgCl₂, 1.0; HEPES, 10 and dextrose, 10. The second or third branches of mesenteric arteries were cleaned of fat and connective tissues, and dissected into small pieces (200~300 μ m in diameter, 4~5 mm in length) under a microscope. Small ring segments were mounted vertically between two thin wires in organ baths (3.0 ml) filled with HEPES solution, gassed with 100% O₂ and maintained at 37°C. Muscle strips were stretched passively by imposing the optimal resting tension (which was evaluated as maximum tension developed by 70 mM K⁺ solution), 0.5 g, which was maintained throughout the experiment as previously described [11]. Each preparation was equilibrated for more than 90 min before permeabilization.

Permeabilization and tension recording

The relaxing solution containing (in mM) potassium methanesulfonate, 74.1; MgATP, 4.5; EGTA, 1.0; PIPES, 30, and creatine phosphate, 10 were neutralized to pH 7.4 with KOH at 25°C as previously described [12]. The free Ca²⁺ concentration was calculated using a computer program and expressed as the negative logarithm (pCa). In the pCa solution, 10 mM EGTA was used to buffer the desired concentration of free Ca²⁺. Ionic strength was constant at 200 mM by adjusting the concentration of potassium methanesulfonate. Tissues were permeabilized by incubating with 30 μ M β -escin in the relaxing solution for 50 min at 27°C. Isometric contractions were recorded using a com-

puterized data acquisition system (PowerLab8/30, AD Instruments, Castle Hill, NSW, Australia).

Drugs

The drugs and chemicals were obtained from the following sources: NaF, H1152, β -escin, ML-7 and Ro31-8220 from Sigma Chemicals (St. Louis, MO, USA); guanosine-5'-O-(3-triphosphate) (GTP γ S) from Roche (Indianapolis, IN, USA); Y27632 from Santa Cruz Biotechnology (Santa Cruz, CA, USA); guanosine-5'-O-(2-thiodiphosphate); (GDP β S) from Enzo Life Sciences (Plymouth Meeting, PA, USA). Stock solutions of NaF, H1152, Y27632, GTP γ S, GDP β S and Ro31-8220 were prepared in double distilled water; ML-7 was prepared in ethanol:water (1 : 1) mixture. All other reagents were analytical grade.

Statistical analysis

Data are expressed as mean \pm SEM and were analyzed by repeated measures ANOVA followed by post-hoc Dunnett test or one-way ANOVA, followed by post-hoc Dunnett test. *p* values of less than 0.05 were regarded as significant.

RESULTS

NaF induces calcium sensitization in permeabilized mesenteric arteries

Concentration-response curves were obtained by changing Ca²⁺-controlled solution with free calcium of pCa 9.0~5.5 in the absence or presence of NaF (4.0, 8.0 or 12 mM). As shown in Fig. 1, NaF significantly increased the concen-

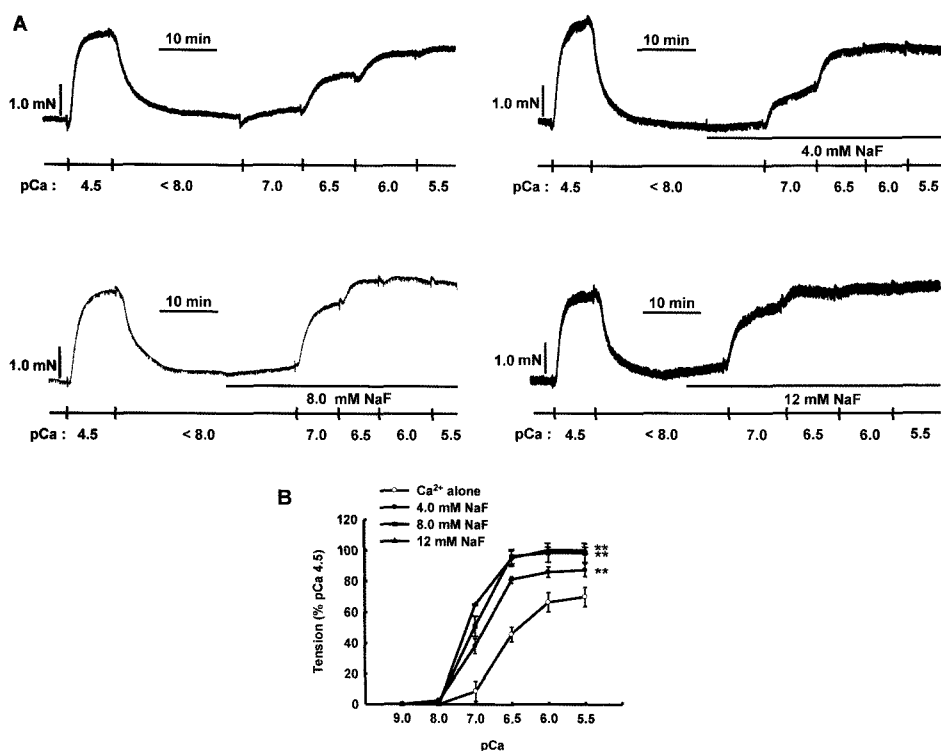


Fig. 1. NaF induces Ca²⁺ sensitization in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to increasing concentration of free calcium after initial high concentration of calcium (pCa 4.5) in the absence or presence of NaF (4.0, 8.0 or 12 mM). Line graphs (B) show the concentration-response curve to increasing concentration of free calcium (pCa 9.0~5.5) in the absence or presence of NaF (4.0, 8.0 or 12 mM) in β -escin-permeabilized mesenteric arteries. Developed tension is expressed as a percentage of the maximum tension induced by pCa 4.5. Data are expressed as means of 5 experiments with vertical bars showing SEM. ***p* < 0.01 vs. Ca²⁺ alone (Repeated measures ANOVA followed by post-hoc Dunnett test).

tration-response curves compared to calcium-alone curve, decreasing the $[Ca^{2+}]$ required to produce half-maximal force (EC_{50}) and increasing the maximum force developed in response to pCa 5.5 (Fig. 1 and Table 1).

Tensions were measured by cumulative addition of NaF (4.0, 8.0 and 16 mM) with calcium clamped at pCa 8.0, pCa 7.0 or pCa 6.5. As shown in Fig. 2, cumulative addition of NaF increased vascular tension in Ca^{2+} -controlled solution at pCa 7.0 or pCa 6.5, but not at pCa 8.0. NaF-induced contraction was reversible by washing with pCa 7.0 solutions (data not shown).

Effect of $GTP\gamma S$ on NaF-induced contraction in permeabilized mesenteric arteries

To examine whether $GTP\gamma S$ has an additive effect on NaF-induced vasoconstriction, the tension elicited by 100 μM

$GTP\gamma S$ in the absence or presence of NaF (4.0 or 8.0 mM) was recorded. As shown in Fig. 3, the tension induced by 4.0 or 8.0 mM NaF were not additive to those by 100 μM $GTP\gamma S$ alone ($p > 0.05$).

Effect of $GDP\beta S$ on NaF-induced contraction in permeabilized mesenteric arteries

To examine whether $GDP\beta S$ has an inhibitory effect on NaF-induced vasoconstriction, we recorded the tension elicited by 8.0 mM NaF before or after treatment with 300 μM $GDP\beta S$. Treatment with $GDP\beta S$, even at a high concentration (1,000 μM) (data not shown), did not inhibit NaF- or $GTP\gamma S$ -induced vasoconstrictions (Fig. 4).

Effect of Rho kinase inhibitors, Y27632 or H1152, on NaF-induced vasoconstriction in permeabilized mesenteric arteries

In order to address whether NaF-induced contraction involves RhoA/Rho kinase signaling pathway in permeabilized mesenteric arteries, the tension elicited by 8.0 mM NaF 30 min after pretreatment with Rho kinase inhibitors H1152 or Y27632 was recorded. NaF (8.0 mM) induced a sustained contraction, which reached plateaus within 20 min and maintained for at least 20 min. In the present study the same results were obtained as previous studies using intact vascular tissues [8,9] that both H1152 and Y27632 dose-dependently decreased vascular contraction in permeabilized mesenteric arteries (Fig. 5). At a high concentration of H1152 or Y27632, NaF-induced contractions were almost completely inhibited.

Table 1. EC_{50} and maximal effect (E_{max}) of the concentration-response curves to Ca^{2+} in the absence or presence of NaF in β -escin-permeabilized rat mesenteric arteries

	EC_{50} (pCa)	E_{max} (% of pCa 4.5)
Ca^{2+} alone	6.6 ± 0.03	69 ± 4.5
4.0 mM NaF	$7.0 \pm 0.01^*$	$87 \pm 4.0^*$
8.0 mM NaF	$7.0 \pm 0.01^*$	$98 \pm 6.2^*$
12 mM NaF	$7.1 \pm 0.06^*$	$100 \pm 2.6^*$

E_{max} is expressed as a percentage of the maximum tension to pCa 4.5. Values are means \pm SEM of 5 experiments for EC_{50} and E_{max} . * $p < 0.01$ vs. Ca^{2+} alone (One-way ANOVA followed by post-hoc Dunnett test).

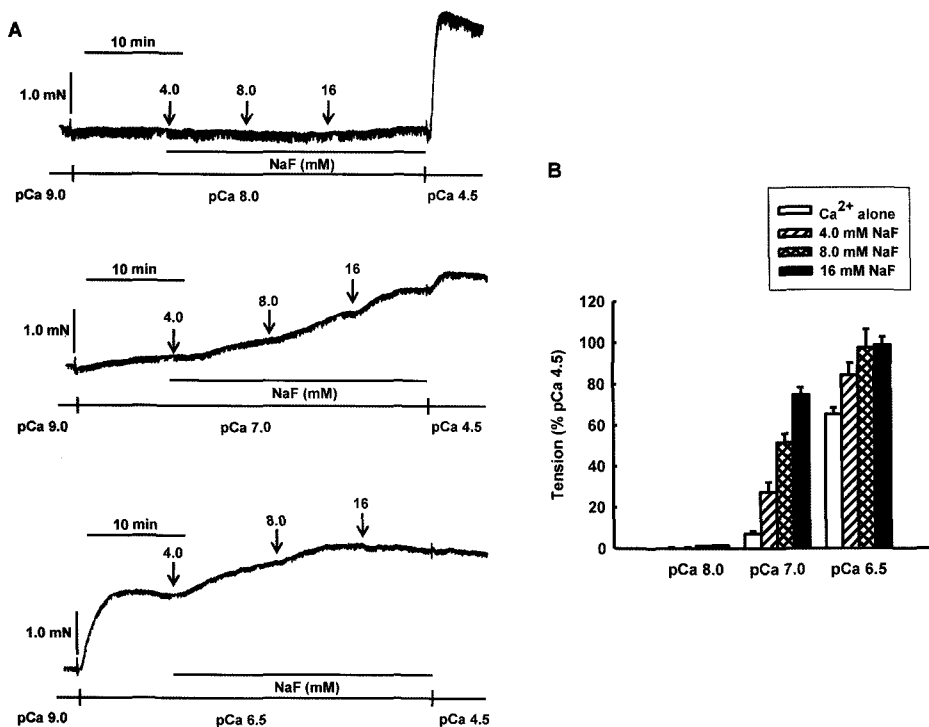


Fig. 2. NaF induces vasoconstriction in Ca^{2+} -controlled solution in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to cumulative addition of NaF (4.0, 8.0 and 16 mM) at constant concentration of calcium of pCa 8.0, 7.0 or 6.5. Bar graphs (B) show the developed tension elicited by cumulative addition of NaF (4.0, 8.0 and 16 mM) at constant concentration of calcium of pCa 8.0, 7.0 or 6.5 in β -escin-permeabilized mesenteric arteries. When NaF (16 mM)-induced contraction reached plateau, the bathing solutions were replaced with pCa 4.5 solution to obtain maximum contraction. Developed tension is expressed as a percentage of the maximum tension to pCa 4.5. Data are expressed as means of 5 experiments with vertical bars showing SEM.

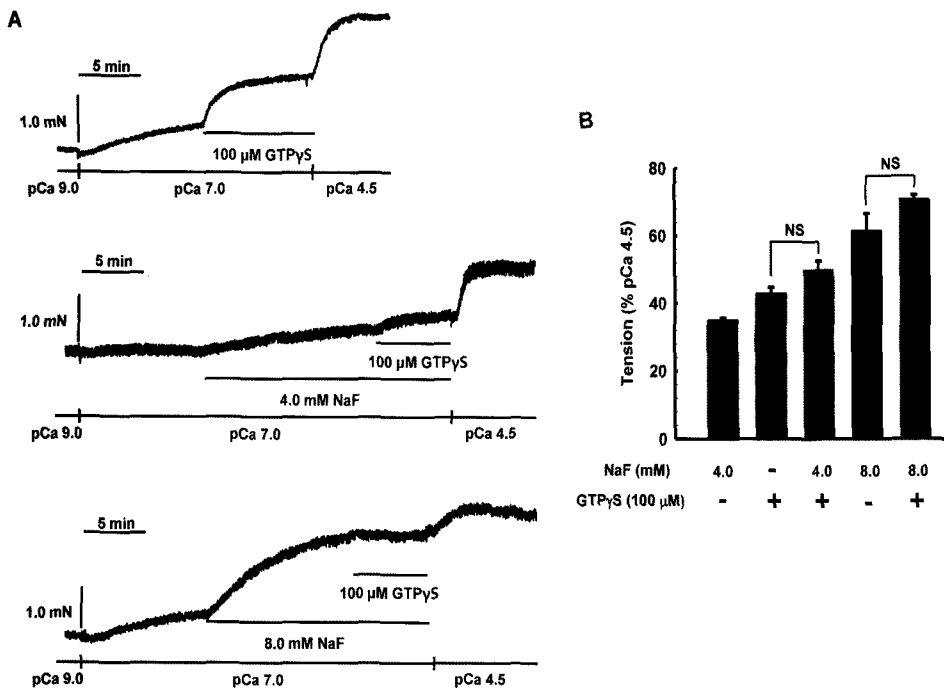


Fig. 3. Effect of GTP γ S on NaF-induced contraction in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to addition of 100 μ M GTP γ S in the absence or presence of NaF (4.0 or 8.0 mM) at constant concentration of calcium of pCa 7.0. Bar graphs (B) show the developed tension elicited by addition of 100 μ M GTP γ S in the absence or presence of NaF (4.0 or 8.0 mM) at constant concentration of calcium of pCa 7.0. When GTP γ S (100 μ M)-induced contraction reached plateau, the bathing solutions were replaced with pCa 4.5 solutions to obtain maximum contraction. Developed tension is expressed as a percentage of the maximum tension to pCa 4.5. Data are expressed as means of 5 experiments with vertical bars showing SEM. NS, not significant ($p > 0.05$) (One-way ANOVA followed by post-hoc Dunnett test).

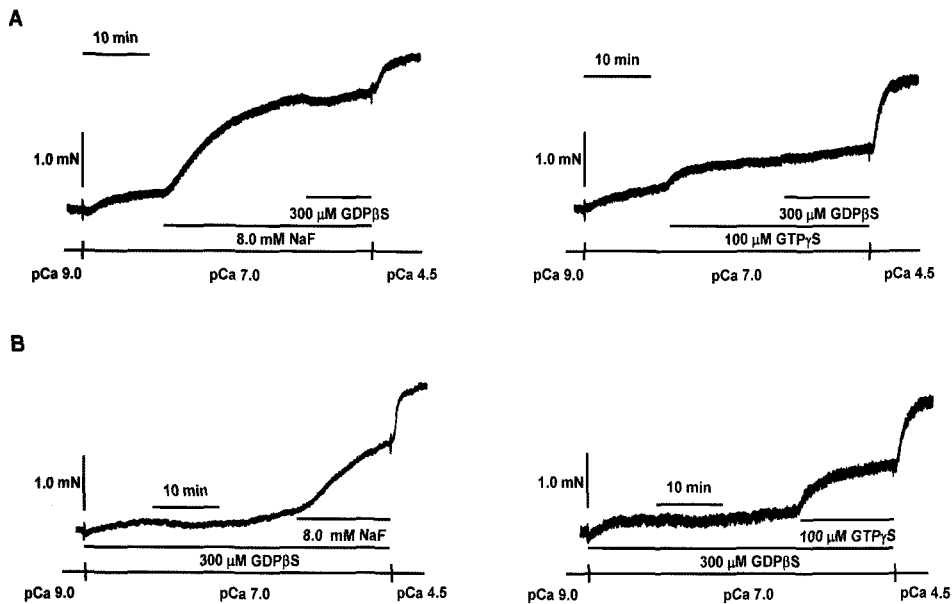


Fig. 4. Effect of GDP β S on NaF-induced contraction in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to addition of 8.0 mM NaF or 100 μ M GTP γ S following 300 μ M GDP β S at constant concentration of calcium of pCa 7.0. Representative traces (B) show tension development when β -escin-permeabilized mesenteric arteries were exposed to addition of 8.0 mM NaF or 100 μ M GTP γ S in the absence or presence of 300 μ M GDP β S at constant concentration of calcium of pCa 7.0. When GTP γ S (100 μ M)- or NaF (8.0 mM)-induced contraction reached plateau, the bathing solutions were replaced with pCa 4.5 solutions to obtain maximum contraction. The traces are representative of four experiments.

Effect of ML-7 or Ro31-8220 on NaF-induced vasoconstriction in permeabilized mesenteric arteries

To examine the involvement of MLCK or PKC on NaF-induced vasoconstriction in permeabilized mesenteric arteries, the effects of each inhibitor, ML-7 or Ro31-8220, on NaF-induced vasoconstriction were compared. As shown in Fig. 6, ML-7 or Ro31-8220 did not significantly inhibit the contraction induced by NaF ($p > 0.05$).

DISCUSSION

This study demonstrates that NaF induces calcium sensitization in a Ca^{2+} -dependent manner in β -escin-permeabilized rat mesenteric arteries. NaF-induced calcium sensitization involves the Rho kinase pathway.

Accumulating evidence demonstrates that agonists induce calcium sensitization in permeabilized smooth muscle tissues of different species. Sphingosylphosphorylcholine (SPC), a biologically active sphingomyelin metabolite, aug-

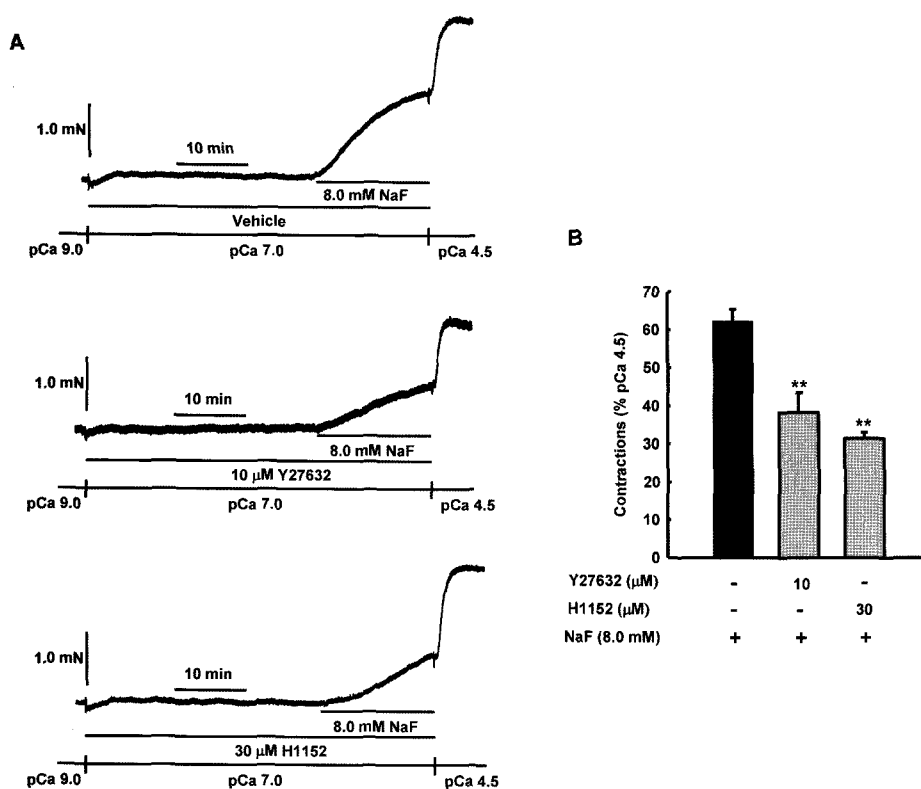


Fig. 5. Effect of Rho kinase inhibitors, Y27632 and H1152 on NaF-induced vasoconstriction in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to addition of 8.0 mM NaF in the absence or presence of Rho kinase inhibitors, Y27632 or H1152, at constant concentration of calcium of pCa 7.0. Bar graphs (B) show the developed tension elicited by addition of 8.0 mM NaF in the absence or presence of Rho kinase inhibitors, Y27632 or H1152, at constant concentration of calcium of pCa 7.0 in β -escin-permeabilized mesenteric arteries. When NaF (8.0 mM)-induced contraction reached plateau, the bathing solutions were replaced with pCa 4.5 solution to obtain maximum contraction. Developed tension is expressed as a percentage of the maximum tension to pCa 4.5. Data are expressed as means of 5 experiments with vertical bars showing SEM. ** $p < 0.01$ vs. vehicle (One-way ANOVA followed by post-hoc Dunnett test).

mented contractile force at pCa 6.3 in β -escin-permeabilized rabbit coronary arteries [12]; a muscarinic agonist induces calcium sensitization in β -escin-permeabilized rat and guinea-pig detrusor smooth muscles [13]; histamine, ET-1 and PDBu induce calcium sensitization in α -toxin-permeabilized rabbit femoral artery [14]; a thromboxane A_2 agonist, U46619-induced contraction involves Ca^{2+} entry and calcium sensitization in rat caudal arterial smooth muscle [15]. However, whether NaF induces calcium sensitization is poorly understood. In a previous study it was reported that NaF enhanced vasoconstriction in intact rat aorta in normal Krebs' solution or in EGTA-treated solution. The present study was able to demonstrate that NaF induces calcium sensitization (Fig. 1). Cumulative addition of NaF increased vascular tension in Ca^{2+} -controlled solution at pCa 7.0 or pCa 6.5, but not at pCa 8.0 (Fig. 2), showing that NaF enhances vasoconstriction in a Ca^{2+} -dependent manner.

In smooth muscles, the major mechanism for calcium sensitization is inactivation of MLCP. There are two main pathways known to control independently the activity of MLCP. One of them is RhoA/ Rho kinase signaling pathway. The involvement of RhoA in agonist-induced calcium sensitization has been reported in many tissues such as vas deferens, cerebral vasculatures, intestine and airways [6,16-18]. RhoA also causes a concentration-dependent calcium sensitization in β -escin-permeabilized smooth muscles [19]. U46619-induced Ca^{2+} sensitization involves Rho kinase mediated phosphorylation of MYPT1 at Thr855, but not Thr697, in rat caudal arterial smooth muscle [15]. Rho kinase is responsible for the calcium sensitization during arterial myogenic response through phosphorylation of MYPT1^{Thr855}

[16]. In a previous study we observed that NaF dose-dependently increased the amount of GTP RhoA and the phosphorylation level of CPI17 and MYPT1 which are downstream effectors of Rho kinase. Furthermore, both H1152 and Y27632 dose-dependently decreased vascular contraction and the phosphorylation level of CPI17 and MYPT1 induced by NaF in intact rat aorta (unpublished data). The present study also obtained the same results that both H1152 and Y27632 decreased vascular contraction in β -escin-permeabilized mesenteric arteries (Fig. 5). These results suggest that NaF is an activator of Rho kinase signaling pathway during vascular contraction in permeabilized mesenteric arteries.

Another signaling pathway for calcium sensitization is the inactivation of MLCP mediated by PKC and CPI17. PDBu-induced calcium sensitization which involves PKC/CPI17 can be almost completely inhibited by pretreatment with a PKC inhibitor GF109203X but slightly inhibited by a Rho kinase inhibitor Y27632 [14]. NaF-induced vasoconstriction was not inhibited by pretreatment with a PKC inhibitor Ro31-8220 (Fig. 6). PKC-induced Ca^{2+} sensitization may be absent in permeabilized arteries where CPI-17 can be washed out [20]. In the experimental conditions in this study, PDBu also did not produce significant tension in the permeabilized mesenteric arteries (data not shown), suggesting that some signaling molecules involved in PKC pathway may be missed in rat mesenteric arteries after permeabilization. PKC, at least in the current experiment, is not a main pathway in NaF-induced calcium sensitization in β -escin-permeabilized mesenteric arteries so that the involvement PKC in NaF-induced vasoconstriction can not be ruled out. NaF-induced vasoconstriction was not in-

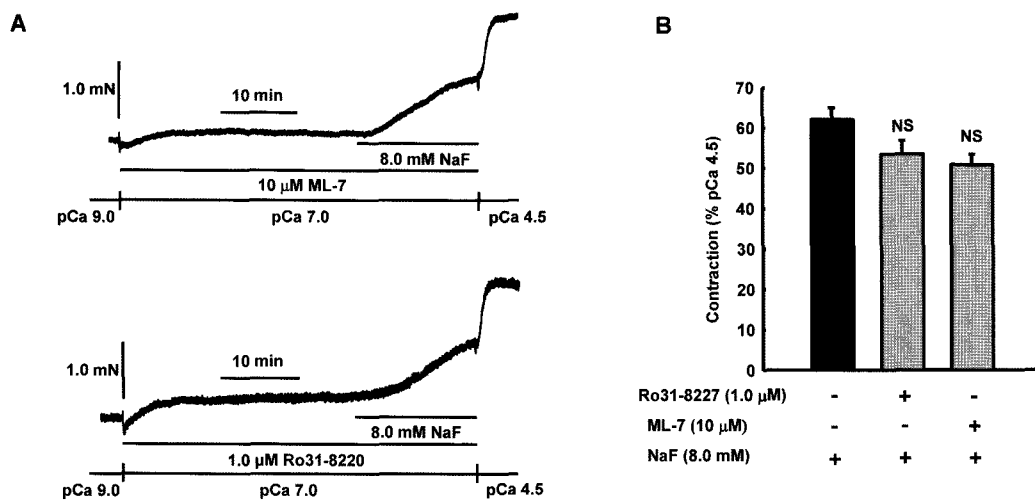


Fig. 6. Effect of ML-7 or Ro31-8220 on NaF-induced vasoconstriction in β -escin-permeabilized mesenteric arteries. Representative traces (A) show tension development when β -escin-permeabilized mesenteric arteries were exposed to addition of 8.0 mM NaF in the absence or presence of MLCK inhibitor ML-7 or PKC inhibitor Ro31-8220 at constant concentration of calcium of pCa 7.0. Bar graphs (B) show the developed tension elicited by addition of 8.0 mM NaF in the absence or presence of MLCK inhibitor ML-7 or PKC inhibitor Ro31-8220 at constant concentration of calcium of pCa 7.0 in β -escin-permeabilized mesenteric arteries. When NaF (8.0 mM)-induced contraction reached plateau, the bathing solutions were replaced with pCa 4.5 solution to obtain maximum contraction. Developed tension is expressed as a percentage of the maximum tension to pCa 4.5. Data are expressed as means of 5 experiments with vertical bars showing SEM. NS, not significant ($p > 0.05$) (One-way ANOVA followed by post-hoc Dunnett test).

hibited by pretreatment with ML-7, either (Fig. 6), indicating that MLCK is not a main pathway in NaF-induced calcium sensitization in β -escin-permeabilized mesenteric arteries. In the present study we examined the involvement of Ca^{2+} on NaF-induced contraction in permeabilized rat mesenteric arteries. NaF-induced vasoconstriction was not inhibited by pretreatment with a sarcoplasmic reticular Ca^{2+} -ATPase inhibitor cyclopiazonic acid (data not shown), suggesting that NaF induces vasoconstriction regardless of Ca^{2+} release from sarcoplasmic reticulum.

It is known that $GTP\gamma S$ activates both the membrane-associated heterotrimeric G protein and the small G protein, resulting in Ca^{2+} -independent contraction of smooth muscle. Many agonists such as histamine and norepinephrine (NE) induce calcium sensitization in the presence of GTP [6,10], whereas SPC does in the absence of GTP [10]. The facts that NaF induced vasoconstriction in the absence of $GTP\gamma S$ and that $GTP\gamma S$ did not enhance the contraction induced by NaF suggest that $GTP\gamma S$ has no additive effect on NaF-induced contraction (Fig. 3). $GDP\beta S$ reversed agonist- or GTP-induced contraction in many permeabilized tissues such as rabbit femoral artery and guinea pig vas deferens [6,14]. As shown in Fig. 4, $GDP\beta S$ did not inhibit NaF- or $GTP\gamma S$ -induced vasoconstriction. It may be due to a high concentration of $GTP\gamma S$ in this condition because $GDP\beta S$ competes with $GTP\gamma S$ for its binding [21]. Fluoride has been known to be a potent stimulator of G proteins including G_s , G_i and transducin [22-26]. The structural similarity of AlF_4^- to phosphate allows it to bind next to the β -phosphate of GDP and to mimic the terminal γ -phosphate of GTP. This eliminates the normal requirement for GDP-GTP exchange to cause conformational change and consequent activation of G proteins. The bound AlF_4^- cannot be hydrolyzed so that the G protein complex is maintained in its active, dissociated state, thus interrupting its

normal cycling between GDP- and GTP-bound states [27]. Together, it seems likely that NaF and GTP activate signaling pathway in the similar way at a targeting site on G protein. However, the mechanism by which NaF activates G protein remains to be elucidated.

Rho-kinase was first reported to be involved in development of hypertension in 1997. Y27632 decreased the blood pressure of all three hypertensive animal models including spontaneously hypertensive rat (SHR), renovascular hypertensive rats and deoxycorticosterone acetate (DOCA) salt-induced hypertensive rats, while it did a little in normotensive WKY rats [28]. Furthermore, conditional deletion of G12-13 did not alter normal blood pressure but blocked the development of salt-induced hypertension [29]. Rho kinase has been identified as an important target for treatment of several cardiovascular diseases [3,30]. Since NaF is an activator of Rho kinase signaling pathway during vascular contraction, it may be an important chemical tool for development of therapeutic drugs.

In conclusion, NaF induces calcium sensitization in a Ca^{2+} -dependent manner in β -escin-permeabilized rat mesenteric arteries. Rho kinase is the major signaling pathway in NaF induced calcium sensitization.

ACKNOWLEDGEMENTS

This study was supported by grants from the Korea Science and Engineering Foundation (R01-2008-000-12198-0), Ministry of Education, Science and Technology, Republic of Korea, and the Brain Korea 21 Project in 2009. Authors appreciate Professor Young Ho Lee, Department of Physiology, Yonsei University College of Medicine, for his orientation to this experiment.

REFERENCES

- Hirano K. Current topics in the regulatory mechanism underlying the Ca^{2+} sensitization of the contractile apparatus in vascular smooth muscle. *J Pharmacol Sci.* 2007;104:109-115.
- Somlyo AP, Somlyo AV. Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev.* 2003;83:1325-1358.
- Budzyn K, Marley PD, Sobey CG. Targeting Rho and Rho-kinase in the treatment of cardiovascular disease. *Trends Pharmacol Sci.* 2006;27:97-104.
- Murthy KS, Makhlof GM. Fluoride activates G protein-dependent and -independent pathways in dispersed intestinal smooth muscle cells. *Biochem Biophys Res Commun.* 1994;202:1681-1687.
- Bogatcheva NV, Wang P, Birukova AA, Verin AD, Garcia JG. Mechanism of fluoride-induced MAP kinase activation in pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2006;290:L1139-1145.
- Fujita A, Takeuchi T, Nakajima H, Nishio H, Hata F. Involvement of heterotrimeric GTP-binding protein and rho protein, but not protein kinase C, in agonist-induced Ca^{2+} sensitization of skinned muscle of guinea pig vas deferens. *J Pharmacol Exp Ther.* 1995;274:555-561.
- Yoshimura H, Jones KA, Perkins WJ, Kai T, Warner DO. Calcium sensitization produced by G protein activation in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol.* 2001;281:L631-638.
- Jeon SB, Jin F, Kim JL, Kim SH, Suk K, Chae SC, Jun JE, Park WH, Kim IK. A role for Rho kinase in vascular contraction evoked by sodium fluoride. *Biochem Biophys Res Commun.* 2006;343:27-33.
- Yang E, Jeon SB, Baek I, Chen ZA, Jin Z, Kim IK. 17beta-estradiol attenuates vascular contraction through inhibition of RhoA/Rho kinase pathway. *Naunyn Schmiedebergs Arch Pharmacol.* 2009;380:35-44.
- Todoroki-Ikeda N, Mizukami Y, Mogami K, Kusuda T, Yamamoto K, Miyake T, Sato M, Suzuki S, Yamagata H, Hokazono Y, Kobayashi S. Sphingosylphosphorylcholine induces Ca^{2+} -sensitization of vascular smooth muscle contraction: possible involvement of rho-kinase. *FEBS Lett.* 2000;482:85-90.
- Ryu SK, Ahn DS, Cho YE, Choi SK, Kim YH, Morgan KG, Lee YH. Augmented sphingosylphosphorylcholine-induced Ca^{2+} -sensitization of mesenteric artery contraction in spontaneously hypertensive rat. *Naunyn Schmiedebergs Arch Pharmacol.* 2006;373:30-36.
- Choi SK, Ahn DS, Lee YH. Comparison of contractile mechanisms of sphingosylphosphorylcholine and sphingosine-1-phosphate in rabbit coronary artery. *Cardiovasc Res.* 2009;82:324-332.
- Durlu-Kandilci NT, Brading AF. Involvement of Rho kinase and protein kinase C in carbachol-induced calcium sensitization in beta-escin skinned rat and guinea-pig bladders. *Br J Pharmacol.* 2006;148:376-384.
- Niuro N, Koga Y, Ikebe M. Agonist-induced changes in the phosphorylation of the myosin-binding subunit of myosin light chain phosphatase and CPI17, two regulatory factors of myosin light chain phosphatase, in smooth muscle. *Biochem J.* 2003;369:117-128.
- Chiba Y, Takeyama H, Sakai H, Misawa M. Effects of Y-27632 on acetylcholine-induced contraction of intact and permeabilized intrapulmonary bronchial smooth muscles in rats. *Eur J Pharmacol.* 2001;427:77-82.
- Johnson RP, El-Yazbi AF, Takeya K, Walsh EJ, Walsh MP, Cole WC. Ca^{2+} sensitization via phosphorylation of myosin phosphatase targeting subunit at threonine-855 by Rho kinase contributes to the arterial myogenic response. *J Physiol.* 2009;587:2537-2553.
- Otto B, Steusloff A, Just I, Aktories K, Pfister G. Role of Rho proteins in carbachol-induced contractions in intact and permeabilized guinea-pig intestinal smooth muscle. *J Physiol.* 1996;496:317-329.
- Gong MC, Iizuka K, Nixon G, Browne JP, Hall A, Eccleston JF, Sugai M, Kobayashi S, Somlyo AV, Somlyo AP. Role of guanine nucleotide-binding proteins-ras-family or trimeric proteins or both-in Ca^{2+} sensitization of smooth muscle. *Proc Natl Acad Sci U S A.* 1996;93:1340-1345.
- Wilson DP, Sunjar M, Kiss E, Sutherland C, Walsh MP. Thromboxane A2-induced contraction of rat caudal arterial smooth muscle involves activation of Ca^{2+} entry and Ca^{2+} sensitization: Rho-associated kinase-mediated phosphorylation of MYPT1 at Thr-855, but not Thr-697. *Biochem J.* 2005;389:763-774.
- Kitazawa T, Takizawa N, Ikebe M, Eto M. Reconstitution of protein kinase C-induced contractile Ca^{2+} sensitization in triton X-100-demembrated rabbit arterial smooth muscle. *J Physiol.* 1999;520:139-152.
- Kobayashi S, Somlyo AP, Somlyo AV. Guanine nucleotide- and inositol 1,4,5-trisphosphate-induced calcium release in rabbit main pulmonary artery. *J Physiol.* 1988;403:601-619.
- Antonny B, Sukumar M, Bigay J, Chabre M, Higashijima T. The mechanism of aluminum-independent G-protein activation by fluoride and magnesium. 31P NMR spectroscopy and fluorescence kinetic studies. *J Biol Chem.* 1993;268:2393-2402.
- Blackmore PF, Exton JH. Studies on the hepatic calcium-mobilizing activity of aluminum fluoride and glucagon. Modulation by cAMP and phorbol myristate acetate. *J Biol Chem.* 1986;261:11056-11063.
- Cockcroft S, Taylor JA. Fluoroaluminates mimic guanosine 5'-[gamma-thio]triphosphate in activating the polyphosphoinositide phosphodiesterase of hepatocyte membranes. Role for the guanine nucleotide regulatory protein Gp in signal transduction. *Biochem J.* 1987;241:409-414.
- Gilman AG. Guanine nucleotide-binding regulatory proteins and dual control of adenylate cyclase. *J Clin Invest.* 1984;73:1-4.
- Kanaho Y, Moss J, Vaughan M. Mechanism of inhibition of transducin GTPase activity by fluoride and aluminum. *J Biol Chem.* 1985;260:11493-11497.
- Bigay J, Deterre P, Pfister C, Chabre M. Fluoroaluminates activate transducin-GDP by mimicking the gamma-phosphate of GTP in its binding site. *FEBS Lett.* 1985;191:181-185.
- Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature.* 1997;389:990-994.
- Wirth A, Benyo Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, Orsy P, Horvath B, Maser-Gluth C, Greiner E, Lemmer B, Schütz G, Gutkind JS, Offermanns S. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med.* 2008;14:64-68.
- Lohn M, Plettenburg O, Ivashchenko Y, Kannt A, Hofmeister A, Kadereit D, Schaefer M, Linz W, Kohlmann M, Herbert JM, Janiak P, O'Connor SE, Ruetten H. Pharmacological characterization of SAR407899, a novel rho-kinase inhibitor. *Hypertension.* 2009;54:676-683.