Inhibition of AT\textsubscript{1} Receptor Internalization by Concanavalin A Blocks Angiotensin II-induced ERK Activation in Vascular Smooth Muscle Cells

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Recent studies of \(\beta\)-adrenergic receptor suggest that agonist-promoted receptor internalization may play an important role in extracellular signal-regulated kinase (ERK) activation by G protein-coupled receptors. In the present study, we explored the effects of angiotensin II (Ang II) type-1 receptor (AT\textsubscript{1}) internalization on Ang II-induced activation of ERK using the receptor internalization blocker concanavalin A (ConA) and the carboxyl terminus-truncated receptor mutants with impaired internalization. ConA inhibited AT\textsubscript{1} receptor internalization without affecting ligand binding to the receptor, Ang II-induced generation of second messengers, and activation of tyrosine kinases Src and Pyk2 in vascular smooth muscle cells (VSMC). ConA blocked ERK activation evoked by Ang II and the calcium ionophore A23187. Impairment of AT\textsubscript{1} receptor internalization by truncating the receptor carboxyl terminus did not affect Ang II-induced ERK activation. ConA induced proteolytic cleavage of the epidermal growth factor (EGF) receptor at carboxyl terminus and abolished Ang II-induced transactivation of the EGF receptor, which is critical for ERK activation by Ang II in VSMC. ConA also induced proteolysis of erbB-2 but not platelet-derived growth factor receptor. Thus, ConA blocks Ang II-induced ERK activation in VSMC through a distinct mechanism, the ConA-mediated proteolysis of the EGF receptor.

Angiotensin II (Ang II),\textsuperscript{2} a major effector peptide of the renin-angiotensin system, is believed to play a critical role in the pathogenesis of cardiovascular remodeling associated with hypertension, heart failure, and atherosclerosis (1). Ang II binds to at least two types of receptor with high affinity, designated AT\textsubscript{1} and AT\textsubscript{2} receptors (2). We and others (3, 4) previ-ously cloned the AT\textsubscript{1} receptor that belongs to the superfamily of heterotrimeric G protein-coupled receptors (GPCRs). The AT\textsubscript{1} receptor activates phospholipase C\textsubscript{b} through the G\textsubscript{q} protein to generate inositol trisphosphate (IP\textsubscript{3}) and diacylglycerol, which in turn releases calcium from intracellular stores and activates protein kinase C, respectively (5, 6). At present, the AT\textsubscript{1} receptor is thought to mediate almost all the known physiological and pathological effects of Ang II on the target cells such as vascular smooth muscle cells (VSMC) (7–9), cardiomyocytes (5), cardiac fibroblasts (10), and renal mesangial cells (11).

Since the AT\textsubscript{1} receptor is a key mediator in the biologic mechanisms of the renin-angiotensin system, there has been considerable interest in defining its signaling pathways that mediate the cardiovascular physiology. In cultured VSMC, Ang II activates extracellular signal-regulated kinase (ERK), which is an obligatory step for the Ang II-induced cellular hypertrophy of VSMC (12). We and others recently reported that Ang II-induced calcium-dependent transactivation of the epidermal growth factor (EGF) receptor plays a predominant role in the ERK activation by Ang II in VSMC (13) and in cardiac fibroblasts (14). Src family kinase and Pyk2, a recently identified calcium-sensitive tyrosine kinase, seem to be involved in the EGF receptor transactivation by Ang II in VSMC (15).

Recent studies of the \(\beta\)-adrenergic receptor suggest that agonist-promoted receptor internalization plays an important role in ERK activation (16). In Rat 1a fibroblasts, the GPCR-mediated activation of ERK is sensitive to four mechanistically distinct inhibitors of clathrin-mediated endocytosis, concanavalin A (ConA), hypertonic medium, depletion of intracellular potassium, and monodansylcadaverine (17). In addition, expression of dominant inhibitory mutants of \(\beta\)-arrestin1 or dynamin, which attenuate agonist-induced receptor internalization, inhibits \(\beta\)-adrenergic receptor-mediated ERK activation (16, 18). Since GPCRs appear to internalize through a clathrin-mediated process and AT\textsubscript{1} receptor internalization is blocked by ConA (19), we performed studies to examine the possible requirement of AT\textsubscript{1} receptor internalization for the receptor signaling using the receptor internalization blocker ConA and the receptor mutants with impaired internalization. We obtained evidence that AT\textsubscript{1} receptor internalization is not required for the early signaling events and the downstream ERK activation by the receptor in VSMC. Furthermore, we found that ConA blocked Ang II-induced activation of ERK through a distinct mechanism, the ConA-mediated proteolysis of the EGF receptor in VSMC.

EXPERIMENTAL PROCEDURES

Materials—Ang II and [\(\text{Sar}^1, \text{Ile}^8\)]-Ang II were obtained from Peninsula Laboratories. Protein A-Sepharose was purchased from Amersham...
Pharmacia Biotech. Polyvinylidene difluoride membranes were obtained from Millipore. \[^{125}\text{I}]\text{NaI}, [\gamma-\text{P}]\text{ATP}, \text{and } [\text{H}]\text{inosine 1,4,5-
triphosphate (IP\textsubscript{3})} \text{radioisotope assay kits were purchased from NEN Life Science Products. Polyclonal antibodies against Src (SRC-2) and EGF receptor were obtained from Santa Cruz Biotechnology. Monoclonal anti-Py\textsubscript{2}, anti-EGF receptor, and anti-phosphotyrosine (PY20) antibodies were obtained from Transduction Laboratories. Src family kinases (AT\textsubscript{1A}) kit and EGF receptor were purchased from Upstate Biotechnology Inc. Polyclonal antibodies against phospho-specific ERK and ERK and alkaline phosphatase-conjugated secondary antibodies and reagents for chemiluminescence detection were purchased from New England Biolabs. All other reagents were from Sigma.

**Cell Culture—VSMC** were prepared from the thoracic aorta of 12-week-old Harlan Sprague-Dawley rats (Charles River Breeding Laboratories) by the explant method and cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, penicillin, and streptomycin as described previously. Subcultured VSMC from passages 3 through 15 were used in the experiments. Subconfluent VSMC were serum-starved in plain medium for 48 h. Chinese hamster ovary (CHO) K1 cells were obtained from American Type Culture Collection and cultured in Ham’s F-12 medium supplemented with 10% fetal calf serum at 37 °C in a humidified incubator with 5% CO\textsubscript{2}.

**Radioligand Binding Assay—Monolodinated \[^{125}\text{I}][\text{Sar}^1, \text{Ile}^8]\text{-Ang II} was prepared by the lactoperoxidase method and purified by reverse-phase high performance liquid chromatography as described previously.\(^{21}\) Determination of receptor affinity and density and measurement of the surface receptor binding capacity were performed as described previously.\(^{19}\)

**Measurement of IP\textsubscript{3} Production—Cells** were subcultured into 12-well plates and, when nearly confluent, were stimulated with 100 nM Ang II with ice-cold phosphate-buffered saline containing 1 mM Na\textsubscript{3}VO\textsubscript{4} and subcloned into the expression vector pRc/CMV and stably expressed in inserting a stop codon. The wild type or mutated AT\textsubscript{1A} cDNA was acid residues in the carboxyl terminus, respectively, were prepared by previously.\(^{22}\) In the presence of ConA, which is shown in Fig. 1.

**Measurement of IP\textsubscript{3} Production—Cells** were subcultured into 12-well plates and, when nearly confluent, were stimulated with 100 nM Ang II for 15 s. The IP\textsubscript{3} mass generated in 15 s was measured by radioreceptor binding assay as described previously.\(^{19}\)

**Calcium Measurement—Ang II—mediated changes in intracellular calcium concentration were determined with Fura-2/AM as described previously.\(^{22}\)**

**Stable Expression of Wild Type and Truncated AT\textsubscript{1A} Receptors—** The truncated AT\textsubscript{1A} receptor mutants T318 and T328 lacking 42 or 32 amino acid residues in the carboxyl terminus, respectively, were prepared by inserting a stop codon. The wild type or mutant AT\textsubscript{1A} cDNA was subcloned into the expression vector pRc/CMV and stably expressed in CHO cells as described previously.\(^{23}\)

**Immunoprecipitation and Immunoblotting—Cells** were washed twice with ice-cold phosphate-buffered saline containing 1 mM Na\textsubscript{2}VO\textsubscript{4} and then lysed on ice in Nonidet P-40 lysis buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 1 mM Na\textsubscript{3}VO\textsubscript{4}, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, and protease inhibitors). The extract was clarified by centrifugation at a full speed in a micro-centrifuge. The clarified lysates were incubated sequentially (4 h for each incubation at 4 °C) with antibodies as indicated and protein A-Sepharose. The immunoprecipitates were collected and washed four times with the lysis buffer. For immunoblotting, whole cell lysates or immunoprecipitates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was probed with various primary antibodies as indicated and detected using the ECL system with alkaline phosphatase-conjugated secondary antibodies according to the manufacturer’s protocol.

**Immune Complex Tyrosine Kinase Assay—** For measuring the activities of Src family kinases, cell lysates prepared in Nonidet P-40 lysis buffer were precleared with protein A-Sepharose and control IgG from normal rabbit serum, and the lysates were immunoprecipitated with a polyclonal antibody (SRC-2), which recognizes the carboxyl-terminal sequence of the Src family members Src, Fyn, and Yes. Immunoprecipitates were washed four times with Nonidet P-40 lysis buffer and once with phosphate-buffered saline and resuspended in 15 μl of a buffer solution containing 40 mM Hepes, pH 7.0, 2% (v/v) glycerol, 0.02% Nonidet P-40. Kinase reactions were initiated by the addition of 100 μM ATP, 25 mM MgCl\textsubscript{2}, 5 mM MnCl\textsubscript{2}, 50 μM Na\textsubscript{2}VO\textsubscript{4}, and 10 μCi of [\gamma-\text{P}]\text{ATP} in the presence of 300 μM Src family kinase specific peptide (KEVKEIGETGYGVKK) in a total volume of 35 μl. After incubation at 30 °C for 10 min, peptide phosphorylation was stopped by the addition of 15 μl of 50% acetic acid, and the reaction mixture was then applied onto P-81 phosphocellulose filter paper. Papers were washed four times in 0.75% phosphoric acid, washed once with acetic, dried, and then counted in a scintillation counter.
response to the agonist Ang II in VSMC. Pretreatment of the cells with ConA virtually blocked the Ang II-induced internalization of AT1 receptor in VSMC (Fig. 1A). However, ConA treatment did not affect Ang II-induced IP₃ formation and calcium mobilization in VSMC (Fig. 1, B and C). In addition, ConA did not alter the AT1 receptor binding number (520 ± 70 fmol/mg of protein with ConA versus 505 ± 59 fmol/mg of protein without ConA treatment) and the receptor binding affinity to 125I-[Sar¹, Ile⁸]-Ang II in VSMC (K_D = 0.72 ± 0.06 nM with ConA versus 0.68 ± 0.07 nM without ConA treatment). These results with ConA indicate that AT1 receptor-mediated generation of second messengers occurs in the absence of the receptor internalization.

**ConA Blocks ERK Activation by Ang II and Calcium Ionophore A23187 in VSMC**—In VSMC, ERK activation by Ang II is calcium-dependent (26). The ERK is activated upon phosphorylation of a Thr and a Tyr residue in a TEY motif (27). Specific antibody against phosphorylated ERK detects the activated form of ERK by immunoblotting. Using this phospho-specific antibody, we performed immunoblot analysis to determine the effect of ConA on Ang II-induced ERK activation in VSMC. As shown in Fig. 2, treatment of ConA virtually abolished the ERK activation by both Ang II (100 nM) (Fig. 2A) and calcium ionophore A23187 (10 μM) (Fig. 2B). In contrast, ConA did not affect the ERK activation evoked by the protein kinase C activator phorbol 12-myristate 13-acetate (Fig. 2B). Thus, ConA interrupts signaling pathways to ERK activation downstream of calcium. Since Ang II-induced activation of ERK is calcium-dependent in VSMC (26), these results suggest that internalization of the AT1 receptor may not be involved in the ERK activation by Ang II in VSMC.

**Internalization of AT1 Receptor Is Not Required for ERK Activation by Ang II**—To determine whether internalization of AT1 receptor is required for the ERK activation, we have constructed truncated mutants of the AT1A receptor and stably expressed them in CHO cells. Truncation mutants T318 and T328 lack 42 or 32 amino acid residues in the carboxyl terminus of the AT1A receptor, respectively. We reported that the dissociation constants (K_D) for 125I-[Sar¹, Ile⁸]-Ang II among the wild type and the truncated mutants were similar, indicating that the wild type and the mutants possessed similar ligand binding affinity (23). As shown in Fig. 3A, Ang II (100 nM) induced rapid and profound internalization of the wild type AT1A receptor in transfected CHO cells, whereas the Ang II-induced receptor internalization was impaired in the truncated mutants (T318 and T328). We compared the ERK activation by Ang II among the wild type and the truncated mutants of AT1A receptors. As shown in Fig. 3B, the ERK is fully activated by Ang II in CHO cells stably expressing the wild type AT1A receptor or the truncated mutant T318. The expression level of the mutant T318 is about 1.7-fold that of the wild type receptor (23). Denatimetric analysis revealed that the extent of ERK2 phosphorylation by the mutant T318 is 2.28-fold that of the wild type receptor (Fig. 3C). This may be due to the high expression level of receptor and more receptor remaining on the plasma membrane in cells expressing the mutant T318 during Ang II stimulation as shown in Fig. 3A. We also observed that ERK was fully activated by Ang II in CHO cells expressing the mutant T328 (data not shown). These results indicate that the Ang II-induced receptor internalization and the ERK activation are two distinct receptor functions. AT1 receptor internalization is not required for the ERK activation by Ang II.
ConA Does Not Affect Ang II-induced Activation of Pyk2 and Src Family Kinases in VSMC—It has been recently reported that Src family kinases and the calcium-sensitive proline-rich tyrosine kinase (Pyk2) play important roles in the ERK activation by Ang II (28, 29). We have recently reported that Src family kinases and Pyk2 may mediate the Ang II-induced transactivation of the EGF receptor in VSMC (15). Pyk2 tyrosine phosphorylation is associated with an increase in its own kinase activity (30). To determine the effect of ConA on activation of Pyk2 by Ang II, VSMC were pretreated with 0.25 mg/ml ConA for 30 min, and Ang II-induced tyrosine phosphorylation of Pyk2 was measured as shown in Fig. 4A. Ang II (100 nM) induced a rapid (1 min) tyrosine phosphorylation of Pyk2, and this effect was not altered by ConA pretreatment in VSMC.

We determined the effect of ConA on Src family kinase activation by Ang II. VSMC were pretreated with or without 0.25 mg/ml ConA for 30 min as indicated and then stimulated with 100 nM Ang II for 1 min. Cell lysates were immunoprecipitated with the polyclonal SRC-2 antibody, which recognizes the common carboxyl-terminal sequence of the Src, Fyn, and Yes. The immunoprecipitable kinase activity was determined as described under “Experimental Procedures.” Data are the mean ± S.D. of three separate experiments.

ConA Induces Proteolysis of EGF Receptor in VSMC—The above results indicate that ConA blocks Ang II-induced activation of ERK through a mechanism independent of the AT_{1} receptor internalization in VSMC. Instead, ConA interrupts the signaling pathways downstream of calcium mobilization, Pyk2, and Src family kinases. We recently reported that Ang II activates ERK through a calcium-dependent transactivation of the EGF receptor in VSMC (13). To determine whether ConA affects the Ang II-induced transactivation of the EGF receptor, we measured tyrosine phosphorylation of the EGF receptor by Ang II in ConA-treated VSMC as shown in Fig. 5B. VSMC were pretreated with or without 0.25 mg/ml ConA for 30 min then either left unstimulated (−) or stimulated with Ang II (100 nM) or EGF (100 ng/ml) for 2 min. Lysates were directly subjected to SDS-PAGE and immunoblotting with an anti-EGF receptor antibody that recognizes the carboxyl terminus of EGF receptor at amino acid residue 996 to 1022. B–D, VSMC were pretreated with or without 0.25 mg/ml ConA for 30 min then either left unstimulated (−) or stimulated with Ang II (100 nM) or EGF (100 ng/ml) for 2 min. Lysates were directly subjected to SDS-PAGE and immunoblotting with an anti-EGF receptor antibody against the receptor carboxyl-terminal region (amino acid residues 1005–1016) (B), an antibody against the carboxyl terminus of erbB-2 (C), or an antibody against the carboxyl-terminal region (residues 1013–1025) of the PDGF receptor type β (PDGF-R_{β}) (D). Results shown are representative immunoblots of three separate experiments.
reacts with rat EGF receptor. Surprisingly, the intact EGF receptor was barely detected in ConA-treated VSMC when compared with that in control cells (Fig. 5A, bottom panel). These results suggest that ConA may induce degradation or proteolysis of the EGF receptor. Alternatively, ConA may induce post-modification of the EGF receptor, which may interfere with the immunoprecipitation of the EGF receptor by the polyclonal anti-EGF receptor antibody we used in a nature condition. To confirm the effect of ConA on the EGF receptor degradation, VSMC were pretreated with or without 0.25 mg/ml ConA for 30 min and then stimulated with 100 Ang II or 100 ng/ml EGF for 2 min. Cell lysates were directly subjected to SDS-PAGE and analyzed by the polyclonal antibody (Santa Cruz) against the carboxyl-terminal region (amino acid residues 1005–1016) of the EGF receptor. As shown in Fig. 5B, the intact EGF receptor was barely detected by the antibody in ConA-treated VSMC when compared with that in control cells. Ang II or EGF did not affect the degradation of the EGF receptor by ConA treatment (Fig. 5B). The same blot was stripped and reprobed with a monoclonal anti-EGF receptor antibody (Transduction Laboratory), which recognizes the carboxyl terminus of the EGF receptor at amino acid residues 996 to 1022, or an EGF receptor antisemur (32) (kindly provided by Dr. Stanley Cohen), which was raised with the whole molecule of mouse liver EGF receptor and reacts with the rat EGF receptor. Similar results were obtained when these two different anti-EGF receptor antibodies were employed in the immunoblotting (data not shown). These data indicate that ConA could induce degradation or proteolysis of the EGF receptor in VSMC.

The EGF receptor subfamily member erbB-2 but not erbB-3 or -4 is expressed in VSMC. ConA treatment also resulted in a decrease of the erbB-2 protein in VSMC (Fig. 5C). The erbB-2 was detected by an antibody (Santa Cruz) against the carboxyl terminus of the protein. In contrast, PDGF receptor type β, which is expressed abundantly in VSMC (33), remained intact during ConA treatment (Fig. 5D). The PDGF receptor type β was detected by an antibody (Upstate Biotechnology) against the receptor carboxyl-terminal region (residues 1013–1025).

To identify proteolytic cleavage fragment of the EGF receptor, cell lysates from ConA-treated VSMC were subjected to SDS-PAGE (7 or 10% gel) and analyzed by the polyclonal antibody (Santa Cruz) against the carboxyl-terminal region (amino acid residues 1005–1016) of the EGF receptor. As shown in Fig. 6A, ConA induced an appearance of a 26-kDa fragment in a time-dependent manner, which coincides with a decrease in the level of the native EGF receptor. Fig. 6B shows that divalent succinyl ConA did not cause a decrease of the native EGF receptor and appearance of the 26-kDa fragment. This suggests that clustering of surface membrane proteins including the EGF receptor by ConA binding may be responsible for the EGF receptor proteolysis. Finally, we determined the effects of other two chemically distinct inhibitors of clathrin-mediated endocytosis, such as hyperosmolar sucrose (34) and phenylarsine oxide (35), on proteolysis of the EGF receptor in VSMC. Both hyperosmolar sucrose and phenylarsine oxide inhibited the AngII-induced internalization of AT1 receptor in VSMC (data not shown). As shown in Fig. 6C, ConA caused proteolysis of the EGF receptor, which produces a 26-kDa EGF receptor fragment in VSMC. In contrast, treatment of VSMC with sucrose (0.45 M) or phenylarsine oxide (20 μM) did not affect the integrity of the EGF receptor. These data strongly indicate that ConA induces proteolysis of the EGF receptor in VSMC, probably by cleaving the receptor carboxyl terminus at a site before amino acid residue 1005.

Five sites of in vivo autophosphorylation have been identified in the EGF receptor: three major (Tyr1082, Tyr1148, and Tyr1173) and two minor sites (Tyr992 and Tyr1086) (36–39). These sites bind a variety of downstream signaling proteins that contain Src homology 2 domains, including Shc (40) and phospholipase Cγ (41), thus leading to ERK activation. Deletion of the carboxyl-terminal region that contains these auto-phosphorylation sites by ConA will abolish the EGF-mediated mitogenic signal such as activation of mitogen-activated protein kinase ERK. Thus, we determined ERK activation by EGF in ConA-treated VSMC. As shown in Fig. 7, treatment of VSMC with 0.25 mg/ml ConA for 30 min abolished the ERK activation by EGF. The activation of ERK was determined by a phosho-specific antibody to ERK.

**DISCUSSION**

In the present study, we explored the effects of AT1 receptor internalization on the receptor signaling using the receptor internalization blocker ConA and the receptor mutants (T318 and T328) with impaired internalization. We have obtained evidence that AT1 receptor internalization is not required for Ang II-induced generation of second messengers (IP3 and calcium) and downstream activation of Pyk2, Src family kinases,
and ERK in VSMC. Furthermore, we found that ConA blocked Ang II-induced ERK activation in VSMC through a distinct mechanism, the ConA-mediated proteolysis of EGF receptor.

Recent studies indicate that internalization of β2-adrenergic receptor is required for the receptor-mediated ERK activation (16). Attenuation of the receptor internalization by inhibitors of clathrin-mediated endocytosis or by expression of dominant inhibitory mutants of β-arrestin 1 or dynamin inhibits β2-adrenergic receptor-mediated ERK activation (16–18). However, several lines of evidence suggest that this concept may not be applied to the Gq-coupled AT1 receptor. First, the mechanism of AT1 receptor internalization appears to be different from that of β2-adrenergic receptor. Internalization of β2-adrenergic receptor can be inhibited by the tyrosine kinase inhibitor herbimycin A and a selective Src family kinase inhibitor PP1 (42). However, we found that neither herbimycin A and PP1 nor tyrosine phosphatase inhibitor vanadate can attenuate the inhibitory mutants of AT1 receptor internalization (17). The ERK activation evoked by these agonists for GPCRs is mainly mediated by the transactivation of the EGF receptor in the Rat-1 cells (45). Thus, ConA-mediated proteolysis of the EGF receptor other than the inhibition of the GPCR internalization seems to be the common mechanism for the ConA to suppress the ERK activation.

The lectin ConA binds specifically to terminal mannose residues on cell surface glycoproteins and glycolipids and modulates their lateral mobility differently in different cell types (46). ConA polyclonally activates T cells (47). On the other hand, ConA inhibits internalization of EGF receptor and the receptor-mediated mitogenic effects without affecting EGF binding and EGF-stimulated changes in pH, calcium, and levels of inositol phosphates in NIH 3T3 cells (48, 49). Five sites of in vivo autophosphorylation have been identified in the EGF receptor: three major (Tyr1068, Tyr1148, and Tyr1173) and two minor sites (Tyr992 and Tyr1086) (36–39). These sites bind a variety of downstream signaling proteins that contain Src homology 2 domains, including Shc (40) and phospholipase Cγ (41), thus leading to ERK activation. In the present study, we found that ConA could induce proteolytic cleavage of the EGF receptor carboxyl terminus at a site before amino acid residue 1005 without affecting the EGF binding to the receptor in VSMC (3275 ± 395 cmap with ConA versus 3003 ± 360 cmap without ConA treatment). Proteolytic cleavage to remove the EGF receptor carboxyl-terminal region that contains these three major and two minor autophosphorylation sites will abolish the EGF mitogenic signal such as ERK activation. In fact, ConA did block the EGF-induced ERK activation in VSMC (Fig. 7). It has been reported that these EGF receptor autophosphorylation sites are also required for the binding of the receptor carboxyl terminus to the adaptor protein 2, thus mediating the receptor internalization (50). It is interesting to determine whether the proteolytic cleavage of the EGF receptor carboxyl terminus is involved in the ConA-mediated inhibition of the EGF receptor internalization.

In summary, we have obtained substantial evidence indicating that AT1 receptor internalization is not required for the early signaling events and the downstream ERK activation by the receptor in VSMC. Furthermore, we found that ConA, an AT1 receptor internalization blocker, suppressed Ang II-induced activation of ERK through a distinct mechanism, the ConA-mediated proteolysis of the EGF receptor in VSMC.

FIG. 7. Concanavalin A abolishes ERK activation by EGF in VSMC. VSMC were pretreated with or without 0.25 μg/ml ConA for 30 min then stimulated with 100 ng/ml EGF for 5 min. Lysates were directly subjected to immunoblotting with antibody against phosphorylated ERK (top panel). The blots were stripped and reprobed with anti-ERK antibody (bottom panel).
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