The Journal of Veterinary Medical Science

FULL PAPER

Pharmacology

# Angiotensin II, a unique vasoactive agent dissociates myosin light chain phosphorylation from contraction

**Takashi HIRANO[1)], Takeharu KANEDA[2)], Hiroshi OZAKI[1)] and Masatoshi HORI[1)]***

[1)]Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

[2)]Laboratory of Veterinary Pharmacology Nippon Veterinary and Life Science University, Kyonan-cho 1-chome, Musashino, Tokyo 180-862, Japan

*J. Vet. Med. Sci.*
80(2): 219–224, 2018
doi: 10.1292/jvms.17-0415

Received: 27 July 2017
Accepted: 11 December 2017
Published online in J-STAGE: 20 December 2017

**ABSTRACT.** Angiotensin II (100 nM) induced bi-phasic increases in cytosolic $Ca^{2+}$ level ($[Ca^{2+}]_i$) through the activation of angiotensin II type 1 receptor. Pharmacological examinations using 10 $\mu$M verapamil, 30 $\mu$M $La^{3+}$, and 1 $\mu$M thapsigargin indicated that the first phase of the $[Ca^{2+}]_i$-increase was mediated by $Ca^{2+}$ release from sarcoplasmic reticulum (SR) and $Ca^{2+}$ influx independently of voltage dependent $Ca^{2+}$ channel (VDC). In contrast, the second phase of $[Ca^{2+}]_i$-increase was mediated by $Ca^{2+}$ influx through VDC. Although both $[Ca^{2+}]_i$ and myosin light chain (MLC)-phosphorylation at the first phase was apparently exceeded the threshold for contraction as estimated by high $K^+$-induced responses, there was no appreciable contraction, indicating the dissociation between MLC phosphorylation and force during this phase. In contrast, the second phase of $[Ca^{2+}]_i$ was associated with the increases in both MLC phosphorylation and force. These results suggest that angiotensin II is a unique agonist which dissociates MLC-phosphorylation from muscle force during the $Ca^{2+}$ releases from SR.

**KEY WORDS:** angiotensin II, contraction, cytosolic $Ca^{2+}$, myosin light chain, rat aorta

Angiotensin II is one of the most potent vasoconstrictors and vascular smooth muscle cell mitogens [5, 10]. Many studies on vascular biology and pharmacology revealed that angiotensin II plays a pivotal role in the physiology and pathology of the vessel wall [2, 8, 21, 22].

Signal transduction pathway of angiotensin II-stimulation has been well examined using vascular smooth muscle cells [11, 14]. Activation of vascular smooth muscle cells by angiotensin II, which mainly binds to angiotensin II type 1 ($AT_1$) receptors, results in the phospholipase C-mediated generation of two second messenger, inositol triphosphate ($IP_3$) to release $Ca^{2+}$ from sarcoplasmic reticulum (SR) and diacylglycerol (DG) to activate protein kinase C (PKC). Angiotensin II also induces protein tyrosine kinase activation, which in turn activates phospholipase Cγ [19] and other protein kinases, and phosphorylates contractile proteins [25, 30].

In *in vitro* studies, angiotensin II has been shown to elicit relatively small contraction distinct from other vascular contractile agents since it induces only a transient and smaller amplitude of contraction during the continuous incubation irrespective of a very small $EC_{50}$ values. Due to such smaller responses, not so large quantity of papers have been reported so far. Furthermore, among the papers, only a few reports have analyzed the relationship between the changes in cytosolic $Ca^{2+}$ level ($[Ca^{2+}]_i$) and myosin light chain (MLC) phosphorylation in the angiotensin II-stimulated vascular smooth muscles [9, 23]. Thus, the purposes of this study was to correlate the muscle force with $[Ca^{2+}]_i$ and MLC-phosphorylation in the angiotensin II-stimulated rat aorta. We found that phasic contraction induced by angiotensin II was composed of two phases and the first phase of $[Ca^{2+}]_i$ transient, which is mediated by $Ca^{2+}$ release form stored site, and the subsequent MLC phosphorylation was not associated with force generation.

## MATERIALS AND METHODS

### *Preparations, solutions and measurement of muscle force and $[Ca^{2+}]_i$ simultaneously*

All animal procedures were performed in accordance with the guidelines of the University of Tokyo. Male Wistar rats (200–250 g) were euthanized by a sharp blow on the neck and exsanguination. Segments of the thoracic aorta were isolated and cut into helical strips (2 mm wide, 10 mm long) for measurement of $[Ca^{2+}]_i$. The isolated muscle rings or strips were placed in physiological salt solution (PSS) containing (in mM); NaCl, 136.9; KCl, 5.4; $CaCl_2$, 1.5; $MgCl_2$, 1.0; $NaHCO_3$, 23.8 and glucose,

*Correspondence to: Hori, M.: ahori@mail.ecc.u-tokyo.ac.jp

5.5. Ethylene diamine tetraacetic acid (EDTA, 1 $\mu$M) was also added to remove contaminating heavy metal ions, which catalyze oxidation of organic chemicals in PSS. The endothelium was removed by gently rubbing the intimal surface with the flat face of a pair of forceps moistened with PSS. The high concentration of KCl (72.7 mM) solution was prepared by replacing NaCl with equimolar KCl in PSS. All solutions had a pH of 7.4 when saturated with a 95% $O_2$–5% $CO_2$ mixture at 37°C. Muscle tension was recorded isometrically with a force-displacement transducer. Each muscle strips were attached to a holder in a special organ bath (2 m*l*) with resting tension of 10 mN, and equilibrated for 30 to 60 min to obtained a stable contractility induced by 72.7 mM KCl.

Simultaneous measurement of muscle force and $[Ca^{2+}]_i$ was performed as reported by Sato *et al.* [24] with fluorescent $Ca^{2+}$ indicator, fura-PE3, which was less dye-leakage than fura-2. Rat aortic strips were treated with PSS containing acetoxymethylester of fura-PE3 (fura-PE3/AM, 5 $\mu$M) and less cytotoxic detergent, cremophor EL (0.02%), for 4–5 hr at room temperature. After loading of fura-PE3/AM, the muscle strips were washed with PSS at 37°C for 20 min to remove uncleaved fura-PE3/AM and held horizontally in a temperature-controlled organ bath (7 m*l*). One end of muscle strips was connected to a force-displacement transducer to monitor muscle force. Experiments were performed with a bio-fluorimeter (CAF-110, Japan Spectroscopic, Tokyo, Japan), and the ratio of 500 nm fluorescence excited at 340 nm (F340) to that excited at 380 nm (F380) (F340/F380) was used as indicator of $[Ca^{2+}]_i$. The resting $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ at 5 min after application of 72.7 mM KCl were taken as 0% and 100%, respectively.

### *MLC-phosphorylation*

The amount of MLC-phosphorylation was measured according to the modified method described by Word *et al*. [31]. Strips of rat aorta were held isometrically under a resting tension force of 10 mN on stainless steel holders and quickly frozen in dry-ice acetone with 10% trichloroacetic acid (TCA). The strips were then incubated for several hours in the acetone/TCA solution until the temperature of the solution became around 0°C, and were then crushed in the liquid nitrogen. The crushed muscle powder was suspended in10% TCA and 10 mM dithiothreitol (DTT) (200–400 $\mu l$). The homogenate was centrifuged at 10,000 × g for 10 min and the pellet was washed with 500 $\mu l$ diethyl ether several times to remove TCA. The pellet was then suspended in urea-glycerol buffer (25–75 $\mu l$) containing 20 mM Tris, 23 mM Glycine, 8 M urea, 10 mM DTT and 0.04% Bromophenol blue, and was centrifuged at 10,000 × g for 15 min. The supernatant was used as a sample.

The phosphorylated MLC was separated using Glycerol polyacrylamide gel electrophoresis (PAGE), the MLC or phosphorylated $Ser^{19}$ MLC was measured by western blot using anti-MLC antibody (donated by Dr. JT Stull).

### *Chemicals*

*The following chemicals were used*: angiotensin II, thapsigargin (Sigma-Aldrich, St. Louis, MO, U.S.A.), cremophor EL, DTT, TCA, EDTA (Nacalai Tesque, Kyoto, Japan), KRH594 (gently donated by KISSEI Pharmaceutical Co., Ltd., Nagano, Japan) and fura-PE3/AM (Texas fluorescence laboratories, Austin, TX, U.S.A.).

### *Stastics*

The numerical data were expressed as mean ± standard error. Differences between mean values were evaluated by Student's *t*-test and, where appropriate, analysis of variance (one-way ANOVA; Bonferroni's test) was performed.

## RESULTS

As shown in Fig. 1, angiotensin II (100 nM) induced bi-phasic increases in $[Ca^{2+}]_i$ in rat aorta. The first phasic increase in $[Ca^{2+}]_i$ was accompanied by a very slow-rise in muscle force. After the first phase of the increase in $[Ca^{2+}]_i$, the second phase of $[Ca^{2+}]_i$-increase with spontaneous oscillation was observed. The second phase was accompanied by a large and sustained increase in muscle force. After 6–15 min, spontaneous oscillatory increases in $[Ca^{2+}]_i$ suddenly stopped and the sustained level of $[Ca^{2+}]_i$ decreased toward the resting level of $[Ca^{2+}]_i$ even in the continuous presence of angiotensin II. Angiotensin II (10 nM or 100 nM)-induced contractions were completely inhibited by selective $AT_1$ receptor antagonists, 1 $\mu$M losartan or 10 nM KRH-594 (data not shown).

We then examined the relationship between $[Ca^{2+}]_i$, MLC-phosphorylation and muscle force in the rat aorta stimulated with 72.7 mM KCl or 100 nM angiotensin II. As shown in Fig. 2A, in the muscles stimulated by 72.7 mM KCl for 5 sec, MLC-phosphorylation and muscle force was increased in response to the increase in $[Ca^{2+}]_i$ to 130.59 ± 9.82%. At 15 sec, MLC-phosphorylation further increased irrespective of decrease in $[Ca^{2+}]_i$ to 109.47 ± 2.71% with a further increase in muscle force.

In the rat aorta stimulated with angiotensin II (100 nM), $[Ca^{2+}]_i$ at the first and the second phases was almost identical (82.3 ± 6.8% at 30 sec and 80.1 ± 3.9% at 2 min) which was significantly less than high $K^+$-induced increase in $[Ca^{2+}]_i$ at 5 and 15 sec. In response to these $[Ca^{2+}]_i$ increases, MLC-phosphorylation levels at 30 sec and 2 min increased to 71.2 ± 2.5% and 71.7 ± 4.1%, respectively, whose values were significantly greater than those in high $K^+$ stimulated muscle. Unexpectedly, the level of contraction was very small 30 sec after the addition of angiotensin II (Fig. 2B).

Next, we attempted to characterize the $Ca^{2+}$ source due to angiotensin II (100 nM)-stimulation. Figure 3 shows the effects of verapamil, a voltage dependent $Ca^{2+}$ channel blocker, $La^{3+}$, an inorganic nonselective $Ca^{2+}$ channel blocker, thapsigargin, an inhibitor of capacitative $Ca^{2+}$ entry and the combination of these agents on the $[Ca^{2+}]_i$ and contraction. In the presence of 10 $\mu$M verapamil, the second phase of $[Ca^{2+}]_i$ was disappeared. 30 $\mu$M $La^{3+}$ in the presence of verapamil also eliminated the second phase of $[Ca^{2+}]_i$ (Fig. 3C). Furthermore, the combination treatment with $La^{3+}$, verapamil and 1 $\mu$M thapsigargin abolished either

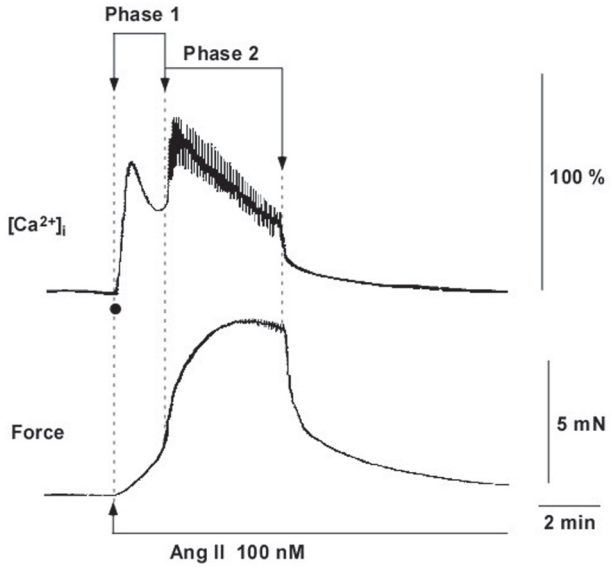


**Fig. 1.** Effect of angiotensin II (Ang II, 100 nM) on $[Ca^{2+}]_i$ (upper trace) and muscle force (lower trace) in rat aorta. The resting $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ at 5 min after application of 72.7 mM KCl were taken as 0% and 100%, respectively. Typical traces are shown from 5−7 experiments.

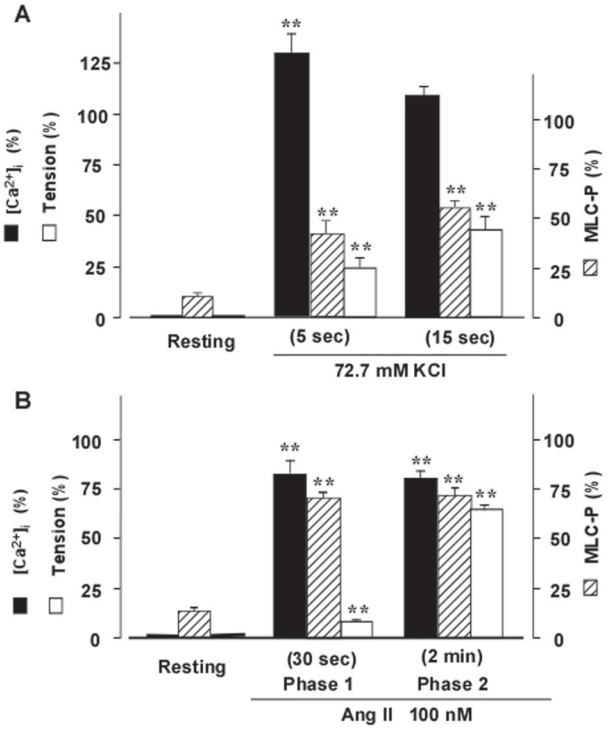


**Fig. 2.** Relationship between $[Ca^{2+}]_i$ (closed column), MLC-phosphorylation (hatched column) and muscle force (open column) in aorta stimulated with 72.7 mM KCl (A) and 100 nM angiotensin II (Ang II, B). Values were obtained at 5 and 15 sec after stimulation with 72.7 mM KCl or at 30 sec and 2 min after the stimulation with 100 nM angiotensin II. The resting $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ at 5 min after application of 72.7 mM KCl were taken as 0% and 100%, respectively. Muscle force was normalized against the contraction at 5 min after application of 72.7 mM KCl. MLC-phosphorylation (MLC-P) was shown by % of phosphorylated MLC against total MLC. Each column represents means ± S.E.M of 4−6 experiments. **: Significantly different from resting state ($P$<0.01).

the first and the second phase of $[Ca^{2+}]_i$. As for the contractile responses, even in the absence of the transient increase in $[Ca^{2+}]_i$ under the condition of the presence of verapamil, $La^{3+}$ and thapsigargin, angiotensin II induced first slowly developed contraction followed by a small sustained contraction, suggesting that at least a sustained contraction is mediated at least by $Ca^{2+}$-independent mechanism.

We further measured the MLC-phosphorylation level of the rat aorta stimulated with 100 nM angiotensin II in the presence or absence of $Ca^{2+}$ release from SR and correlated of $[Ca^{2+}]_i$ with force. As shown in Fig. 4, in the presence of verapamil plus $La^{3+}$, angiotensin II induced phasic increase in $[Ca^{2+}]_i$ reaching its peak (66.0 ± 5.1%) after about 30 sec. This $[Ca^{2+}]_i$-increase was associated with an increase in MLC phosphorylation (41.3 ± 2.8%). However, only a very small contraction was observed in response to the increase in MLC phosphorylation. Even in the absence of $Ca^{2+}$ release under the condition in which tissues were treated with verapamil, $La^{3+}$ and thapsigargin, angiotensin II increased MLC phosphorylation at 30 sec and 2 min. As was shown in the presence of verapamil and $La^{3+}$, angiotensin II again induced only a very small contraction with a significant increase in MLC phosphorylation.

## DISCUSSION

In the rat aorta, angiotensin II (100 nM) induced bi-phasic increases in $[Ca^{2+}]_i$. The first component consisted of a transient increase in $[Ca^{2+}]_i$. The second component consisted of a relatively sustained increase in $[Ca^{2+}]_i$ on which spike-like, oscillatory increases in $[Ca^{2+}]_i$ were superimposed. Several min after the start of the second phase, $[Ca^{2+}]_i$ spontaneously returned to the resting level even in the presence of angiotensin II. Since the AT-receptors are well known to be rapidly down-regulated [1, 20], cessation of $[Ca^{2+}]_i$ increase may be due to the down-regulation of AT-receptors. We also found that the angiotensin II-induced contraction was completely inhibited by the $AT_1$ receptor antagonists, 1 $\mu$M losartan [7] or 10 nM KRH-594 [28], supporting the

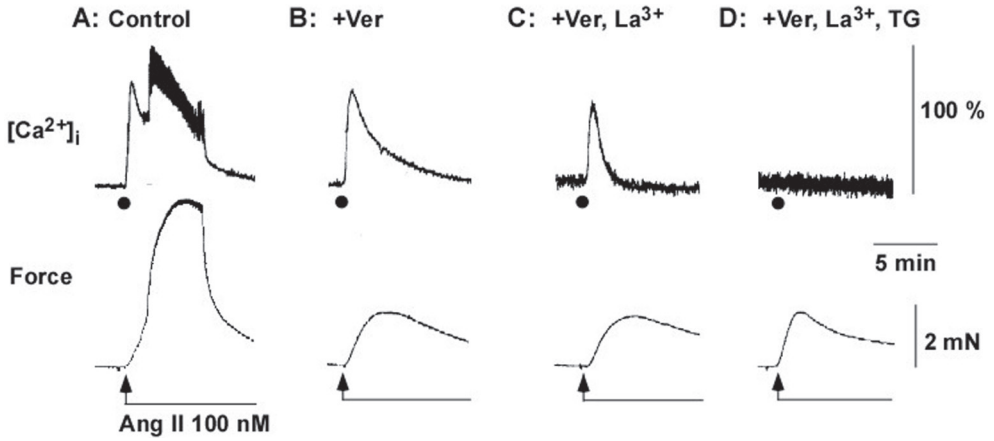


**Fig. 3.** $[Ca^{2+}]_i$ (upper trace) and muscle force (lower trace) increases in rat aorta induced by 100 nM angiotensin II (Ang II) in the absence (A) or presence of 10 $\mu$M verapamil (B), 10 $\mu$M verapamil + 30 $\mu$M $La^{3+}$ (C), or 10 $\mu$M verapamil + 30 $\mu$M $La^{3+}$ + 1 $\mu$M thapsigargin (D). The resting $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ at 5 min after application of 72.7 mM KCl were taken as 0% and 100%, respectively. Typical traces are shown from 3−4 experiments.

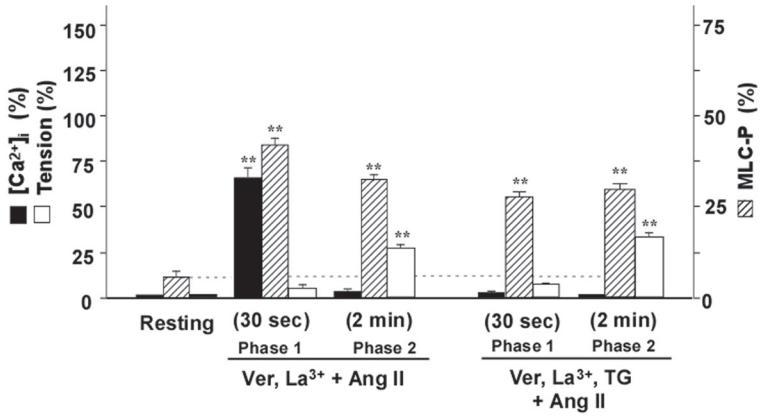


**Fig. 4.** Relationship between $[Ca^{2+}]_i$ (closed column), MLC-phosphorylation (hatched column) and muscle force (open column) in aorta stimulated with 100 nM angiotensin II (Ang II). Values were obtained at 30 sec and 2 min after the stimulation with angiotensin II in the absence 10 $\mu$M verapamil + 30 $\mu$M $La^{3+}$ or 10 $\mu$M verapamil + 30 $\mu$M $La^{3+}$ + 1 $\mu$M thapsigargin. The resting $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ at 5 min after application of 72.7 mM KCl were taken as 0% and 100%, respectively. Muscle force was normalized against the contraction at 5 min after application of 72.7 mM KCl. MLC-phosphorylation (MLC-P) was shown by % of phosphorylated MLC against total MLC. Each column represents means ± S.E.M. of 4−6 experiments. **: Significantly different from resting state ($P$<0.01).

results by Schutzer *et al.* [27] in rat aortic tissue and by Castoldi *et al*. [6] in cultured rat aortic smooth muscle cells.

We next characterize the mechanisms of angiotensin II-induced increases in $[Ca^{2+}]_i$, Addition of an inhibitor of voltage dependent $Ca^{2+}$ channel, verapamil, completely abolished the second phase of $[Ca^{2+}]_i$-increase without affecting the maximum amplitude of the first phase of $[Ca^{2+}]_i$ increase, indicating that the second phase of $[Ca^{2+}]_i$ increase by angiotensin II is mediated through voltage dependent $Ca^{2+}$ channels. Since the combination treatment with verapamil and $La^{3+}$, an inorganic $Ca^{2+}$ channel blocker, decreased the duration of the first phase $[Ca^{2+}]_i$-increase without affecting the maximum amplitude, $Ca^{2+}$ influx pathway other than voltage-dependent $Ca^{2+}$ channels, such as capacitative $Ca^{2+}$ entry [32], may partly be involved in the first phase of the $[Ca^{2+}]_i$-increase (Fig. 3B and 3C and our unpublished observation). Data also indicated that treatment with verapamil, $La^{3+}$ and thapsigargin, a $Ca^{2+}$ pump inhibitor, abolished the first phase of the $[Ca^{2+}]_i$-increase due to 100 nM angiotensin II in the absence of external $Ca^{2+}$ (Fig. 3D). This result suggests that the first phase of $[Ca^{2+}]_i$ increase is mediated by $Ca^{2+}$ release from SR. Consisted with these observations, it has been reported that angiotensin II increases $IP_3$ formation following activation of phospholipase $C_\beta$ in cultured vascular smooth muscle cells [12, 26].

The relationship between MLC-phosphorylation and muscle force revealed that the level of the increases in $[Ca^{2+}]_i$ and MLC phosphorylation at 30 sec after the stimulation with angiotensin II (100 nM) was not reflected in the muscle force as shown in Fig. 2B. The same results were obtained in the conditions in which $Ca^{2+}$ influx had been inhibited by the presence of verapamil and $La^{3+}$ or the presence of verapamil, $La^{3+}$ and thapsigargin (Fig. 4B). In contrast, in the muscle stimulated with 72.7 mM KCl, increased MLC-phosphorylation at 5 and 15 sec, whose values are smaller than those in the angiotensin II-induced responses at 30 sec, was associated with contraction. These contradictory results obtained in angiotensin II- and high $K^+$-induced responses can not be explained by the possibility that the dissociation between MLC-phosphorylation and muscle force is due to the delay of force-transmission by elastic component, since the angiotensin II-induced responses were much slower than the high $K^+$-induced responses.

Brizzolara-Gourdie and Webb [4] reported that angiotensin II stimulates cAMP formation to induce vasodilation in rat aorta. However, this is not the case with rat aorta, since enzyme-immunoassay showed that angiotensin II (100 nM) did not increase cAMP content (data not shown). Furthermore, angiotensin II did not inhibit 72.7 mM KCl- or 1 $\mu$M noradrenaline-induced contraction. These results suggest that MLC-phosphorylation 30 sec after stimulation with angiotensin II dissociates with muscle force by a cAMP-independent mechanisms. Tansey *et al*. [29] reported that phosphatase inhibitor, okadaic acid, inhibited carbachol-induced contractions without decreasing MLC phosphorylation in bovine trachea. Moreover, phorbol esters increased MLC phosphorylation and actin remodeling in bovine lung endothelium without increased contraction [3]. These results suggest the possibility that, like okadaic acid, angiotensin II may phosphorylates indirectly or directly some unknown regulatory proteins to dissociate MLC-phosphorylation and contraction in vascular smooth muscle. Further experiments are required to clarify this point.

The levels of phosphorylated MLC at 30 sec or 2 min after stimulation with angiotensin II (100 nM) were significantly higher than those of the response to 72.7 mM KCl at 5 and 15 sec. However, $[Ca^{2+}]_i$ at 30 sec or 2 min after addition of angiotensin II was significantly lower than those of 72.7 mM KCl. In addition, $[Ca^{2+}]_i$-independent and MLC-phosphorylation dependent contraction was induced by angiotensin II in the presence of verapamil, $La^{3+}$ and thapsigargin (Fig.4). These results suggest that angiotensin II sensitizes the MLC-phosphorylation step at a given $[Ca^{2+}]_i$. This $Ca^{2+}$ sensitization might be mediated by inhibition of protein phosphatase through rhoA/ROCK pathway [16] and/or through PKC/PCI-17 pathway [18].

We have previously shown the dissociation between the increase in $[Ca^{2+}]_i$ due to $Ca^{2+}$ release from SR and muscle force in the rat aorta stimulated with $P_2$ purinergic receptors [17]. In this report, we have demonstrated that ATP-induced increase in $[Ca^{2+}]_i$ was not associated with the increment of MLC phosphorylation, indicating the presence of localization of $[Ca^{2+}]_i$ that is not able to couple with contractile proteins [15]. In contrast, verapamil inhibited noradrenaline-induced contraction with decreases of MLC-P in rat aorta [13], indicating that there is no dissociation between contraction and MLC-P. At present, we have no explanation for the marked difference between purinoceptor and α-adrenaline agonists and angiotensin II regarding the correlation between MLC phosphorylation and contraction, and it is also necessary to clarify this point in a future study.

In conclusion, angiotensin II induces contraction by an influx of $Ca^{2+}$ through VDC but not $Ca^{2+}$ release from SR. Angiotensin II is a unique receptor-agonist to dissociate MLC-phosphorylation from muscle force during the $[Ca^{2+}]_i$-increase due to $Ca^{2+}$ releases from SR.

ACKNOWLEDGMENTS. This work was partly supported by Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan (The University of Tokyo). We thank Dr. James T. Stull (University of Texas, Southwestern Medical Center at Dallas) and KISSEI Pharmaceutical Co., Ltd. for supplying us with MLC antibody and KRH-594, respectively.

## REFERENCES

1. Anderson, K. M., Murahashi, T., Dostal, D. E. and Peach, M. J. 1993. Morphological and biochemical analysis of angiotensin II internalization in cultured rat aortic smooth muscle cells. *Am. J. Physiol.* **264**: C179–C188. [Medline] [CrossRef]
2. Azuma, H., Niimi, Y. and Hamasaki, H. 1992. Prevention of intimal thickening after endothelial removal by a nonpeptide angiotensin II receptor antagonist, losartan. *Br. J. Pharmacol.* **106**: 665–671. [Medline] [CrossRef]
3. Bogatcheva, N. V., Verin, A. D., Wang, P., Birukova, A. A., Birukov, K. G., Mirzopoyazova, T., Adyshev, D. M., Chiang, E. T., Crow, M. T. and Garcia, J. G. 2003. Phorbol esters increase MLC phosphorylation and actin remodeling in bovine lung endothelium without increased contraction. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **285**: L415–L426. [Medline] [CrossRef]
4. Brizzolara-Gourdie, A. and Webb, J. G. 1997. Angiotensin II potentiates vasodilation of rat aorta by cAMP elevating agonists. *J. Pharmacol. Exp. Ther.* **281**: 354–359. [Medline]
5. Campbell-Boswell, M. and Robertson, A. L. Jr. 1981. Effects of angiotensin II and vasopressin on human smooth muscle cells in vitro. *Exp. Mol. Pathol.* **35**: 265–276. [Medline] [CrossRef]
6. Castoldi, G., Di Gioia, C. R., Pieruzzi, F., D'Orlando, C., Van De Greef, W. M., Busca, G., Sperti, G. and Stella, A. 2003. ANG II increases TIMP-1 expression in rat aortic smooth muscle cells in vivo. *Am. J. Physiol. Heart Circ. Physiol.* **284**: H635–H643. [Medline] [CrossRef]
7. Chiu, A. T., McCall, D. E., Price, W. A., Wong, P. C., Carini, D. J., Duncia, J. V., Wexler, R. R., Yoo, S. E., Johnson, A. L. and Timmermans, P. B. 1990. Nonpeptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.* **252**: 711–718. [Medline]
8. Flavahan, S., Chang, F. and Flavahan, N. A. 2016. Local renin-angiotensin system mediates endothelial dilator dysfunction in aging arteries. *Am. J. Physiol. Heart Circ. Physiol.* **311**: H849–H854. [Medline] [CrossRef]
9. Fukuda, T., Kuroda, T., Kono, M., Hyoguchi, M., Tajiri, S., Tanaka, M., Mine, Y. and Matsui, T. 2016. Adenine attenuates the Ca(2+) contraction-signaling pathway via adenine receptor-mediated signaling in rat vascular smooth muscle cells. *Naunyn Schmiedebergs Arch. Pharmacol.* **389**: 999–1007. [Medline] [CrossRef]

10. Geisterfer, A. A. T., Peach, M. J. and Owens, G. K. 1988. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circ. Res.* **62**: 749–756. [Medline] [CrossRef]
11. Griendling, K. K., Tsuda, T., Berk, B. C. and Alexander, R. W. 1989. Angiotensin II stimulation of vascular smooth muscle cells. Secondary signalling mechanisms. *Am. J. Hypertens.* **2**: 659–665. [Medline] [CrossRef]
12. Griendling, K. K., Ushio-Fukai, M., Lassègue, B. and Alexander, R. W. 1997. Angiotensin II signaling in vascular smooth muscle. New concepts. *Hypertension* **29**: 366–373. [Medline] [CrossRef]
13. Hori, M., Sato, K., Sakata, K., Ozaki, H., Takano-Ohmuro, H., Tsuchiya, T., Sugi, H., Kato, I. and Karaki, H. 1992. Receptor agonists induce myosin phosphorylation-dependent and phosphorylation-independent contractions in vascular smooth muscle. *J. Pharmacol. Exp. Ther.* **261**: 506–512. [Medline]
14. Islam, M. Z., Van Dao, C., Miyamoto, A. and Shiraishi, M. 2017. Rho-kinase and the nitric oxide pathway modulate basilar arterial reactivity to acetylcholine and angiotensin II in streptozotocin-induced diabetic mice. *Naunyn Schmiedebergs Arch. Pharmacol.* **390**: 929–938. [Medline] [CrossRef]
15. Karaki, H., Ozaki, H., Hori, M., Mitsui-Saito, M., Amano, K., Harada, K., Miyamoto, S., Nakazawa, H., Won, K. J. and Sato, K. 1997. Calcium movements, distribution, and functions in smooth muscle. *Pharmacol. Rev.* **49**: 157–230. [Medline]
16. Khromov, A., Choudhury, N., Stevenson, A. S., Somlyo, A. V. and Eto, M. 2009. Phosphorylation-dependent autoinhibition of myosin light chain phosphatase accounts for $Ca^{2+}$ sensitization force of smooth muscle contraction. *J. Biol. Chem.* **284**: 21569–21579. [Medline] [CrossRef]
17. Kitajima, S., Harada, K., Hori, M., Ozaki, H. and Karaki, H. 1996. Dissociation of $P_2$ purinoceptor-mediated increase in intracellular $Ca^{2+}$ level from myosin light chain phosphorylation and contraction in rat aorta. *Br. J. Pharmacol.* **118**: 543–548. [Medline] [CrossRef]
18. Kitazawa, T. 2010. G protein-mediated $Ca^{2+}$-sensitization of CPI-17 phosphorylation in arterial smooth muscle. *Biochem. Biophys. Res. Commun.* **401**: 75–78. [Medline] [CrossRef]
19. Marrero, M. B., Schieffer, B., Bernstein, K. E. and Ling, B. N. 1996. Angiotensin II-induced tyrosine phosphorylation in mesangial and vascular smooth muscle cells. *Clin. Exp. Pharmacol. Physiol.* **23**: 83–88. [Medline] [CrossRef]
20. Oppermann, M., Freedman, N. J., Alexander, R. W. and Lefkowitz, R. J. 1996. Phosphorylation of the type 1A angiotensin II receptor by G protein-coupled receptor kinases and protein kinase C. *J. Biol. Chem.* **271**: 13266–13272. [Medline] [CrossRef]
21. Owens, G. K. 1989. Control of hypertrophic *versus* hyperplastic growth of vascular smooth muscle cells. *Am. J. Physiol.* **257**: H1755–H1765. [Medline]
22. Powell, J. S., Clozel, J. P., Müller, R. K. M., Kuhn, H., Hefti, F., Hosang, M. and Baumgartner, H. R. 1989. Inhibitors of angiotensin-converting enzyme prevent myointimal proliferation after vascular injury. *Science* **245**: 186–188. [Medline] [CrossRef]
23. Rembold, C. M. and Murphy, R. A. 1990. Latch-bridge model in smooth muscle: $[Ca^{2+}]_i$ can quantitatively predict stress. *Am. J. Physiol.* **259**: C251–C257. [Medline] [CrossRef]
24. Sato, K., Ozaki, H. and Karaki, H. 1988. Changes in cytosolic calcium level in vascular smooth muscle strip measured simultaneously with contraction using fluorescent calcium indicator fura 2. *J. Pharmacol. Exp. Ther.* **246**: 294–300. [Medline]
25. Sauro, M. D., Sudakow, R. and Burns, S. 1996. In vivo effects of angiotensin II on vascular smooth muscle contraction and blood pressure are mediated through a protein tyrosine-kinase-dependent mechanism. *J. Pharmacol. Exp. Ther.* **277**: 1744–1750. [Medline]
26. Schelling, J. R., Nkemere, N., Konieczkowski, M., Martin, K. A. and Dubyak, G. R. 1997. Angiotensin II activates the beta 1 isoform of phospholipase C in vascular smooth muscle cells. *Am. J. Physiol.* **272**: C1558–C1566. [Medline] [CrossRef]
27. Schutzer, W. E., Xue, H., Reed, J. F., Roullet, J. B., Anderson, S. and Mader, S. L. 2000. Angiotensin II enhances beta-adrenergic receptor-mediated vasorelaxation in aortas from young but not old rats. *Am. J. Physiol. Heart Circ. Physiol.* **279**: H2807–H2814. [Medline] [CrossRef]
28. Tamura, K., Okuhira, M., Mikoshiba, I. and Hashimoto, K. 1997. In vitro pharmacological properties of KRH-594, a novel angiotensin II type 1 receptor antagonist. *Biol. Pharm. Bull.* **20**: 850–855. [Medline] [CrossRef]
29. Tansey, M. G., Hori, M., Karaki, H., Kamm, K. E. and Stull, J. T. 1990. Okadaic acid uncouples myosin light chain phosphorylation and tension in smooth muscle. *FEBS Lett.* **270**: 219–221. [Medline] [CrossRef]
30. Touyz, R. M. and Schiffrin, E. L. 1997. Angiotensin II regulates vascular smooth muscle cell pH, contraction, and growth via tyrosine kinase-dependent signaling pathways. *Hypertension* **30**: 222–229. [Medline] [CrossRef]
31. Word, R. A., Casey, M. L., Kamm, K. E. and Stull, J. T. 1991. Effects of cGMP on $[Ca^{2+}]_i$, myosin light chain phosphorylation, and contraction in human myometrium. *Am. J. Physiol.* **260**: C861–C867. [Medline] [CrossRef]
32. Zhu, X., Jiang, M., Peyton, M., Boulay, G., Hurst, R., Stefani, E. and Birnbaumer, L. 1996. *trp*, a novel mammalian gene family essential for agonist-activated capacitative $Ca^{2+}$ entry. *Cell* **85**: 661–671. [Medline] [CrossRef]