Activation of Akt/protein kinase B after stimulation with angiotensin II in vascular smooth muscle cells

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Takahashi, Tomosaburo, Takeshi Taniguchi, Hiroaki Konishi, Ushio Kikkawa, Yuichi Ishikawa and Mitsuhito Yokoyama. Activation of Akt/protein kinase B after stimulation with angiotensin II in vascular smooth muscle cells. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1927–H1934, 1999.—Involvement of Akt/Protein kinase B (PKB), a serine/threonine kinase with a pleckstrin-homology domain, in angiotensin II (ANG II)-induced signal transduction was investigated in cultured vascular smooth muscle cells (VSMC). Stimulation of the cells with ANG II led to a marked increase in the kinase activity of Akt/PKB, which coincided with Ser-473 phosphorylation. ANG II-stimulated Akt/PKB activation was rapid, concentration dependent, and inhibited by the AT1-receptor antagonist CV-11974, but not by pertussis toxin. Akt/PKB activity was stimulated by the Ca2+ ionophore ionomycin, suggesting the possible involvement of Ca2+ in ANG II-stimulated Akt/PKB activation. However, blockade of Ca2+ mobilization by BAPTA-AM only partially inhibited ANG II-stimulated Akt/PKB activation. ANG II-stimulated Akt/PKB activation was inhibited by the tyrosine kinase inhibitors genistein and herbimycin A and by the phosphatidylinositol 3-kinase (PI3K) inhibitors wortmannin and LY-294002. These results indicate that ANG II stimulates Akt/PKB activity via AT1 receptors in VSMC and that the activities of tyrosine kinase and PI3K are required for this activation.

angiotsin II type 1 receptor; signal transduction; vascular biology

ANGIOTENSIN II (ANG II) is the main peptide hormone of the renin-angiotensin system and plays an key regulatory role in the regulation of blood pressure and circulating volume. In addition, ANG II has been shown to play an important role in the development of various cardiovascular diseases characterized by the accumulation of vascular smooth muscle cells (VSMC), such as hypertension, atherosclerosis, and restenosis after balloon angioplasty (19). Recent evidence suggests that the regulation of not only cell growth but also cell death by apoptosis could be an important determinant of vessel structure and lesion formation (6, 18, 21, 25). In a cell culture system, ANG II stimulates hypertrophic growth and migration of VSMC (19). Moreover, ANG II is a potent antiapoptotic factor capable of reversing the apoptotic effects of serum withdrawal and nitric oxide (46). However, molecular mechanisms responsible for growth promoting and antiapoptotic actions of ANG II have not been fully understood.

ANG II acts via a high-affinity cell-surface receptor called the ANG II type 1 (AT1) receptor. This receptor is a heterotrimeric G protein-coupled receptor with seven transmembrane helices and has been reported to be coupled to either a pertussis toxin-insensitive G protein of the Gq subfamily or a pertussis toxin-sensitive G protein of the Gi subfamily (19). However, some of the intracellular signals mediated by the AT1 receptor are similar to the signaling pathways activated by receptor tyrosine kinases such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) receptors. For instance, ANG II induces the activation of the ras protooncogene product Ras (13, 45, 49), the tyrosine and threonine phosphorylation and activation of mitogen-activated protein (MAP) kinases (56), the expression of early growth-response genes such as c-fos, c-jun, and c-myc (28, 40, 41, 53), and the tyrosine phosphorylation of multiple intracellular proteins, including the focal adhesion-associated protein paxillin, focal adhesion kinase, and the Crk-associated substrate p130 Cas (36, 44, 52, 57, 58). Recently, phosphatidylinositol 3-kinase (PI3K) has also been shown to be activated in response to ANG II in VSMC (48). Although the role of PI3K in intracellular signaling is underscored by its implication in a plethora of biological responses, relatively little is known about the downstream elements of PI3K.

Akt/protein kinase B (PKB), also called RAC (related to A and C)-protein kinase, is a serine/threonine kinase that contains a pleckstrin-homology domain in its NH2-terminal end region and a catalytic domain closely related to both cAMP-dependent protein kinase and protein kinase C (PKC), and it is also the cellular homolog of the product of the retroviral oncogene v-akt (4, 8, 26). Kinase activity of Akt/PKB has been shown to be stimulated by growth factors acting through receptor tyrosine kinases such as PDGF, EGF, and insulin receptors, and the activation of Akt/PKB by these growth factors has been shown to be mediated by PI3K (7, 9, 16, 31). Recently, a signaling pathway from PI3K to Akt/PKB was implicated in some cellular responses of PI3K including suppression of apoptosis (12, 27, 29, 34). However, little is yet known about the participation of Akt/PKB in signaling pathways for seven-transmembrane G protein-coupled receptors, especially for G proteins of the Gq subfamily-coupled receptors.

In the present study, we first examined whether Akt/PKB activity was stimulated in response to ANG II in VSMC. This report shows that, via AT1 receptors,
ANG II stimulates the kinase activity and Ser-473 phosphorylation of Akt/PKB in a pertussis toxin-insensitive fashion in VSMC. Evidence is also provided showing that the activities of tyrosine kinase and PI3K are required for this activation. These results suggest that Akt/PKB could play a role in ANG II-induced signal transduction in VSMC.

MATERIALS AND METHODS

Materials. ANG II, ionomycin, phorbol 12-myristate 13-acetate (PMA), pertussis toxin, and wortmannin were obtained from Sigma (St. Louis, MO). 1,2-Bis(2-aminophenoxy)ethane-N,N',N''-tetraacetic acid acetoxyethyl ester (BAPTA-AM) and herbimycin A were from Life Technologies (Rockville, MD). LY-294002 and genistein were from Calbiochem (San Diego, CA). The sheep polyclonal antibody against BAPTA-AM was from Upstate Biotechnology (Lake Placid, NY). The rabbit polyclonal phospho-specific Akt/PKB antibody, Akt/PKB was from Upstate Biotechnology (Lake Placid, NY). LY-294002 and genistein were from Calbiochem (San Diego, CA). The sheep polyclonal antibody against BAPTA-AM and herbimycin A were from Life Technologies (Rockville, MD). LY-294002 and genistein were from Calbiochem (San Diego, CA). The sheep polyclonal antibody against BAPTA-AM was from Upstate Biotechnology (Lake Placid, NY). The rabbit polyclonal phospho-specific Akt/PKB antibody, Akt/PKB was from Upstate Biotechnology (Lake Placid, NY). LY-294002 and genistein were from Calbiochem (San Diego, CA). The sheep polyclonal antibody against BAPTA-AM and herbimycin A were from Life Technologies (Rockville, MD). LY-294002 and genistein were from Calbiochem (San Diego, CA).

Preparation of cell lysates and immunoprecipitation kinase assay. The quiescent VSMC were stimulated as indicated. The cells were then washed twice with ice-cold phosphate-buffered saline and lysed into buffer A (50 mM Tris- HCl, pH 7.5, 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM sodium fluoride, 10 mM sodium β-glycerophosphate, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 0.1% 2-mercaptoethanol) containing 1 µM microcystin LR (Research Biochemicals International). After insoluble materials were removed by centrifugation at 15,000 rpm for 20 min, protein concentrations in the supernatants were normalized using a protein assay (Bio-Rad). As whole cell extracts, lysates were directly added to 5× sample buffer (1:4 vol/vol) for SDS-polyacrylamide gel electrophoresis (PAGE) and boiled at 100°C for 5 min. For immunoprecipitation, after the lysates (500 µg total protein) were preabsorbed with 15 µl of a 1:1 slurry of protein G-Sepharose for 30 min at 4°C, the lysates were incubated with sheep polyclonal anti-Akt/PKB antibody (2 µg) for 2 h at 4°C. Immunocomplexes were immunoprecipitated for 1 h at 4°C with 40 µl of the 1:1 slurry of protein G-Sepharose. The immunocomplexes were washed three times with buffer B (50 mM Tris-HCl, pH 7.5, 0.03% Brij 35, 0.1 mM EGTA, and 0.1% 2-mercaptoethanol), and once with assay dilution buffer (20 mM MOPS, pH 7.2, 25 mM sodium β-glycerophosphate, 1 mM sodium orthovanadate, and 1 mM dithiothreitol). The beads were resuspended in 30 µl of kinase reaction mixture (assay dilution buffer containing 25 mM MgCl2, 170 µM ATP, 1 µg histone H2B (Boehringer Mannheim), and 1 µCi [γ-32P]ATP) and incubated at 30°C for 30 min. Kinase reactions were stopped by the addition of 7 µl of 5× sample buffer, after which the samples were boiled for 5 min at 100°C and electrophoresed on 15% SDS-polyacrylamide gels. The gels were stained with Coomassie blue, and dried, and radioactivities were analyzed using a bio-imaging analyzer (Fuji BAS2000).

Immunoblot analysis. Samples were subjected to 10% SDS-PAGE, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Millipore). Blots were incubated with rabbit polyclonal phospho-specific Akt/PKB antibody or total Akt/PKB antibody, and the primary antibodies were detected using horseradish peroxidase-labeled donkey anti-rabbit IgG, followed by enhanced chemiluminescence (Amersham Life Science).

Statistical analysis. Where applicable, results were expressed as means ± SE. Statistical analysis was performed with unpaired Student’s t-test or ANOVA when appropriate,
and differences were considered to be significant when the probability value was <0.05.

RESULTS

Effects of ANG II and PDGF on kinase activity and Ser-473 phosphorylation of Akt/PKB in VSMC. To examine the effect of ANG II on kinase activity of Akt/PKB, cultured VSMC were treated with either ANG II (100 nM) or PDGF BB homodimer (PDGF-BB; 20 ng/ml), and kinase activity of Akt/PKB was measured by immunoprecipitation kinase assay with histone H2B as a substrate. As shown in Fig. 1, A and B, stimulation of the cells with ANG II led to a marked increase in the kinase activity of Akt/PKB. As has already been reported in other cell types (16), PDGF also stimulated kinase activity of Akt/PKB in VSMC, and this activation was more pronounced than that by ANG II. Because Akt/PKB has been shown to be activated by phospholipid binding and by phosphorylation within the activation loop at Thr-308 and within the COOH terminus at Ser-473 (22, 23), we assessed the effect of ANG II on the phosphorylation state of Akt/PKB at Ser-473 by immunoblotting with phospho-specific Akt/PKB antibody that recognized Akt/PKB only when phosphorylated at Ser-473. Stimulation of VSMC with ANG II or PDGF caused a marked increase in the phosphorylation state at Ser-473, whereas total Akt/PKB proteins were not altered by treatment with ANG II or PDGF (Fig. 1C). These results indicate that, in VSMC, ANG II stimulates the kinase activity of Akt/PKB and that this activation is coincident with Ser-473 phosphorylation.

Time course and dose response of ANG II- and PDGF-stimulated kinase activity of Akt/PKB. The effects of ANG II and PDGF on kinase activities of Akt/PKB were time and concentration dependent. ANG II-stimulated Akt/PKB activation could be detected within 2 min after addition of ANG II, reached a maximum at ~5 min, and then declined (Fig. 2A). Half-maximum and maximum effects were achieved at 10 and 100 nM of ANG II, respectively (Fig. 2B). On the other hand, activation of Akt/PKB by PDGF was maximal at 5–10 min and then slowly declined (Fig. 2C) and was dose dependent up to 200 ng/ml (Fig. 2D).

Effects of AT1- and AT2-receptor antagonists and pertussis toxin on ANG II-stimulated kinase activity of Akt/PKB. ANG II receptors have been classified into AT1 and AT2 subtypes (19). To determine which ANG II receptor subtype mediates Akt/PKB activation, VSMC were pretreated with either the AT1-receptor antago-

Fig. 2. Time course and dose response of ANG II- or PDGF-stimulated kinase activity of Akt/PKB. Cultured VSMC were treated with 100 nM ANG II (A) or 20 ng/ml PDGF (C) for various periods of time as indicated or treated for 5 min with various concentrations of ANG II (B) or PDGF (D) as indicated. Insets: anti-Akt/PKB immunoprecipitates were subjected to in vitro kinase assay with histone H2B as a substrate. Radioactivities of phosphorylated histone H2B were quantitated. Values are means ± SE of 3 independent trials and are expressed as percentages of unstimulated levels. Insets are representative of 3 independent trials. *P < 0.05 vs. control.
nist CV-11974 or the AT$_2$-receptor antagonist PD-123319. ANG II-stimulated Akt/PKB activation was completely inhibited by CV-11974 (200 nM), but not by PD-123319 (200 nM), whereas both antagonists by themselves had no effect on Akt/PKB activity (Fig. 3). These results indicated that the stimulatory effect of ANG II on Akt/PKB activity was mediated by AT$_1$ receptors. The AT$_1$ receptor has been shown to be linked to a pertussis toxin-insensitive G protein of the G$_q$ subfamily or a pertussis toxin-sensitive G protein of the G$_i$ subfamily (19). Pretreatment with 500 ng/ml of pertussis toxin for 5 h neither affected basal Akt/PKB activity nor inhibited the response to ANG II (Fig. 3).

We have previously shown that this protocol of treatment with pertussis toxin resulted in almost complete ADP ribosylation of the $\alpha$-subunit of the G protein of the G$_i$ subfamily in VSMC (45, 55). These results suggest that a G protein of G$_q$ subfamily is involved in this reaction.

Effects of PMA and ionomycin on kinase activity of Akt/PKB. Via a pertussis toxin-insensitive G protein of the G$_q$ subfamily, the activation of AT$_1$ receptor induces a phospholipase C-mediated phosphoinositide hydrolysis, which causes PKC activation and intracellular Ca$^{2+}$ mobilization (19). Whereas the activation of PKC by phorbol ester has been reported to have almost no effect on the enzymatic activity of Akt/PKB (16, 31), the involvement of Ca$^{2+}$ on Akt/PKB activation remains to be elucidated. We next examined whether intracellular Ca$^{2+}$ mobilization or PKC activation was involved in Akt/PKB activation in VSMC. The Ca$^{2+}$ ionophore ionomycin (1 µM) significantly stimulated kinase activity of Akt/PKB, whereas the PKC-activating phorbol ester PMA (100 nM) had a minimal effect on Akt/PKB activity (Fig. 4). These results suggested that Ca$^{2+}$ mobilization was involved in ANG II-stimulated Akt/PKB activation in VSMC.

Effects of BAPTA-AM on kinase activity of Akt/PKB stimulated by ANG II and ionomycin. Next, to assess the involvement of Ca$^{2+}$ mobilization in ANG II-stimulated Akt/PKB activation, we examined the effect of the membrane-permeable Ca$^{2+}$ chelator BAPTA-AM on ANG II-stimulated Akt/PKB activation. The blockade of Ca$^{2+}$ mobilization by BAPTA-AM (25 µM) only partially inhibited ANG II-stimulated Akt/PKB activation (Fig. 5). Under the same conditions, pretreatment with BAPTA-AM effectively inhibited ionomycin-stimulated Akt/PKB activation, but not basal Akt/PKB activity (Fig. 5), and indeed suppressed ANG II-mediated Ca$^{2+}$ mobilization in these cells (data not shown). These results suggest that Ca$^{2+}$ mobilization was partially
involved in ANG II-induced Akt/PKB activation, whereas a Ca\(^{2+}\)-independent pathway could also exist. Effects of tyrosine kinase inhibitors on ANG II-induced Akt/PKB activation. In VSMC, ANG II has been shown to activate the multiple tyrosine kinase pathways that, by analogy with growth factors, are important in mediating the multiple cellular responses of ANG II (5, 20, 57). We next examined whether tyrosine kinase activity was required for ANG II-induced Akt/PKB activation by using the tyrosine kinase inhibitors genistein and herbimycin A. These inhibitors are chemically and mechanically distinct, are highly specific for protein tyrosine kinases (1, 17, 37, 59), and are shown to inhibit ANG II-stimulated overall tyrosine phosphorylation and protein synthesis without affecting ANG II binding to the AT\(_1\) receptor, phospholipase C activation, and MAP kinase activation (35, 51). As shown in Fig. 6, both genistein (100 µM) and herbimycin A (3 µM) inhibited activation of Akt/PKB by ANG II, although the inhibitory effect of herbimycin A was less than that of genistein. These inhibitors did not affect the basal kinase activity of Akt/PKB. These results suggest the involvement of tyrosine kinase(s) in ANG II-stimulated Akt/PKB activation.

Effects of PI3K inhibitors on kinase activity of Akt/PKB stimulated by ANG II, PDGF, and ionomycin. PI3K is shown to be necessary and sufficient for growth factor-dependent activation of Akt/PKB, although an additional pathway independent of PI3K for activation of Akt/PKB has been proposed (14, 22, 23). We then tested the effect of two structurally unrelated PI3K inhibitors, wortmannin and LY-294002, on ANG II-induced Akt/PKB activation. Wortmannin is a fungal metabolite that has been characterized as a specific inhibitor of PI3K at nanomolar concentrations (3, 43, 50). LY-294002 is another specific inhibitor of PI3K at low micromolar concentrations, but it has no inhibitory effect against phosphatidylinositol 4-kinase or a number of intracellular serine/threonine or tyrosine kinases at a 50 µM concentration (50, 60). As shown in Fig. 7, both wortmannin (100 nM) and LY-294002 (50 µM) completely inhibited ANG II-stimulated kinase activity of Akt/PKB. These inhibitors also inhibited Akt/PKB...
Akt/PKB in signaling pathways for the Gq protein in VSMC. Little is known regarding the participation of Gi protein-coupled thrombin receptors in platelets (15), PKB activation. These reports include the activation by G-protein-coupled receptors, and only a few reports have demonstrated the G protein-coupled, receptor-mediated Akt/PKB activation. These reports include the activation by G, protein-coupled thrombin receptors in platelets (15), Gs and Gi protein-coupled β3-adrenoreceptors in adipocytes (39), and G protein-coupled receptors in phagocytes (54). In VSMC, ANG II-induced Akt/PKB activation was pertussis toxin insensitive, implying that a G protein of the Gs subfamily is involved in AT1 receptor-mediated Akt/PKB activation. Via a G protein of the Gq subfamily, AT1-receptor activation leads to the production of two second messengers, inositol trisphosphate and diacylglycerol, that induce the release of Ca²⁺ from intracellular storage and PKC activation. The activity of Akt/PKB was stimulated by the Ca²⁺ ionophore ionomycin but not by the PKC-activating phorbol ester PMA. These results suggest that Ca²⁺, but not phorbol ester-responsive PKC, was involved in ANG II-stimulated Akt/PKB activation, although it is still possible that activation of Akt/PKB could occur via atypical PKC, which is not activated by PMA in VSMC (42). However, blockade of Ca²⁺ mobilization by BAPTA-AM, which effectively inhibited ionomycin-induced Akt/PKB activation, only partially inhibited ANG II-stimulated Akt/PKB activation. These results suggest that both Ca²⁺-dependent and -independent mechanisms may be involved in ANG II-stimulated Akt/PKB activation in VSMC.

ANG II-stimulated Akt/PKB activation was inhibited by the tyrosine kinase inhibitors genistein and herbimycin A, suggesting that tyrosine phosphorylation may be involved in this process. It has been shown that activated forms of c-Src, v-Src, or SrcY527F activate Akt/PKB in fibroblasts (10) and that ANG II activates c-Src in VSMC (24), suggesting the possible involvement of c-Src in ANG II-induced Akt/PKB activation in VSMC. However, the inhibitory effect of genistein, which is a broad-spectrum inhibitor of tyrosine kinases as well as Src-family tyrosine kinases (1), is more potent than that of herbimycin A, which is a relatively specific inhibitor of Src-family tyrosine kinases (17, 59). Thus the responsible tyrosine kinase(s) for ANG II-stimulated Akt/PKB activation remains obscure.

Growth factors such as PDGF and EGF were reported to activate Akt/PKB, and this growth factor-dependent Akt/PKB activation was shown to be prevented by wortmannin, expression of PDGF-receptor mutants that cannot interact with PI3K, and expression of dominant negative mutants of PI3K (2, 7, 16, 31). On the other hand, expression of a constitutive active mutant of PI3K results in the activation of Akt/PKB (15, 30, 38). PI3K is necessary and sufficient for growth factor-dependent activation of Akt/PKB. However, an additional pathway for activation of Akt/PKB that is independent of PI3K could exist, because cellular stress such as heat shock and hyperosmolality, β3-adrenoreceptor stimulation, and elevation of intracellular cAMP level have been shown to activate Akt/PKB through a pathway independent of PI3K (33, 39, 47). Treatment of VSMC with two structurally unrelated PI3K inhibitors, wortmannin and LY-294002, not only completely inhibited ANG II-stimulated activation of Akt/PKB but also reduced the basal kinase activity of Akt/PKB. These results indicate that PI3K activity is necessary for both ANG II-stimulated activa-

**DISCUSSION**

In the present study, we first demonstrated that ANG II stimulated the kinase activity and Ser-473 phosphorylation of Akt/PKB via a G protein-coupled AT1 receptor in VSMC. Little is known regarding the participation of Akt/PKB in signaling pathways for the Gs protein-coupled receptors, and only a few reports have demonstrated the G protein-coupled, receptor-mediated Akt/PKB activation. These reports include the activation by G, protein-coupled thrombin receptors in platelets (15), Gs and Gi protein-coupled β3-adrenoreceptors in adipocytes (39), and G protein-coupled receptors in phagocytes (54). In VSMC, ANG II-induced Akt/PKB activation was pertussis toxin insensitive, implying that a G protein of the Gs subfamily is involved in AT1 receptor-mediated Akt/PKB activation. Via a G protein of the Gq subfamily, AT1-receptor activation leads to the production of two second messengers, inositol trisphosphate and diacylglycerol, that induce the release of Ca²⁺ from intracellular storage and PKC activation. The activity of Akt/PKB was stimulated by the Ca²⁺ ionophore ionomycin but not by the PKC-activating phorbol ester PMA. These results suggest that Ca²⁺, but not phorbol ester-responsive PKC, was involved in ANG II-stimulated Akt/PKB activation, although it is still possible that activation of Akt/PKB could occur via atypical PKC, which is not activated by PMA in VSMC (42). However, blockade of Ca²⁺ mobilization by BAPTA-AM, which effectively inhibited ionomycin-induced Akt/PKB activation, only partially inhibited ANG II-stimulated Akt/PKB activation. These results suggest that both Ca²⁺-dependent and -independent mechanisms may be involved in ANG II-stimulated Akt/PKB activation in VSMC.

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**Fig. 7. Effects of phosphatidylinositol 3-kinase inhibitors on kinase activity of Akt/PKB stimulated by ANG II, PDGF, or ionomycin.**}

Cultured VSMC were pretreated with or without 50 µM LY-294002 or 100 nM wortmannin for 20 min. Cells were subsequently treated with 100 nM ANG II, 20 ng/ml PDGF, or 1 µM ionomycin for 5 min. A: anti-Akt/PKB immunoprecipitates were subjected to in vitro kinase assay with histone H2B as a substrate. Experiment represents 1 of 3 independent trials that gave nearly identical results. B: radioactivities of phosphorylated histone H2B were quantitated. Values are means ± SE of 3 independent trials and are expressed as percentages of unstimulated levels. *P < 0.05 vs. control. †P < 0.05 vs. ANG II-stimulated Akt/PKB activation. ‡P < 0.05 vs. PDGF-stimulated Akt/PKB activation. §P < 0.05 vs. ionomycin-stimulated Akt/PKB activation.

**A**}

**B**

Akt/PKB activity (%) of Control

Control ANG II PDGF Ionomycin Control ANG II PDGF Ionomycin Control ANG II PDGF Ionomycin Control ANG II PDGF Ionomycin

LY-294002 Wortmannin

LY-294002 Wortmannin

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tion of Akt/PKB and maintenance of basal Akt/PKB kinase activity in VSMC. Furthermore, Akt/PKB activation by PDGF or ionomycin was significantly inhibited by these inhibitors, although there existed a small degree of increase by PDGF in Akt/PKB activity in wortmannin-treated cells. These results indicate a predominant role of PI3K in both PDGF- and ionomycin-induced Akt/PKB activation in VSMC, whereas it is possible that a wortmannin-insensitive mechanism may be partially involved in PDGF-induced Akt/PKB activation.

At present, the exact role of Akt/PKB in ANG II action is unclear. It is becoming apparent that Akt/PKB plays various roles in cell regulation, including growth-factor-induced survival and growth promotion (11, 22). Therefore, it is possible that ANG II-stimulated Akt/PKB activation has a potential role in the processes that regulate VSMC survival and hypertrophy, although we can only speculate at this point.

In summary, the results of the present study provide the first demonstration of a potential role of Akt/PKB in ANG II actions in VSMC. Identification of upstream and downstream elements of Akt/PKB activation will be an important issue in understanding the role of Akt/PKB in ANG II actions and in fully clarifying the signaling pathways of ANG II in VSMC.

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