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Angiotensin II Stimulates Protein Kinase D–Dependent Histone Deacetylase 5 Phosphorylation and Nuclear Export Leading to Vascular Smooth Muscle Cell Hypertrophy

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Abstract

Background—Angiotensin II (Ang II) induces the phenotypic modulation and hypertrophy of vascular smooth muscle cells (VSMCs), which is implicated in the pathogenesis of hypertension, atherosclerosis, and diabetes. In this study, we tested the hypothesis that histone deacetylases 5 (HDAC5) and its signal pathway play a role in Ang II–induced VSMC hypertrophy.

Methods and Results—VSMCs were isolated from the thoracic aortas of male Sprague-Dawley rats and treated with Ang II. We found that Ang II rapidly stimulated phosphorylation of HDAC5 at Serine259/498 residues in a time- and dose-dependent manner. Ang II receptor-1, protein kinase C, and protein kinase D1 (PKD1) mediated HDAC5 phosphorylation. Furthermore, we observed that Ang II stimulated HDAC5 nuclear export, which was dependent on its PKD1-dependent phosphorylation. Consequently, both inhibiting PKD1 and HDAC5 Serine259/498 to Alanine mutant significantly attenuated Ang II–induced myocyte enhancer factor-2 (MEF2) transcriptional activity and protein synthesis in VSMCs.

Conclusion—These findings demonstrate for the first time that PKD1-dependent HDAC5 phosphorylation and nuclear export mediates Ang II–induced MEF2 activation and VSMC hypertrophy, and suggest that PKD1 and HDAC5 may emerge as potential targets for the treatment of pathological vascular hypertrophy.

Keywords

angiotensin; vascular smooth muscle cells; histone deacetylases 5; protein kinase D; hypertrophy

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Disclosures
None.

The renin-angiotensin system is a central component of the physiological and pathological responses of cardiovascular system.[1,2] Angiotensin II (Ang II), the primary effecting hormone in this system, plays critical roles in mediating cardiovascular diseases such as hypertension, atherosclerosis, and diabetes.[3] Mounting evidence shows that Ang II activation of the Ang II receptor 1 (AT_1) contributes to pathological vascular remodeling, largely by stimulating vascular smooth muscle cells (VSMCs) hypertrophy.[4–7] However, the molecular mechanisms by which Ang II stimulates VSMC hypertrophy are not fully understood.

Histone acetylation/deacetylation has emerged as a fundamental mechanism for the control of gene expression.[8,9] Histone acetyltransferases stimulate transcription through acetylation of histones, resulting in relaxation of nucleosomes; but histone deacetylases (HDACs) deacetylate histone and repress transcription by condensing the chromatin. In particular, class II HDACs have been shown to interact with myocyte enhancer factor 2 (MEF2) and play an important role in the repression of cardiac hypertrophy.[10–13] For example, mutant mice lacking either HDAC5 or HDAC9 develop extremely enlarged hearts in response to pathological signals.[14,15] However, little is known of the role of class II HDACs in VSMC hypertrophy.

PKD1, also called PKCμ, is a newly identified serine/threonine kinase.[16,17] PKD1 is mainly activated by a signal pathway, which involves phospholipase C activation, production of diacylglycerol, and activation of classical/novel protein kinase C (PKC).[16] PKC-mediated phosphorylation of 2 conserved serine residues (Ser744 and Ser748) in the activation loop of PKD1 is essential for its activation.[18] PKD1 activation results in its autophosphorylation at the Ser916 site.[16] In addition, binding of diacylglycerol to the regulatory domain of PKD1 contributes both to PKD1 activation and to PKD1 subcellular localization.[16] Important discoveries have been made regarding the roles of PKD1 in cell growth, survival, motility, and protein trafficking.[16,19,20] Most recently it has been proposed that PKD1 may control gene transcription via the regulation of class II HDACs in T lymphocytes and in cardiac cells.[21–23] However, the specific substrates and function for PKD1 in VSMCs remain unclear.

Here, we describe that Ang II rapidly stimulates HDAC5 phosphorylation in rat aortic VSMCs. Moreover, PKD1, which is activated in a PKC-dependent manner after Ang II stimulation, mediates HDAC5 phosphorylation that subsequently leads to HDAC5 nuclear export and to the MEF2 dependent transcriptional activation. Based on our findings, we suggest that PKD1 and HDAC5 are implicated in Ang II–induced VSMC hypertrophy.

Materials and Methods

The materials and methods used in this study are fully described in the supplemental material (available online at http://atvb.ahajournals.org). Briefly, primary cultures of VSMCs were obtained from the thoracic aortas of male Sprague-Dawley rats. Growth arrested VSMCs were stimulated with Ang II as indicated. Western Blot Analysis was performed in total cell lysates, and HDAC5 subcellular localization study was performed in

VSMCs infected with GFPtagged HDAC5 using a fluorescence microscope. VSMC hypertrophy was analyzed by [^{3}H]leucine incorporation.

Results

Ang II Induces HDAC5 Phosphorylation in VSMCs

To examine the potential role of HDAC5 in Ang II signaling and function, we first studied the phosphorylation of HDAC5 at Ser259/498 residues in VSMCs in response to Ang II stimulation. Phosphorylation of HDAC5 was determined by using a phosphospecific HDAC5 antibody, which recognizes HDAC5 only when phosphorylated at Ser259/498.[21] Exposure to VSMCs to Ang II (100 nmol/L) rapidly induced phosphorylation of HDAC5 within 45 seconds (Figure 1A). HDAC5 phosphorylation reached a maximum between 2 and 40 minutes and returned to basal line after 90 minutes (Figure 1A). This response was dose-dependent, with a threshold of 1 nmol/L and a maximum effect occurring at 100 nmol/L Ang II (Figure 1B). The levels of HDAC5 and β-actin expression were detected by Western blots using the antibodies for HDAC5 and β-actin, respectively. During the course of Ang II stimulation, there was no significant change of HDAC5 expression in VSMCs, and the levels of β-actin showed the equal loading in each samples (Figure 1).

AT1 Receptor Mediates Ang II–Induced HDAC5 Phosphorylation

Two types of Ang II receptors, AT_1 and AT_2, were identified in VSMCs, which belong to the superfamily of G protein– coupled receptors. To determine which subtype of Ang II receptors mediates HDAC5 phosphorylation in VSMCs, we examined the effect of specific receptor antagonists on HDAC5 phosphorylation. Cells were pretreated for 30 minutes with either losartan (5 μmol/L), a specific antagonist for AT1, or PD123319 (10 μmol/L), an antagonist for AT2, and then stimulated with Ang II (100 nmol/L) for 5 minutes. As shown in Figure 2A, losartan completely blocked Ang II– induced HDAC5 phosphorylation, whereas PD123319 had no effect. These results suggest that AT1, but not AT2, mediates Ang II–induced HDAC5 phosphorylation in rat VSMCs.

PKCs Are Involved in Ang II–Induced HDAC5 Phosphorylation

To determine whether AT1-mediated PKC activation is involved in Ang II–induced HDAC5 phosphorylation in VSMCs, we examined the effect of PKC inhibitors on HDAC5 phosphorylation. Quiescent cells were pretreated with general PKC inhibitors GF109203X (0.3, 1, 3 μmol/L) or Gö6983 (0.3, 1, 3 μmol/L) for 30 minutes before exposure to Ang II (100 nmol/L) for 5 minutes. As shown in Figure2B and 2C, both GF109203X and Gö6983 dose-dependently blocked HDAC5 phosphorylation. In addition, we found that the phosphatidylinositol-3-kinase (PI3K) inhibitors wortmannin and LY294002, calcium chelator BAPTA/AM, or calmodulin kinase (CaMK) inhibitors KN62 and KN93, had no effects on Ang II–induced HDAC5 phosphorylation (data not shown). Together, these results suggest that PKC, but not PI3K- and calcium-dependent signal pathways, is involved in the Ang II–stimulated HDAC5 phosphorylation in VSMCs.

PKD Specifically Mediates Ang II–Induced HDAC5 Phosphorylation

Ang II Induces PKC-Dependent PKD Activation in VSMCs—Because PKC activation leads to PKD phosphorylation and activation in several cell types,[16] we decided to examine the potential role of PKD in Ang II–induced HDAC5 phosphorylation in VSMCs. We first observed that Ang II induced PKD1 phosphorylation both at Ser744/748 (activation sites) and at Ser916 (autophosphorylation site) in a time- and dose-dependent manner (Figure3A and 3B), which is resembled to the patterns of HDAC5 phosphorylation (Figure 1). Furthermore, PKC inhibitors GF109203X and Gö6983 dose-dependently inhibited Ang II–stimulated PKD1 activation (Figure3C and 3D), which is consistent with the notion that PKD1 activation is PKC-dependent.[16] Again, PI3K- and calcium-dependent signal pathways are not involved in the Ang II– stimulated PKD1 activation in VSMCs (data not shown).

PKD Inhibitor Gö6976 Blocked Ang II–Induced HDAC5 Phosphorylation—The rapid and prominent phosphorylation of HDAC5 and PKD1 by Ang II prompted us to examine whether activation of PKD1 contributed to Ang II–induced HADC5 phosphorylation. Gö6976 has been reported to inhibit both PKD1 activation and calcium-dependent PKC activation.[24] Because we have shown that Ang II–induced PKD activation and HDAC5 phosphorylation are calcium-independent, Gö6976 is useful as PKD inhibitor for our studies. VSMCs were pretreated with various concentrations of Gö6976 for 30 minutes, followed by stimulation with Ang II (100 nmol/L) for 5 minutes. As shown in Figure 3E, Gö6976 dose-dependently inhibited Ang II– triggered HDAC5 phosphorylation in VSMCs, suggesting that PKD1 is involved in this process.

Knockdown PKD1 by siRNA Attenuated Ang II–Induced HDAC5 Phosphorylation—To substantiate the role of PKD1 in Ang II–induced HDAC5 phosphorylation, we knocked down endogenous PKD1 in VSMCs using siRNA. Transfection of PKD1 siRNA in rat VSMCs significantly reduced PKD1 protein expression without affecting the expression of HDAC5 and β-actin (Figure 3F). Silencing PKD1 by siRNA significantly inhibited Ang II– induced HDAC5 phosphorylation (Figure 3F), indicating that PKD1 is required for HDAC5 phosphorylation by Ang II in VSMCs.

PKD1 Kinase-Negative Mutant Inhibited Ang II–Induced HDAC5 Phosphorylation—To further determine whether PKD1 activation mediates HDAC5 phosphorylation by Ang II, we generated and tested adenovirus expressing GFP-tagged PKD1 kinase-negative mutant (GFP-PKD1-KN) on Ang II–induced HDAC5 phosphorylation. As shown in Figure 3G, infection of VSMCs with adenoviruses encoding GFP-PKD1-KN resulted in robust expression of GFP-PKD1-KN. Ang II–induced HDAC5 phosphorylation was significantly inhibited by GFPPKD1- KN overexpression, whereas a control adenovirus encoding GFP had no effect. In addition, to demonstrate PKD1 is downstream of PKC signaling, we examined whether PMA-induced phosphorylation and nuclear export of HDAC5 are attenuated by inhibition of PKD1. Indeed, overexpression of GFP-PKD1-KN markedly decreased PMA-induced phosphorylation (supplemental data). Taken together, these results strongly suggest that PKD1 mediates Ang II–induced HDAC5 phosphorylation in VSMCs.

Ang II Stimulates HDAC5 Nuclear Export via PKD-Dependent Phosphorylation

To determine the consequence of HDAC5 phosphorylation, we studied the effect of Ang II on HDAC5 subcellular localization in VSMCs. We infected VSMCs with adenovirus expressing GFP-tagged HDAC5-WT. As shown in Figure 4A, before treatment of Ang II, GFP-HDAC5 was located primarily in the nuclei of VSMCs, which allow us to conveniently assess the possible nuclear export of HDAC5. HDAC5 nuclear export was seen at 1 hour after Ang II stimulation (Figure 4A). Striking nuclear export of HDAC5 in the cells was observed by 2 hours after addition of Ang II and maintained for several hours (Figure 4A). After 6 hours of Ang II treatment, GFP-HDAC5 was gradually shuttled back to the nuclei from cytoplasm (Figure 4A). These results clearly demonstrate that Ang II stimulates the nucleocytoplasmic shuttling of HDAC5 in VSMCs.

To further define the signaling pathways leading to Ang II–induced nuclear export of HDAC5, we examined the effects of PKC inhibitors and activators on nuclear export of HDAC5 in VSMCs. Inhibition of PKCs by GF109203X and Gö6983 blocked Ang II–induced HDAC5 nuclear export, whereas PKC activator PMA (200 nmol/L for 3 hours treatment) strongly stimulated HDAC5 nuclear export (Figure 4B), suggesting PKCs are involved in HDAC5 nuclear export in VSMCs. Consistent with the critical role of PKD1 in Ang II–induced HDAC5 phosphorylation, PKD inhibitor Gö6976 (1 μmol/L) also blocked Ang II–induced HDAC5 nuclear export (Figure 4B). Taken together, these results suggest that a PKC-PKD1–dependent pathway stimulates nuclear export of HDAC5 in VSMCs.

To determine whether the phosphorylation of HDAC5 at Ser259/498 residues is required for Ang II–induced HDAC5 nuclear export, we studied subcellular localization of GFPHDAC5-S/A mutant. VSMCs were infected with adenoviruses encoding GFP, GFP-HDAC5-WT, or GFP-HDAC5-S/A. In basal condition without Ang II stimulation, both GFPHDAC5-WT and GFP-HDAC5-S/A were localized in the nuclei of VSMCs, whereas GFP alone was distributed in both nuclei and cytoplasm (Figure 4C). After Ang II stimulation for 3 hours, GFP-HDAC5-WT was shuttled to cytoplasm from nuclei (Figure 4C). In contrast, GFP-HDAC5-S/A remained in the nuclei after Ang II stimulation, suggesting the requisite role of phosphorylation at Ser259/498 residues for HDAC5 nuclear export (Figure 4C). No significant localization change of GFP was observed in the cells transfected with adenovirus encoding GFP, verifying the specificity for HDAC5 nucleocytoplasmic shuttling in response to Ang II. Moreover, knocking down PKD1 expression by siRNA also greatly attenuated PMA-induced nuclear export of HDAC5 in VSMCs (supplemental data).

PKD1 and HDAC5 Are Involved in Ang II– Induced MEF2 Transcriptional Activity

To determine the potential role of HDAC5 in Ang II regulation of MEF2 transcriptional activation in VSMCs, we transfected VSMCs with 3×MEF2-luciferase reporter plasmid and then infected adenovirus encoding LacZ or Flagtagged HDAC5-S/A. Ang II significantly increased MEF2 transcriptional activity in VSMCs (Figure 5A). Interestingly, HADC5-S/A mutant abolished such increase of MEF2 transcriptional activation by Ang II (Figure 5A), suggesting nuclear-retaining HDAC5 negatively regulates Ang II–stimulated MEF2 transcriptional activity. Furthermore, PKD1 was also involved in Ang II–induced MEF2 transcriptional activity because pretreatment of the cells with PKD inhibitor Gö6976 for 30 minutes significantly inhibited Ang II–induced MEF2 transcriptional activation in VSMCs (Figure 5B).

PKD1 and HDAC5 Are Implicated in Ang II–Stimulated VSMC Hypertrophy

To further gain insights into the functional role of PKD1 and HDAC5 in Ang II signaling, we examined whether PKD1 and HDAC5 are involved in Ang II–stimulated VSMC hypertrophy. VSMCs were infected cells with adenoviruses encoding GFP, GFP-HDAC5-S/A or GFP-PKD1-KN, or pretreated cells with PKD inhibitor Gö6976. As shown in Figure 6, Ang II significantly increased [^{3}H]leucine incorporation. Overexpression of GFP-HDAC5-S/A and GFP-PKD1-KN significantly inhibited Ang II–stimulated [^{3}H]leucine incorporation (Figure6A and 6B). Similarly, inhibiting PKD1 by Gö6976 suppressed Ang II–induced [^{3}H]leucine incorporation (Figure 6C). These results suggest that PKD1 and HDAC5 play an important role for Ang II–induced VSMC hypertrophy.

Discussion

The present study demonstrates that Ang II induces HDAC5 phosphorylation and nuclear export via PKC-PKD1 pathway in VSMCs, which results in an increase of MEF2 transcriptional activity and consequent VSMC hypertrophy. First, we showed that Ang II rapidly and strongly stimulated HDAC5 phosphorylation at Ser259/498 residues in a time- and dose-dependent manner in rat VSMCs. Furthermore, Ang II– induced HDAC5 phosphorylation is mediated through a signal pathway that involves AT1 receptor, PKC and PKD1, and this pathway plays a pivotal role for HDAC5 nuclear export and MEF2 transcriptional activation. Importantly, PKD1- and HDAC5-dependent responses contribute to regulation of Ang II–induced [^{3}H]leucine incorporation into VSMCs to cause cell hypertrophy. In addition, we also found that PKD1-HDAC5 are involved in Ang II–induced smooth muscle α-actin expression (supplemental data). These cumulative observations for the first time reveal a novel role of PKD1 and HDAC5 in Ang II–induced signal transduction and VSMC hypertrophy, which may represent an important mechanism for Ang II effects on vascular remodeling observed in animal models and in human.

Acetylation of chromatin proteins and transcription factors is part of a complex signaling system that is largely involved in the control of gene expression.[25] Histone acetyltransferases and HDACs act in an opposing manner to control the acetylation state of nucleosomal histones.[26,27] The present study uncovers a new role of HDAC5 as a key regulator for Ang II–induced VSMC hypertrophy. We found that Ang II promotes phosphorylation of two serine 259/498 residues in HDAC5, which have been shown to be the docking sites for the 14-3-3 chaperone protein.[28–30] Binding of 14-3-3 to HDAC5 may disrupt its association with MEF2 transcriptional factors and triggers HDAC5 export from the nucleus to the cytoplasm, thus freeing MEF2 to activate subordinate genes that govern VSMC hypertrophic growth.[21,30–32] Inconsistent with this notion, we observed that Ang II induced HDAC5 translocation from the nuclei to cytoplasm, and increased MEF2 transcriptional activity. Mutation of these serine sites to alanine (HDAC5-S/A mutant) blocked HDAC5 nucleocytoplasmic shuttling and MEF2 transcriptional activation in response to Ang II. Furthermore, HDAC5-S/A mutant inhibited Ang II–stimulated increase of [^{3}H]leucine incorporation in VSMCs. It has been shown that the induction of smooth muscle α-actin is involved in Ang II–induced VSMC hypertrophy.[6] Our supplemental data showed that smooth muscle α-actin expression is regulated by PKD1-HDAC5 pathway because both PKD1-KN and HDAC5-S/A attenuated an increase of smooth muscle α-actin mRNA in VSMCs in response to Ang II (supplemental data). These results are consistent with recent report that HDAC5 is involved in platelet-derived growth factor-BB–induced suppression of smooth muscle α-actin expression in VSMCs.[33] Expression of smooth muscle α-actin is controlled by the transcriptional factor, serum response factor, and its coactivator, myocardin.[33] Since HDAC5 directly interacts and inhibits myocardin,[34] the dissociation of myocardin from HDAC5 through PKD1-dependent phosphorylation and nuclear export of HDAC5 might be one of possible mechanisms by which Ang II stimulates smooth muscle α-actin expression. Further studies are needed to understand a potential role of PKD1-HDAC5 pathway in the transcriptional regulation of hypertrophic genes, in particular MEF2-dependent genes. Collectively, our findings demonstrate that the signal-resistant form of HDAC5 functions as a potent repressor of Ang II–induced VSMC hypertrophy, suggesting that phosphorylation of HDAC5 is a requisite step in the process of derepressing VSMC growth genes and that the antihypertrophic action of endogenous HDAC5 in VSMCs is overcome by Ang II–signaling pathways that culminate in nuclear export of this transcriptional repressor in VSMCs.

PKD1 is a member of a family of cytosolic serine/ threonine protein kinases and highly expressed in vascular cells.[20] However, the functional roles of PKD1 in vascular cells are largely unknown. In present study, we showed for the first time that PKD1 mediates Ang II–induced HDAC5 phosphorylation and nuclear export in VSMCs and suggest that PKD1 is implicated in VSMC hypertrophy. The critical role of PKD1 for HDAC5 phosphorylation by Ang II was tested with 3 different strategies in our studies. Either pretreatment cells with PKD inhibitor Gö6976, or siRNA knockdown of PKD1 expression, or infection of adenovirus expressing PKD1-KN, blocked Ang II–induced HDAC5 phosphorylation. Inhibiting PKD1 also abolished Ang II– stimulated HDAC5 nuclear export, MEF2 transcriptional activity, and [^{3}H]leucine incorporation in VSMCs. These results strongly suggest that PKD1 plays a critical role in HDAC5 phosphorylation to VSMC hypertrophy in response to Ang II. In agreement with PKC-dependent PKD1 activation, we also showed that PKC inhibitors GF109203X and Gö6983 blocked PKD1 activation, and abolished Ang II– induced HDAC5 phosphorylation and nuclear export. Moreover, we found that PMA-induced phosphorylation and nuclear export of HDAC5 were attenuated by inhibition of PKD1 (supplemental data), further demonstrating PKD1 is downstream of PKC signaling. In addition, we observed that Ang II– dependent phosphorylation of HDAC5 in VSMCs is resistant to calcium chelator BAPTA/AM, CaMK inhibitors KN93 and KN62. Collectively, our results suggest that the PKC-PKD1 pathway, but not the calcium-dependent CaMK pathway, is involved in Ang II–induced HDAC5 phosphorylation in VSMCs. Taken together, our present findings suggest that PKD1 is critical for mediating Ang II–induced HDAC5 phosphorylation and VSMC hypertrophy.

In summary, we have demonstrated that Ang II induced PKD1-dependent HDAC5 phosphorylation and nuclear export, and PKD1-HDAC5 pathway is involved in Ang II–stimulated MEF2 transcriptional activity and VSMC hypertrophy. Further studies to define the specific genes regulated by PKD1-HDAC5 pathway and the functional role of PKD1 and HDAC5 in experimental animal models promise to provide new insight into the molecular underpinnings of pathological vascular remodeling in hypertension, atherosclerosis, and diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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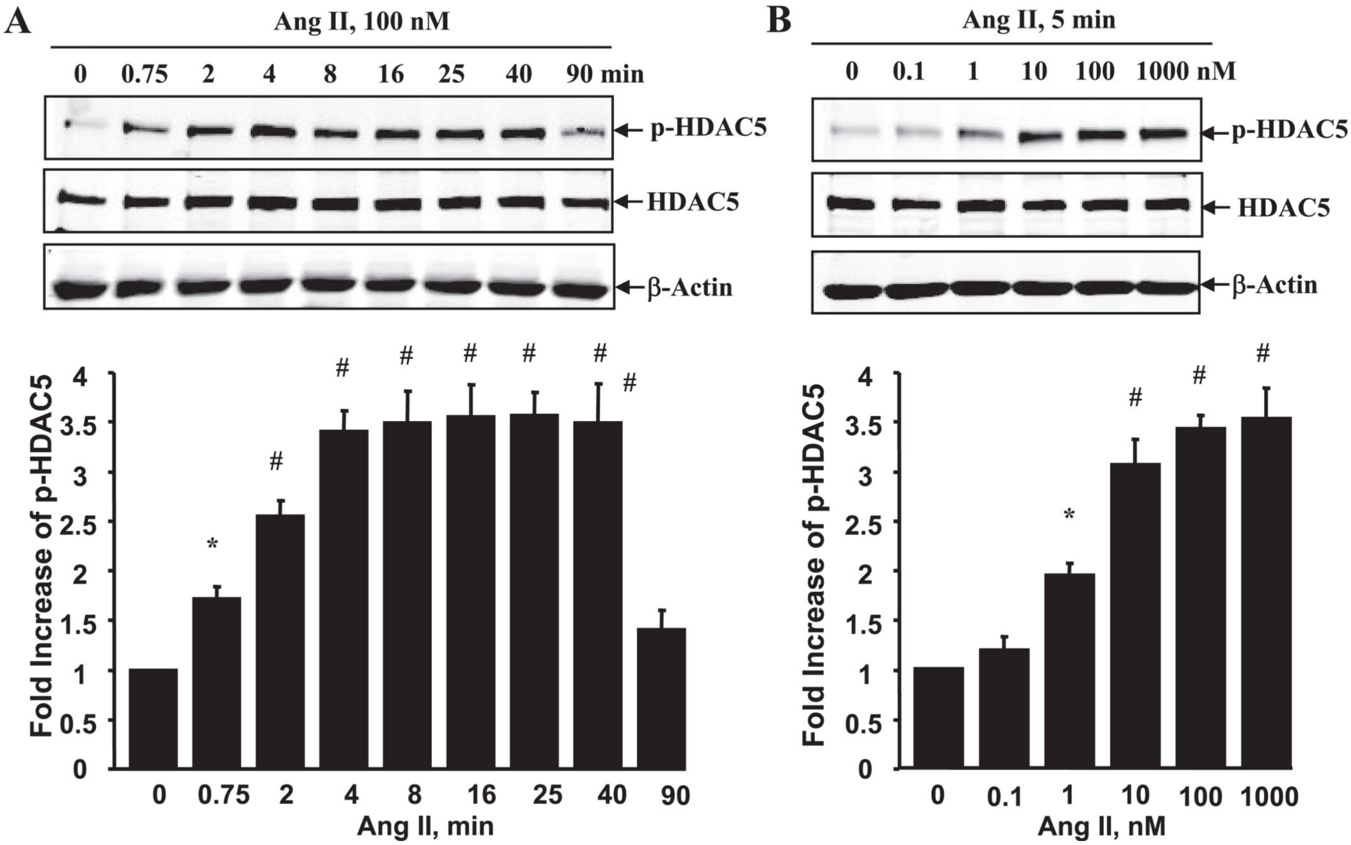

Figure 1.

Ang II stimulates HDAC5 phosphorylation in VSMCs. Rat VSMCs were stimulated with Ang II for various times (A) or at different doses (B). The phosphorylation of HDAC5 (p-HDAC5), expression levels of HDAC5, and β-actin in cell lysates were analyzed by Western blotting. Representative Western blots and statistic data were shown (n=4).
*P<0.05; $^{\#}P$<0.01.

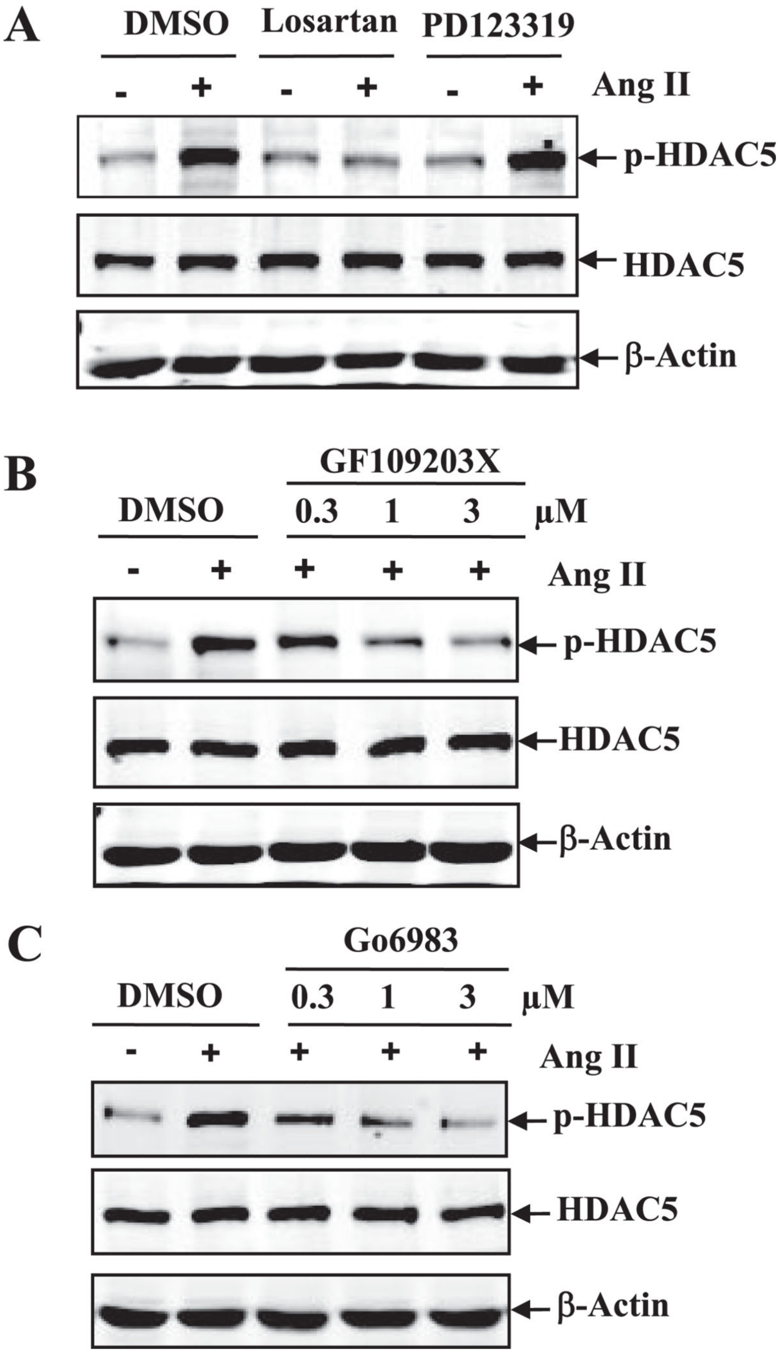

Figure 2.

Ang II receptor AT1 and protein kinase C mediate Ang II–induced HDAC5 phosphorylation. VSMCs were pretreated with Losartan or PD123319 (A), or with different doses of GF109203X (B) or Gö 6983 (C), and then stimulated with Ang II. Representative Western blots (n=3) showed the phosphorylation of HDAC5, expression levels of HDAC5, and β-actin.

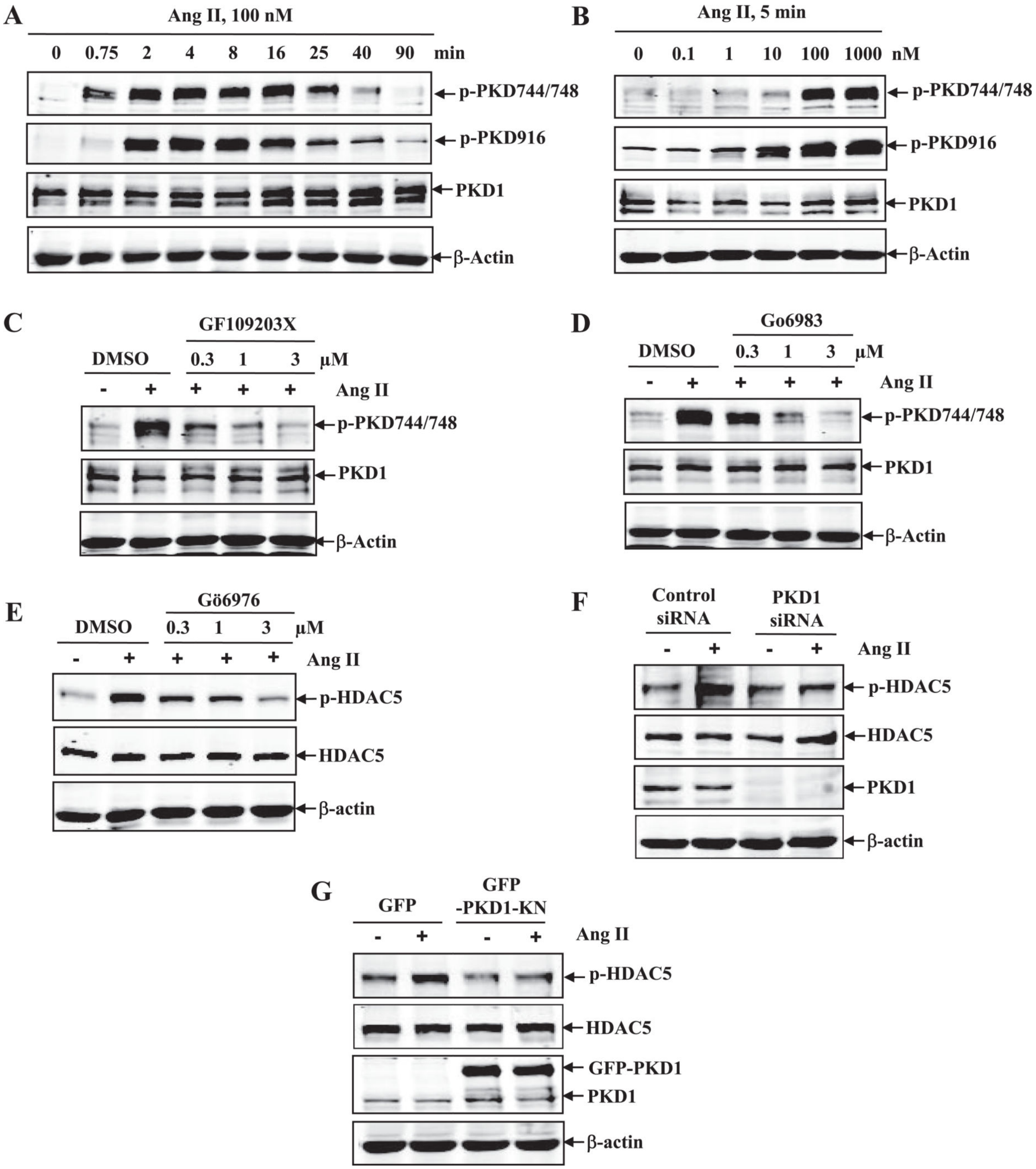

Figure 3.

PKD1 mediates Ang II–stimulated HDAC5 phosphorylation. A–D, VSMCs were stimulated with Ang II in absence or presence of the inhibitors, and Western blots revealed Ang II–induced PKD1 phosphorylation. E–G, VSMCs were pretreated with Gö 6976 (E), or transfected with scrambled siRNA (control) or PKD1 siRNA (F), or infected with adenoviruses encoding GFP alone or GFP-PKD1-KN (G), and then stimulated with Ang II. Western blots (n=3) showed the phosphorylation of HDAC5, expression levels of HDAC5, PKD1, and β-actin.

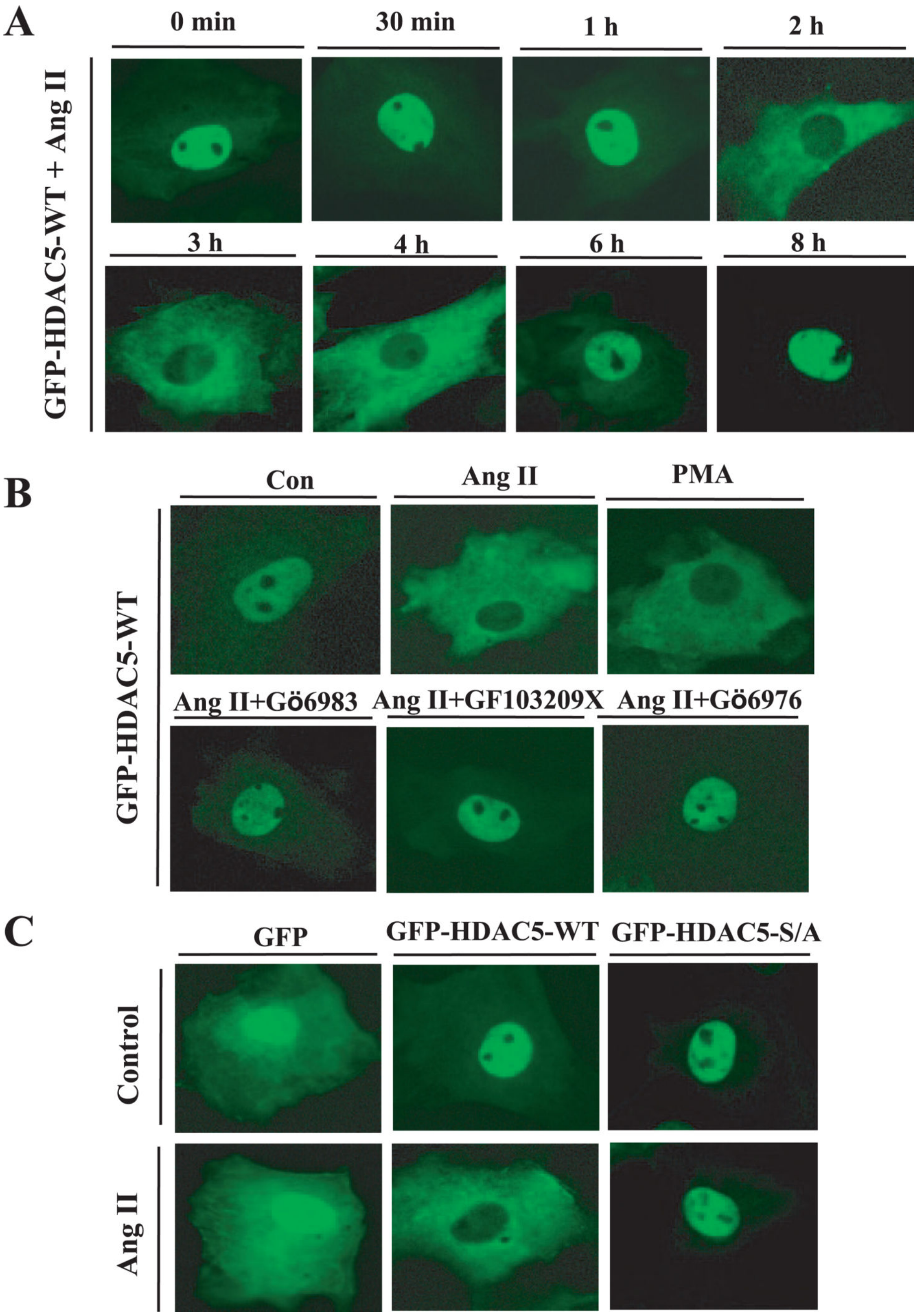

Figure 4.

Ang II stimulates HDAC5 nuclear export through PKC-PKD pathway. VSMCs were infected with adenoviruses encoding GFP-HDAC5 (A and B), and pretreated with the various inhibitors (B), or cells were infected with adenoviruses encoding GFP alone, GFP-HDAC5-WT, or GFP-HDAC5-S/A mutant (C), and then stimulated with Ang II (A-C) or PMA (C). The representative images of GFP fluorescence showed the subcellular localization of the proteins (n=4, magnification, ×60).

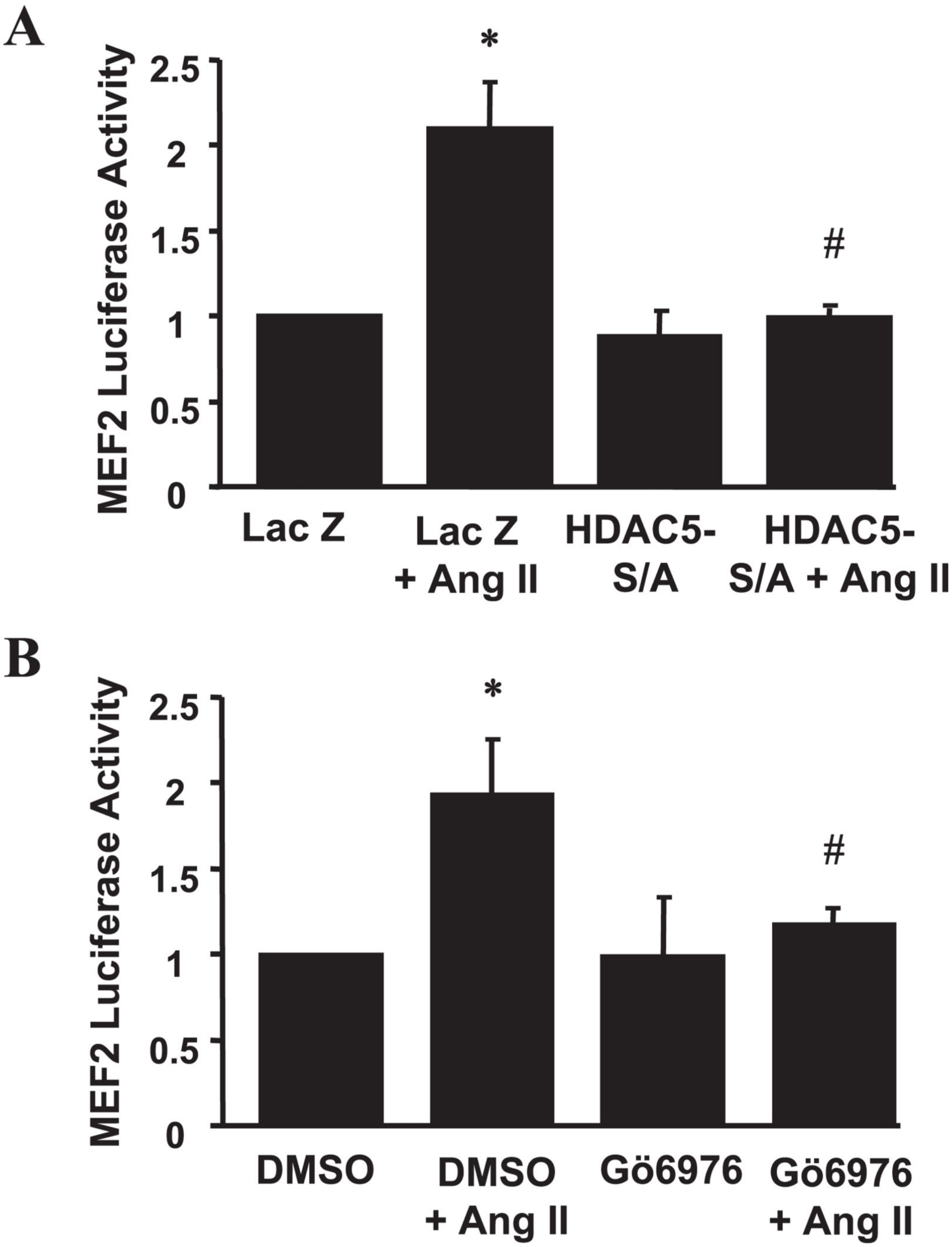

Figure 5.

Ang II–stimulated MEF2 transcriptional activity is PKD- and HDAC5-dependent. VSMCs were transfected with 3×MEF2-luciferase report gene and then infected with adenoviruses encoding LacZ or Flag-HDAC5-S/A mutant (A), or treated with Gö 6976 (B), followed with the stimulation of Ang II. MEF2 luciferase activity was determined (n=4). *$P<0.05$ vs the control without Ang II stimulation; $^{\#}P<0.05$ vs the group treated with Ang II alone.

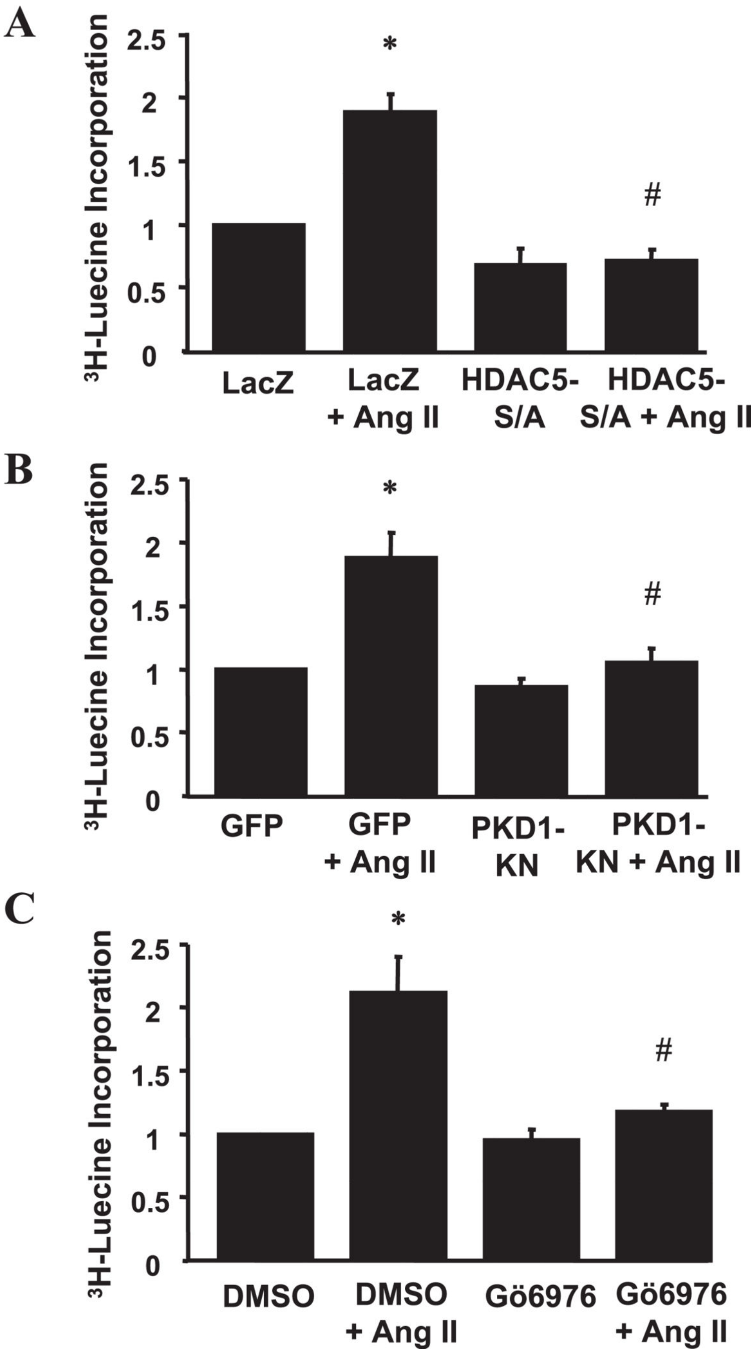

Figure 6.

PKD and HDAC5 are involved in Ang II–stimulated VSMC hypertrophy. A, VSMCs were infected with adenoviruses encoding LacZ or Flag-HDAC5-S/A (A), GFP alone or GFPPKD1-KN (B), or pretreated with Gö 6976 (C), and then incubated with [^{3}H]leucine in the presence or absence of Ang II. [^{3}H]leucine uptake in cells was analyzed (n=4), *P<0.05 vs the control without Ang II stimulation; $^\#P$<0.05 vs the group treated with Ang II alone.