대한물리치료사학회지 제 13권 2호(특집 2)
2006. 6. pp. 137-145

J Pharmacol Sci **101**, 135 – 143 (2006)

Journal of Pharmacological Sciences

Full Paper

Epidermal Growth Factor Induces Vasoconstriction Through the Phosphatidylinositol 3-Kinase-Mediated Mitogen-Activated Protein Kinase Pathway in Hypertensive Rats

Junghwan Kim[1,#], Chang-Kwon Lee[1,#], Hyo-Jun Park[1], Hyo Jin Kim[1], Hyun Ha So[1], Keun Sang Lee[1], Hwan Myung Lee[1], Hui Yul Roh[1], Wahn Soo Choi[2], Tae Kyu Park[3], and Bokyung Kim[1,*]

[1]Department of Physiology and [2]Department of Immunology, College of Medicine, Institute of Biomedical Science and Technology, and [3]Department of Biotechnology, College of Biomedical & Health Science, Konkuk University, Danwol-dong 322, Choongju 380-701, Korea

Received January 10, 2006; Accepted April 14, 2006

Abstract. We investigated whether increased contractile responsiveness to epidermal growth factor (EGF) is associated with altered activation of mitogen-activated protein kinase (MAPK) in the aortic smooth muscle of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. EGF induced contraction and MAPK activity in aortic smooth muscle strips, which were significantly increased in tissues from the DOCA-salt hypertensive rats compared with those from sham-operated rats. AG1478, PD98059, and LY294002, inhibitors of EGF receptor (EGFR) tyrosine kinase, MAPK/extracellular signal-regulated kinase (ERK) kinase, and phosphatidylinositol 3-kinase (PI3K), respectively, inhibited the contraction and the activity of ERK1/2 that were elevated by EGF. Y27632 and GF109203X, inhibitors of Rho kinase and protein kinase C, respectively, attenuated EGF-induced contraction, with no diminution of ERK1/2 activity. Although EGF also elevated the activity of EGFR tyrosine kinase in both sham-operated and DOCA-salt hypertensive rats, the expression and the magnitude of activation did not differ between strips. These results strongly suggest that EGF induces contraction by the activation of ERK1/2, which is regulated by the PI3K pathway in the aortic smooth muscle of DOCA-salt hypertensive rats.

Keywords: epidermal growth factor, hypertension, vasoconstriction, mitogen-activated protein kinase, phosphatidylinositol 3-kinase

Introduction

Epidermal growth factor (EGF), found in urine, platelets, kidneys, and salivary glands, is an important regulator in a variety of cellular systems. EGF binds to EGF receptor (EGFR) tyrosine kinase and subsequently activates the Ras, phosphatidylinositol 3-kinase (PI3K), and phospholipase C (PLC) γ pathways, leading to mitogenic events (1, 2). EGF also stimulates an increase in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) levels (3) and modulates the contractility of vascular and gastric smooth muscles (1, 4, 5).

Smooth muscle contraction is triggered by $[Ca^{2+}]_i$ released from intracellular Ca^{2+} stores and from the extracellular space. In addition to the $[Ca^{2+}]_i$-mediated pathway, a number of intracellular signal molecules, including mitogen-activated protein kinase (MAPK), protein kinase C (PKC), PI3K, and Rho kinase, play important roles in the regulation of smooth muscle contraction (6 – 10).

MAPK is a family of serine/threonine-specific protein kinase, consisting of three isoforms: extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and stress-activated protein kinase/*c-Jun* N-terminal kinase (11 – 13). MAPK plays a central role in the intracellular signal transduction initiated by extracellular stimuli, including growth factors, neurotransmitters, and hormones (14,

[#]These authors contributed equally to this work.
*Corresponding author. bkkim2@kku.ac.kr
Published online in J-STAGE: June 10, 2006
doi: 10.1254/jphs.FP0060021

15). Evidence is accumulating that the MAPK pathway is closely linked to the modulation of the intensity of contraction in vascular smooth muscle (8, 16). Moreover, the inhibition of ERK1/2 diminishes the contractility of smooth muscle cells, indicating that ERK1/2 can contribute to the elevation of contraction (17, 18). EGF increases the activity of ERK1/2 in the vasculature (19). Furthermore, it has been reported that an inhibitor of MAPK attenuated the contraction induced by EGF (20). Although it can be assumed from these results that MAPK may be involved in the EGF-induced contraction in vascular smooth muscle, to our knowledge there is no study showing direct evidence of the involvement of MAPK in EGF-induced contraction.

Elevated blood pressure results in the alteration of vascular properties including morphological and functional changes (21). Increased responsiveness of blood vessels to vasoconstrictors is often associated with an elevation of the systolic blood pressure. The contractile response to receptor agonists, including angiotensin II and phenylephrine, is significantly increased in hypertensive rats (8, 16, 18), which may contribute to the increase in blood pressure in hypertensive rats. It is well established that EGF induces vascular contraction in deoxycorticosterone acetate (DOCA)-salt hypertensive rats but not in normotensive rats (20, 22). This altered response to EGF may be involved in the elevation of blood pressure in hypertensive rats. However, a clear mechanism underlying the EGF-induced increase in responsiveness, especially involving the MAPK pathway, in vascular smooth muscle of hypertensive rats is not fully defined.

In the present study, we investigated whether the increased contractile response to EGF is associated with altered ERK1/2 activation in the aortic smooth muscles of DOCA-salt hypertensive rats. Furthermore, the regulatory mechanism in the ERK pathway activated by EGF in these hypertensive rats was determined.

Materials and Methods

Animals

All experiments were performed in accordance with the institutional guidelines of Konkuk University, Korea. Male Sprague-Dawley rats (6-week-old, 190 – 200 g, n = 72) were uninephrectomized and, after one week, received a silicon rubber implant impregnated with DOCA (200 mg/kg) subcutaneously under intramuscular anaesthesia (35 mg/kg ketamine and 5 mg/kg xylazine). DOCA-salt hypertensive rats were given drinking water solution containing 0.9% NaCl plus 0.2% KCl. A control group (sham-operated rats) was also uninephrectomized and operated upon without receiving an implant. Sham-operated rats were given normal tap water. Systolic blood pressure was directly determined using a pressure transducer (P23XL; Viggo Spectramed, Oxnard, CA, USA) at the common carotid artery, under ketamine/xylazine anaesthesia.

Measurement of isometric contraction

Rats were killed by stunning and bled by cutting carotid arteries. The thoracic aorta was removed and cut into strips (3 × 8 mm). The endothelium was removed by gently rubbing the inner surface of the vessel with a cotton thread moistened with physiological salt solution (PSS: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 5.5 mM glucose, and 0.01 mM ethylenediaminetetraacetic acid [EDTA]). Each strip was attached to a holder with a resting tension of 10 mN. After equilibration for 20 min in PSS, the strips were repeatedly exposed to a 70 mM K^+ solution until the response became stable. The high-concentration K^+ solution was prepared by replacing NaCl in PSS with an equimolar amount of KCl. These solutions were saturated with a mixture of 95% O_2 and 5% CO_2, at 37°C and pH 7.4. Muscle contraction was recorded isometrically with a force-displacement transducer (FT03; Grass, West Warwick, RI, USA) connected to a polygraph system (RPS7C8, Grass).

Measurement of activities of kinases

Aortic strips were isolated as described for the contraction measurement experiments and were snap-frozen in liquid N_2 after treatment with various stimulants for different times. The samples were then homogenized in sample buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM ethylene glycol-*bis*-(*β*-amino ethyl-ether) tetraacetic acid (EGTA), 20 mM *β*-glycerophosphate, 1 mM NaF, 2 mM Na_3VO_4, 5 μg/ml aprotinin, 5 μM leupeptin, 1% Triton X-100, 10% glycerol, 0.3 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol, and 150 mM NaCl. The homogenate was centrifuged at 14,000 × *g* for 10 min at 4°C, and the supernatant was collected (7, 8). Protein concentrations were measured using Bio-Rad DC protein assay reagent (BioRad, Hercules, CA, USA). Proteins were diluted 1:1 (vol:vol) with sodium dodecyl sulfate (SDS) sample buffer containing 40 mM Tris-HCl (pH 6.8), 8 mM EGTA, 4% 2-mercaptoethanol, 40% glycerol, 0.01% bromophenol blue, and 4% SDS, and then boiled for 5 min.

Proteins (50 μg/lane) separated with 5% (EGFR) or 10% (ERK1/2, Akt) SDS-polyacrylamide gel electrophoresis were transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). These

were incubated with phosphate-buffered saline containing 0.05% Tween 20 and 5% non-fat dried milk for 60 min and then incubated with individual antibodies diluted 1:1000 – 5000 overnight at 4°C. Following incubation with horseradish peroxidase-conjugated anti-rabbit IgG (1:1000) for 60 min, the blots were developed using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Buckinghamshire, UK). Antibody-specific bands were quantified using an image analyser (BioRad).

Chemicals

EGF was purchased from Invitrogen (Carlsbad, CA, USA). Polyclonal anti-phosphorylated and anti-non-phosphorylated ERK1/2 antibodies, Triton X-100, and dithiothreitol were purchased from Promega (Madison, WI, USA). Polyclonal anti-phosphorylated and anti-nonphosphorylated EGFR tyrosine kinase and Akt antibodies were purchased from Cell Signaling (Beverly, MA, USA). DOCA, NaF, β-glycerophosphate, Na_3VO_4, aprotinin, leupeptin, endothelin-1, and phenylmethylsulfonyl fluoride were purchased from Sigma (St Louis, MO, USA). PD98059, AG1478, LY294002, Y27632, and GF109203X were purchased from Tocris (Bristol, UK).

Data analysis

The results of experiments are expressed as means ± S.E.M. Unpaired Student's *t*-test was used to compare the data, and $P<0.05$ was considered significantly different.

Results

Increased blood pressure in DOCA-salt hypertensive rats

Four weeks after the silicon rubber implantation, systolic blood pressure was significantly increased in DOCA-salt hypertensive rats (182 ± 4 mmHg, n = 16) relative to sham-operated rats (121 ± 7 mmHg, n = 16).

EGF-induced contraction in aortic smooth muscle

EGF (100 pM to 100 nM) was applied cumulatively to the aortic smooth muscles of sham-operated and DOCA-salt hypertensive rats. EGF at 1 nM induced slight muscle contraction in DOCA-salt hypertensive rats. EGF at 10 and 100 nM increased muscle contraction to 20.2 ± 4.3% (n = 9) and 20 ± 7.0% (n = 9), respectively, of 70 mM K^+-induced contraction in DOCA-salt hypertensive rats (Fig. 1: B and C). In contrast, the aortic strips from sham-operated rats displayed minimal contraction (<1% of 70 mM K^+-induced contraction, n = 6) in response to treatment with EGF (Fig. 1: A and C). Neither basal tone nor EGF-mediated contractions were affected by the equivalent concentration of the vehicle DMSO (0.1%, data not shown). High K^+ (10 – 60 mM) induced sustained contraction in dose-dependent manners in sham-operated and DOCA-salt hypertensive rats. The response to high K^+ was greater in DOCA-salt hypertensive rats compared with controls (Fig. 1D). These

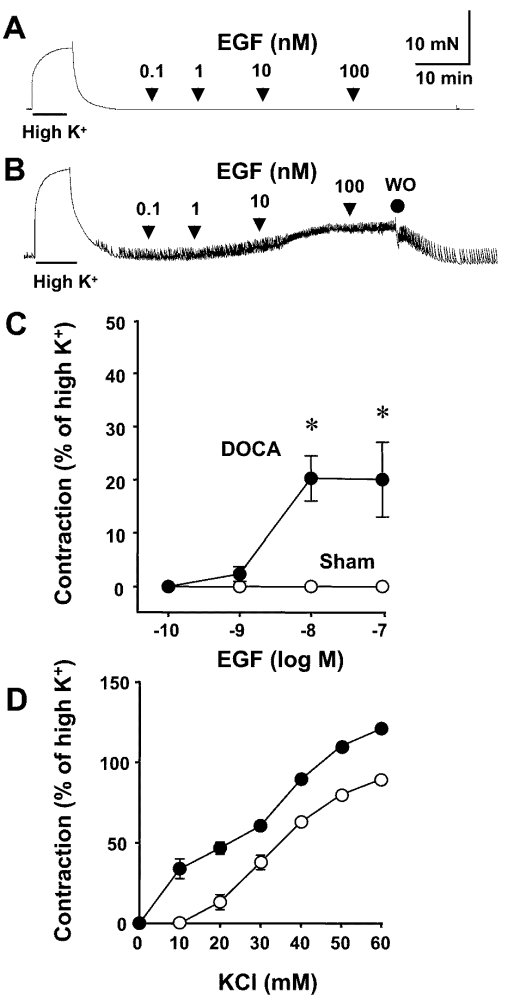

Fig. 1. Contractile effects of EGF on aortic smooth muscle from sham-operated and DOCA-salt hypertensive rats. The muscle strips from sham-operated (A) and DOCA-hypertensive (B) rats were stimulated repeatedly with 70 mM K^+. After the response to high K^+ was determined, EGF (100 pM – 100 nM) was applied cumulatively. Dose-response curves of the contraction induced by EGF (C, n = 6 – 9) and high K^+ (D, n = 6). The contractile levels in response to 70 mM K^+ before treatment with EGF or high K^+ were defined as 100%. *$P<0.05$.

results show that EGF- and high K^+-induced contractions were significantly increased in DOCA-salt hypertensive rats compared with sham-operated rats, which is consistent with earlier studies (22).

Effects of inhibitors on EGF-induced contraction

To determine the involvement of EGFR tyrosine kinase and ERK1/2 in EGF-induced contraction, the effects of inhibitors of these kinases on the contractile responses to EGF were tested in aortic strips. AG1478 (10 μM), an inhibitor of EGFR tyrosine kinase, significantly inhibited EGF (10 nM)-evoked contraction (11.3 ± 2.6% of EGF-induced contraction, n = 4) in DOCA-salt hypertensive rats (Fig. 2: A and C). PD98059 (10 μM), an inhibitor of MAPK/ERK kinase, inhibited the contraction induced by EGF (10 nM) (3.2 ± 1.6% of EGF-induced contraction, n = 6; Fig. 2: B and C) in DOCA-salt hypertensive rats. AG1478 (10 μM) inhibited the myogenic basal tone in DOCA-salt hypertensive rats (Fig. 2D). PD98059 (10 μM) inhibited the basal tone in DOCA-salt hypertensive rats (data not shown). Neither AG1478 nor PD98059 inhibited the basal tone in sham-operated rats (data not shown).

EGF-induced increases in MAPK activity

The results from the mechanical study suggested that EGF-induced contraction may be mediated by the MAPK pathway. Therefore, to determine whether MAPK influences EGF-induced responses, the activity of MAPK was measured in the aortic smooth muscles of sham-operated and DOCA-salt hypertensive rats using phosphorylated ERK1/2 antibody. In the quiescent state without any stimulant, the phosphorylation of ERK1/2 was significantly greater in DOCA-salt hypertensive rat (168.0 ± 11.1%, n = 6, of sham-operated rats). EGF (10 nM) increased the phosphorylation of ERK1/2 in a time-dependent manner in both strips, and maximal phosphorylation of the kinase was recorded at 30 min (Fig. 3A). These increments of ERK1/2 to EGF were greater in strips from DOCA-salt hypertensive rats compared with sham-operated controls (Fig. 3B). The total expression of ERK1/2, measured using non-phosphorylated antibody, in the quiescent and EGF-treated states was not changed in aortic strips from DOCA-salt hypertensive and sham-operated rats (Fig. 3A).

Effects of inhibitors on EGF-induced MAPK activity

PD98059 (10 μM, 15 min) inhibited the activity of ERK1/2 induced by 10 nM EGF in sham-operated and DOCA-salt hypertensive rats (Fig. 3C). Furthermore, AG1478 (10 μM, 15 min) inhibited the activity of

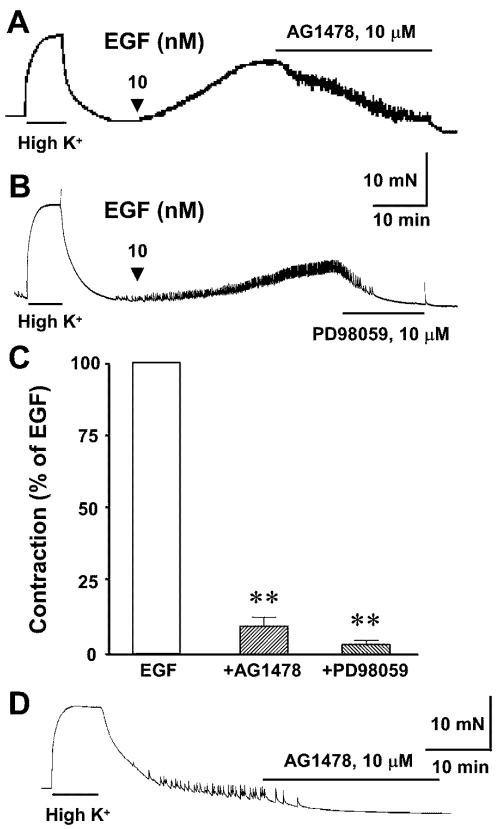

Fig. 2. Effects of inhibitors on EGF-induced contraction in aortic smooth muscle from DOCA-salt hypertensive rats. After the response to 10 nM EGF was determined, 10 μM AG1478 (A) and 10 μM PD98059 (B) were applied. C: Effects of inhibitors on EGF-induced contraction (n = 4 – 6). The contractile level in response to EGF before treatment with inhibitors was defined as 100%. $^{**}P<0.01$. D: The strips in the quiescent state were treated with AG1478 (10 μM) in DOCA-salt hypertensive rats. Results are typical recordings obtained from 3 independent experiments.

ERK1/2 in sham-operated and DOCA-salt hypertensive rats. These inhibitors did not alter the total expression of ERK1/2 (Fig. 3C). Neither basal nor EGF-mediated ERK activities were affected by the equivalent concentration of the vehicle DMSO (0.1%, data not shown).

Involvement of PI3K in EGF-induced responses

It has been reported that EGF receptor tyrosine kinase activates the PI3K and PLCγ pathways (1, 2). To determine the involvement of PI3K and PKC in EGF-induced responses, the effects of kinase inhibitors on the contractile responses and MAPK activity to EGF were tested in sham-operated and DOCA-salt hypertensive rats. LY294002 (10 μM), a PI3K inhibitor,

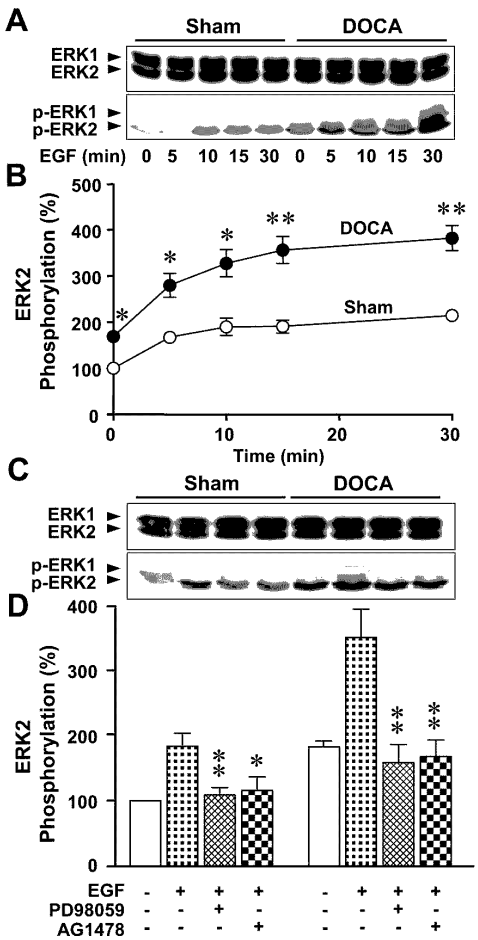

Fig. 3. Effects of EGF on ERK1/2 phosphorylation in the aortic smooth muscles of sham-operated and DOCA-salt hypertensive rats. The strips were prepared as described in the Materials and Methods section. A: ERK1/2 phosphorylation in the quiescent and EGF-stimulated states was demonstrated using an anti-phosphorylated ERK1/2 antibody on strips. Upper panel: Total ERK1/2 expression was measured using an anti-ERK1/2 antibody on strips from both treatment groups. B: The level of ERK phosphorylation was quantified with the relative density of ERK2 (n = 6). The level of phosphorylated ERK2 in the quiescent state in sham-operated rats was defined as 100%. C and D: The strips were stimulated with 10 nM EGF for 15 min and then treated with PD98059 (10 μM) or AG1478 (10 μM) for 15 min. The basal level of phosphorylated ERK2 in sham-operated rats was defined as 100% (n = 4 – 6). *P<0.05, **P<0.01.

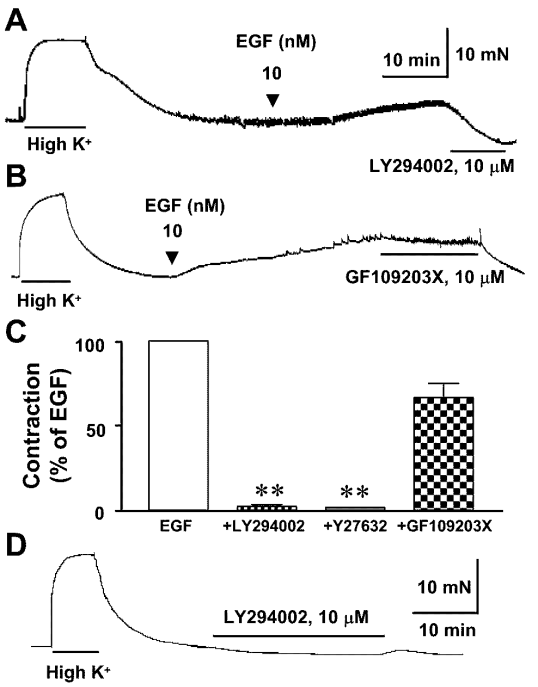

Fig. 4. Effects of inhibitors on EGF-induced contraction and basal tone in aortic smooth muscles of DOCA-salt hypertensive rats. After the maximal response to 10 nM EGF was determined, 10 μM LY294002 (A), 10 μM GF109203X (B), or 10 μM Y27632 (C) was applied. C: Effects of inhibitors on EGF-induced contraction. The contractile level of EGF before treatment with inhibitors was defined as 100% (n = 4 – 5). **P<0.01. D: The strips in the quiescent state were treated with LY294002 (10 μM) in DOCA-salt hypertensive rats. Results are typical recordings obtained from 3 independent experiments.

completely inhibited the contraction induced by 10 nM EGF (Fig. 4A). GF109203X (10 μM), a PKC inhibitor, attenuated EGF-induced contraction in DOCA-salt hypertensive rats (66.5 ± 8.8% of control, n = 4; Fig. 4B). The Rho kinase inhibitor Y27632 (10 μM) completely inhibited the contraction induced by 10 nM EGF (0.7 ± 0.1% of control, n = 4; Fig. 4C). LY294002 slightly attenuated basal tone in DOCA-salt hypertensive rats but not in sham controls (Fig. 4D).

EGF (10 nM, 15 min)-increased phosphorylation of ERK1/2 was significantly inhibited by LY294002 (10 μM) in sham-operated and DOCA-salt hypertensive rats (Fig. 5A). GF109203X (10 μM, 15 min) increased the phosphorylation of ERK1/2 in sham-operated rats, but this was not significant (Fig. 5A). GF109203X (10 μM, 15 min) did not change the phosphorylation of ERK1/2 in DOCA-salt hypertensive rats. EGF (10 nM, 15 min)-induced phosphorylation of ERK1/2 was not inhibited by Y27632 (10 μM, 15 min) in both sham-operated and DOCA-salt hypertensive rats (Fig. 5A). Endothelin-1 (30 nM, 20 min) increased the phosphorylation of ERK1/2 in DOCA-salt hypertensive rats, which was inhibited by PD98059 (10 μM). In contrast,

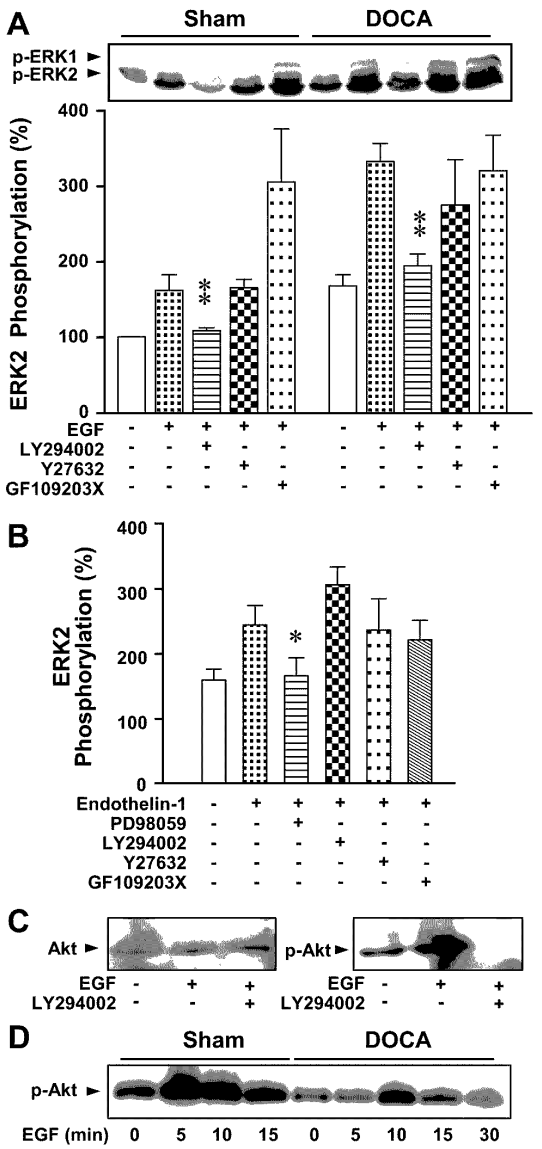

Fig. 5. Effects of inhibitors on EGF-induced ERK1/2 and Akt phosphorylation on the aortic smooth muscles of sham-operated and DOCA-salt hypertensive rats. The strips were stimulated with EGF (10 nM, 15 min) (A) or endothelin-1 (30 nM, 20 min) (B) and then treated with PD98059 (10 μM), LY294002 (10 μM), Y27632 (10 μM), or GF109203X (10 μM) for 15 min. *P<0.05, **P<0.01. C: The strips were stimulated with 10 nM EGF for 15 min and then treated with LY294002 (10 μM) for 15 min in DOCA-salt hypertensive rats. The total expression and activity of Akt was detected using anti-Akt and -phosphorylated Akt antibodies, respectively. D: Time-dependent responses of Akt phosphorylation to EGF (10 nM) in sham-operated and DOCA-salt hypertensive rats. Results are typical recordings obtained from 3 independent experiments.

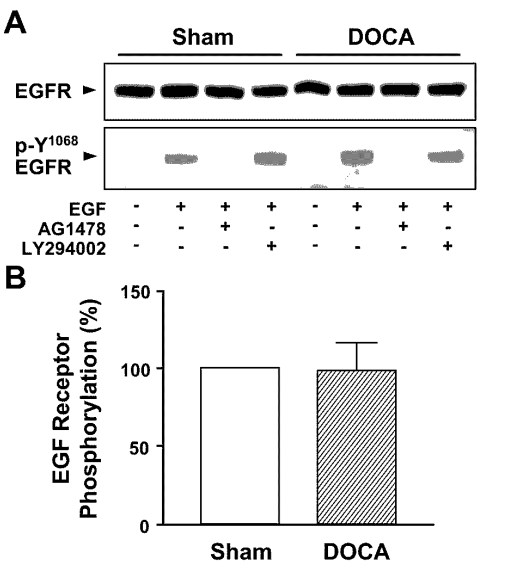

Fig. 6. Effects of EGF and inhibitors on the phosphorylation of EGFR tyrosine kinase in aortic smooth muscles of sham-operated and DOCA-salt hypertensive rats. The strips were stimulated with 10 nM EGF for 15 min and then treated with LY294002 (10 μM) or AG1478 (10 μM) for 15 min. EGFR phosphorylation was demonstrated using an anti-Tyr^{1068} phosphorylated EGFR tyrosine kinase. A: Upper panel: Total ERK1/2 expression was measured using an anti-nonphosphorylated EGFR antibody. B: The level of phosphorylated EGFR in the quiescent state in sham-operated rats was defined as 100% (n = 5).

the increment of ERK1/2 phosphorylation to endothelin-1 was not inhibited by 10 μM LY294002 (Fig. 5B). EGF (10 nM, 10 min) increased the phosphorylation of Akt, which was completely inhibited by LY294002 (10 μM) in hypertensive rats (Fig. 5C). EGF-induced Akt phosphorylation was lower in tissues from DOCA-salt hypertensive rats compared with those from sham-operated rats (Fig. 5D).

EGF-induced increases in EGFR tyrosine kinase activity

To determine whether the change in EGFR activity is associated with the alteration of ERK activation and contraction increment in DOCA-salt hypertensive rats, the expression and activity of EGFR kinase in the aortic smooth muscles were measured using Tyr^{1068} phosphorylated EGFR antibody. In the quiescent state, phosphorylation of EGFR was only slightly detected in both strips. EGF (10 nM, 15 min) significantly increased the EGFR phosphorylation with the magnitude being similar between strips (98 ± 18.8%, n = 5, of sham-operated rats; Fig. 6B). AG1478 (10 μM, 15 min) completely inhibited the receptor phosphorylation evoked by EGF

(10 nM) in both sham-operated and DOCA-salt hypertensive rats (Fig. 6A). LY294002 (10 μM, 15 min) did not alter the receptor phosphorylation evoked by EGF (10 nM) in both strips. In addition, the expression of EGFR, measured using nonphosphorylated antibody, did not change in the quiescent and EGF-treated states in both strips (Fig. 6A).

Discussion

In the present study, we show that EGF increased the activity of ERK1/2 in vascular smooth muscle of sham-operated and DOCA-salt hypertensive rats. Moreover, the inhibitor of ERK1/2 inhibited EGF-induced muscle contraction and ERK1/2 activity in DOCA-salt hypertensive rats. It has been reported that EGF induces contraction (1, 20), and increases the activity of MAPK in vascular smooth muscle (19, 23). Moreover, the response to EGF in contraction and ERK1/2 activity was greater in DOCA-salt hypertensive rats than in sham-operated rats. In the present and previous studies, the change in ERK activity in DOCA-salt hypertensive rats was closely correlated with the results from mechanical study (8, 24). These results strongly suggest that the altered activation of the MAPK pathway is involved in the changed contractile response to EGF in vascular smooth muscle of DOCA-salt hypertensive rats. In the present study, the aortic strips from sham-operated rats displayed minimal contraction in response to treatment with EGF. In contrast, EGF increased ERK activity in sham-operated rats. Although EGF increased the activity of ERK1/2 in sham-operated rats, the maximum elevation of the activity by EGF was similar to the basal level in DOCA-salt hypertensive rats. It can be assumed that the element of ERK1/2 activity by EGF in sham-operated rats may be under the threshold that can elevate the muscle contraction, or it may be involved in cellular processes, including proliferation, migration, and differentiation, but not in elevation of contraction. Moreover, the inhibition of EGFR by a selective inhibitor completely abolished both contraction and ERK1/2 activity mediated by EGF, supporting that EGFR tyrosine kinase induces vasoconstriction through the stimulation of the MAPK pathway in DOCA-salt hypertensive rats. A previous report showed that the expression of EGFR does not differ in DOCA-salt hypertensive and sham-operated rats (23), suggesting that the abnormal responsiveness evoked by EGF is probably regulated by post-receptor events. The present study revealed that the magnitude of EGFR expression and EGF-mediated EGFR activation was similar between sham and DOCA-salt hypertensive rats. These results imply that the increase in the responses to EGF in DOCA-salt hypertensive rats may be mediated by the altered activity of the MAPK pathway.

In a variety of cellular systems, growth factors transfer their signals via intracellular mediators, including the PI3K, Ras, and PLCγ pathways (22, 25). Furthermore, PKC and low molecular weight GTP-binding proteins, such as RhoA and Cdc42, have been identified upstream from ERK1/2 (26). Although inhibitors of PKC and Rho kinase inhibited EGF-induced contraction, these inhibitors did not influence MAPK activity in sham and DOCA-salt hypertensive rats. In contrast, the inhibitor of PI3K strongly inhibited both the contraction and the MAPK activity elicited by EGF in DOCA-salt hypertensive rats. This is consistent with a previous study that showed that PI3K is involved in the MAPK pathway in smooth muscle cells (25, 27). These results indicate that PI3K, but not PKC or Rho kinase, plays a major role in the EGFR-stimulated MAPK pathway in DOCA-salt hypertensive rats. Moreover, these results also show that the EGF-induced contractile response is simultaneously mediated by the MAPK pathway and MAPK-independent pathways that include PKC and Rho kinase (28). Moreover, the inhibitor of PI3K failed to inhibit the ERK1/2 phosphorylation induced by endothelin-1, implying the regulation of MAPK activation by growth factors is different from those by receptor agonists. Moreover, in contrast to the EGF-mediated contraction and MAPK activity that increased during hypertension, the elevation of Akt phosphorylation by EGF was not greater in hypertensive rats compared with control rats. It has been reported that the activity of PI3K was increased, but that of Akt was decreased, in hypertensive rats compared to those in control rats (21, 29). There results imply that PI3K-mediated MAPK activity induced by EGF is not regulated by the Akt pathway. We have previously showed that a vasoconstrictor increases the activity of MAPK and phosphorylation of *h*-caldesmon in aortic strips from sham-operated and DOCA-salt hypertensive rats, which were inhibited by an inhibitor of MAPK (8, 30). Furthermore, the basal tone mediated by MAPK was not associated with the Ca^{2+}-dependent mechanisms in vascular smooth muscle from hypertensive rats (24). These results indicate that MAPK increases the phosphorylation of caldesmon, which causes the elevation of contraction in aortic smooth muscle. It can be assumed from these results that the caldesmon phosphorylation mediated by the MAPK pathway may be involved in the EGF-induced contraction in aortic smooth muscle from DOCA-salt hypertensive rats.

In the present study, the level of MAPK in the quiescent state was significantly increased in the muscle strips of DOCA-salt hypertensive rats than in sham

controls, which is consistent with previous reports on the aortic smooth muscle of hypertensive rats (8, 31, 32). PI3K inhibition slightly attenuated basal tone, similar to a previous report (27). Moreover, inhibitors of EGFR and ERK1/2 attenuated basal tone in DOCA-salt hypertensive rats but not in the sham-operated ones, and the magnitude of inhibition was greater on the EGF-induced response than on the basal tone. These results indicate that basal tone, as well as EGF-induced contraction, in DOCA-salt hypertensive rat is regulated by the PI3K-regulated MAPK activation. Several vasoconstrictors significantly increased the contractility of vascular smooth muscle of DOCA-salt hypertensive rats compared with normotensive rats (18, 33, 34) and also elevated the activity of MAPK (17). Furthermore, the administration of MAPK inhibitor attenuated the blood pressure of hypertensive rats (35). From these results, it can be assumed that the increased MAPK activity in vascular smooth muscle in the quiescent and stimulated states may allow the elevation of blood pressure in DOCA-salt hypertensive rats. These results imply that the increased responsiveness to EGF may contribute to the elevation of blood pressure.

The present study shows that EGF significantly increased the contraction and the activity of ERK1/2 in DOCA-salt hypertensive rats compared to that in sham-operated rats. Furthermore, EGF-induced contraction and ERK activity were activated through EGFR tyrosine kinase and PI3K. In conclusion, EGF induces vasoconstriction through the PI3K-mediated MAPK pathway and the altered activation of the MAPK pathway is involved in the changed contractile response to EGF in vascular smooth muscle of DOCA-salt hypertensive rats.

Acknowledgments

This work was supported by Konkuk University in 2003.

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