Full Paper

Angiotensin II Directly Triggers Endothelial Exocytosis via Protein Kinase C-Dependent Protein Kinase D2 ActivationXiaona Ge¹, Brad Low¹, Mei Liang¹, and Jian Fu^{1,*}¹Center for Biomedical Research, University of Texas Health Center at Tyler, Tyler, Texas 75708, USA

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Abstract. Angiotensin II (AII) has been reported to induce leukocyte adhesion to endothelium through up-regulation of P-selectin surface expression. However, the underlying molecular and cellular mechanisms remain unknown. P-selectin is stored in Weibel-Palade bodies (WPBs), large secretory granules, in endothelial cells. In this study, we examined the role of protein kinase D (PKD), a newly identified regulator of protein transport, in AII-induced WPB exocytosis and the resultant P-selectin surface expression. We demonstrated that PKD2 was rapidly activated by AII in endothelial cells through phosphorylation of the activation loop at Ser744/748. AII-induced PKD2 activation correlated with increased P-selectin surface expression. Furthermore, AII-regulated PKD2 activation is protein kinase C (PKC) α -dependent. Importantly, knock-down of either PKD2 or PKC α expression inhibited AII-mediated P-selectin surface expression and monocyte adhesion. Our findings provide the first evidence that stimulation of P-selectin surface expression via PKC α -dependent PKD2 activation could be an important mechanism in the early onset of AII-initiated endothelial adhesiveness.

Keywords: angiotensin, P-selectin, exocytosis, endothelial cell, protein kinase D (PKD)

Introduction

Angiotensin II (AII), a main effector of the renin-angiotensin system (1), has been known to regulate blood pressure and control fluid/salt balance. However, in the last several years, there has been increasing evidence indicating that AII may also function as a potent pro-inflammatory mediator to induce vascular inflammation, which may lead to the pathogenesis of cardiovascular diseases such as atherosclerosis, myocardial infarction and stroke (2 – 4).

Cell adhesion molecules are key players in vascular inflammation due to their important roles in mediating extravasation of leukocytes (5). The early tethering and rolling of leukocytes along the endothelium require P-selectin surface expression (6). AII perfusion was reported to directly induce rapid and P-selectin-dependent leukocyte rolling, adhesion, and transmigration across the endothelium (7, 8). However, the mechanism of AII-stimulated P-selectin surface expression in endo-

thelial cells is still not elucidated. Leukocyte recruitment to the endothelium has been observed within minutes following infusion with AII (8). Since *de novo* protein synthesis and transport to the plasma membrane usually take hours, the early endothelial adhesiveness induced by AII is likely due to rapid release of P-selectin from pre-formed intracellular stores. Indeed, in endothelial cells, P-selectin is stored in secretory granules such as Weibel-Palade bodies (WPBs) (9). WPBs are endothelial specific secretory granules containing P-selectin and other pro-inflammatory mediators including von Willebrand factor (vWF), IL-8, eotaxin-3, and so on (9). Rapid WPB exocytosis induced by pro-inflammatory stimuli such as thrombin, histamine, and hypoxia plays pivotal roles in thrombosis and inflammation (10, 11). In the present study we examined the effects of AII on endothelial exocytosis and the resultant P-selectin surface expression.

AII has been reported to induce protein kinase C (PKC)-dependent protein kinase D (PKD) activation in several cell types (12 – 14). PKD has recently emerged as an important regulator of membrane trafficking (15 – 20). Studies from several groups have shown that PKD plays important roles in spatial and temporal

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regulation of protein transport and recycling at the plasma membrane (15–20). However, the role of PKD in endothelial membrane trafficking and the regulation of PKD by pro-inflammatory signaling in endothelial cells remain undetermined. PKD is a newly identified serine/threonine protein kinase subfamily currently containing three members PKD1 (PKC μ), PKD2, and PKD3 (PKC ν) (21). PKD activation is dependent on the phosphorylation of Ser744 and Ser748 at the activation loop (21). Activation of PKD has been shown to regulate a variety of cellular responses, including secretion, migration, proliferation, and cell survival (16, 22–24). Signaling through G-protein-coupled receptor has been reported to mediate the phosphorylation of PKD at the activation loop (25–27). Here, we specifically examined the role of PKD in AII-induced P-selectin surface expression in endothelial cells.

Materials and Methods

Antibodies and reagents

Mouse anti-P-selectin monoclonal antibody, rabbit anti-PKC α antibody, and goat anti-actin polyclonal antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). P-selectin function-blocking monoclonal antibody was purchased from R&D systems (Minneapolis, MN, USA). Rabbit anti-PKD2 antibody was from Calbiochem (San Diego, CA, USA). AII, AT1-receptor antagonist telmisartan, AT2-receptor antagonist PD123319, and rabbit antibody against vWF were obtained from Sigma (St. Louis, MO, USA). Rabbit anti-PKD (pSer744/748) was from cell signaling technology (Danvers, MA, USA). TMB substrate solutions and SuperSignal West Pico Chemiluminescent Substrate System were obtained from Pierce Biotechnology (Rockford, IL, USA). EGM-2 was from Cambrex (East Rutherford, NJ, USA). RPMI 1640 was from Invitrogen (Carlsbad, CA, USA).

Cell culture

HPAE (human pulmonary artery endothelial cells), HCAEC (human coronary artery endothelial cells), and HLMVEC (human lung microvascular endothelial cells) cells were obtained from Cambrex and grown in EGM-2 supplemented with 10% fetal bovine serum (FBS). Cells were maintained in an incubator at 37°C in 5% CO₂ and 95% air. Cells from passage 4 or 5 were used in the experiments.

Cell surface ELISA

The expression of P-selectin on the cell surface was measured by the cell surface enzyme-linked immunosorbent assay (ELISA) as described by us previously

(28). Briefly, HPAE cells were seeded and cultured in 24-well plates until confluent. After stimulation with various concentrations of AII, cells were fixed immediately with 1% paraformaldehyde for 10 min at room temperature. After 3 washes with PBS and incubation with blocking buffer (3% BSA in PBS) for 15 min, the cells were incubated with mouse monoclonal antibody against P-selectin (1:200 dilution, 45 min at room temperature). Following washes with PBS, cells were incubated with HRP-conjugated goat anti-mouse antibody (1:4000 dilution, 45 min at room temperature). Peroxidase activity was detected using a 3,3',5,5'-tetramethylbenzidine substrate solution (TMB; Pierce, Rockford, IL, USA) and quantified by reading the plates on a spectrophotometric microplate reader at 450 nm. All assays were done with triplicate samples and repeated three times.

Monocyte adhesion assay

Monocyte adhesion assay was performed as described by Patricia et al. (29). Briefly, HPAE cells were seeded (50,000 cells/well) and grown in gelatin-coated 24-well plates until confluent. Media were then replaced with RPMI 1640 containing 1% FBS. After treatment, 1×10^5 THP-1 cells/well were added and allowed to adhere to endothelial cells with gentle rocking for 10 min at 37°C. The endothelial monolayers were then gently washed with PBS to remove unbound monocytes and fixed with 1% paraformaldehyde/1% glutaraldehyde-PBS solution. Bound monocytes were counted by phase-contrast microscopy. Eight microscopic fields were randomly selected for each experiment and quantified as bound monocytes/microscopic field.

Immunoblotting

Immunoblotting was performed as described by us previously (30). Briefly, cells were lysed in a lysis buffer containing 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerolphosphate, 1 mM Na₃VO₄, 1 mM NaF (pH 7.5), and 1% protease inhibitor mixture (Sigma). Protein concentrations were determined. Cell lysates were analyzed by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. After transfer, membranes were blocked by incubating with 5% (w/v) nonfat dry milk in PBS solution with 0.05% Tween20 for 1 h at room temperature or overnight at 4°C. Membranes were then incubated with the indicated antibodies and developed using the enhanced chemiluminescence method (Pierce). Densitometry analysis was carried out using the Quantity One program (Biorad, Hercules, CA, USA).

siRNA transfection of endothelial cells

Validated PKC α siRNA (Catalog number SI00301308) and PKD2 siRNA (Catalog number SI02224768) were obtained from Qiagen (Valencia, CA, USA). Fluorescein-conjugated negative control siRNA was obtained from Invitrogen. HPAE cells were cultured on 0.2% gelatin-coated plates. Twenty-four hours later, cells (50% confluence) were transfected with 10 nM siRNAs using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol. Slight modification of the protocol was made in order to achieve high transfection efficiency. Namely, 1 μ l/well SilenceMag magnetic beads (Origene, Rockville, MD, USA) was added to the mixture containing the siRNAs and Lipofectamine in opti-MEM (Invitrogen). After adding the mixture to the cells, the plates were placed on a magnetic plate for 15 min and then returned to the CO₂ incubator. Two days after the transfection procedure was accomplished, transfected cells were used for immunoblotting, surface ELISA, or monocyte adhesion assay.

Statistics

All values are expressed as means $\pm$ S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni test. A *P* value <0.05 was considered significant.

Results

AII stimulates P-selectin surface expression in endothelial cells

P-selectin is known to localize in large secretory granules, Weibel-Palade bodies (WPBs), in endothelial cells (9). To determine whether AII can induce the rapid release of P-selectin from WPBs, we examined the effects of AII on P-selectin surface expression in HPAE cells. Cell surface ELISA showed that AII treatment increased surface expression of P-selectin in HPAE cells

in a dose- and time-dependent manner (Fig. 1). The increase of P-selectin expression at the cell surface was observed as early as 5 min after AII stimulation and occurred even at low AII concentration (1 nM). The maximum response was achieved at 100 nM AII concentration. The peak level of P-selectin surface accumulation was achieved at 15 min after AII challenge. Surprisingly, AII at 1 μ M concentration had no effect on P-selectin surface expression. This could be due to induction of inhibitory signals on endothelial exocytosis at higher AII concentration or rapid activation of proteases that may remove P-selectin from the cell surface. Since P-selectin is primarily stored in WPBs, our results suggest that AII can stimulate rapid WPB exocytosis in HPAE cells.

AII induces transient PKD2 activation in endothelial cells

PKD, which can be activated by AII signaling (12–14), has recently emerged as a key regulator of protein transport from the trans-Golgi network to the plasma membrane (15–20). To explore the potential role of PKD in AII-triggered WPB exocytosis in endothelial cells, we first examined the expression and regulation of PKD in endothelial cells. The expression of PKD in endothelial cells was examined by using isoform-specific PKD antibodies (anti-PKD1, PKD2, and PKD3). The activity and isoform-specificity of the antibodies were confirmed using GST recombinant fusion proteins of PKD1, PKD2, and PKD3 (data not shown). Only PKD2 expression was detected in endothelial cells (Fig. 2A). Activation of PKD is dependent on the phosphorylation of two activation loop sites, Ser744 and Ser748, via a PKC-dependent signaling pathway. Using the antibody against the phosphorylated ser744 and 748 of PKD2, we showed that AII induced PKD2 phosphorylation in HPAE cells in a time and dose-dependent fashion (Fig. 2: B and C).

Fig. 1. AII stimulates P-selectin surface expression in HPAE cells. HPAE cells were treated with AII as indicated. Surface expression of P-selectin was quantified by a cell surface ELISA following AII treatment as described in Materials and Methods. Data are presented as means $\pm$ S.E.M. An asterisk indicates a value significantly different from that of control cells (Con) without AII treatment, *P*<0.05.

Fig. 2. PKD Expression and AII-induced PKD2 phosphorylation in endothelial cells. A: cell lysates (20 μ g protein/lane) from HPAE (human pulmonary artery endothelial cells), HCAEC (human coronary artery endothelial cells), and HLMVEC (human lung microvascular endothelial cells) were resolved by SDS-PAGE, transferred onto polyvinylidene difluoride membranes, and immunoblotted with anti-PKD2 antibody. Time course (100 nM) (B) and dose-response (15 min) (C) of AII-induced PKD2 phosphorylation in HPAE cells were assessed. HPAE cells were pre-treated with AII as indicated. Cell lysates were resolved by SDS-PAGE, transferred onto polyvinylidene difluoride membranes, and immunoblotted with anti-PKD (pSer744/748) antibody. The same membrane was stripped and blotted with anti-PKD2 antibody. Representative blots and bar graphs show the change of PKD2 phosphorylation. Results shown are representative of three independent experiments. Data are presented as means $\pm$ S.E.M. An asterisk indicates a value significantly different from that of control cells (Con) without AII challenge, $P < 0.05$.

Role of PKD2 in AII-induced P-selectin surface expression in endothelial cells

To confirm the functional role of PKD2 in AII-regulated P-selectin surface expression, we used a siRNA against PKD2 to knock down its expression in HPAE cells. The effect of the siRNA on P-selectin surface expression was examined. PKD2 siRNA transfection specifically reduced the expression of PKD2 in HPAE cells (Fig. 3A). Knock-down of PKD2 expression caused a significant reduction of AII-induced P-selectin surface expression when compared with that of cells transfected with the control siRNA (Fig. 3B). Our results indicate that PKD2 is an important regulator of P-selectin surface expression in endothelial cells.

AT1 receptor mediates AII-induced PKD2 activation and endothelial exocytosis

To identify the subtype of angiotensin II receptors that mediates AII responses in endothelial cells, we pre-treated HPAE cells with selective AT1- or AT2-receptor antagonists, and their effects on PKD2 activation and P-selectin surface expression were then examined. AII-induced PKD2 activation and P-selectin surface expression were markedly decreased by pre-treatment with the AT1-receptor antagonist telmisartan, but not the AT2-

receptor antagonist PD123,319 (Fig. 4). Thus, AT1-receptor signaling is necessary for AII-induced PKD2 activation and P-selectin surface expression. Our findings provide a new mechanism for AT1 receptor-mediated endothelial inflammatory responses.

PKC α -dependent PKD2 phosphorylation and P-selectin surface expression following AII challenge

PKC has been reported to mediate various cellular responses of AII (31–36). We have shown that PKC α activation by thrombin mediates P-selectin surface expression in endothelial cells (28). PKC α is highly expressed in endothelial cells (37). Here, we examined whether AII-induced PKD activation and P-selectin surface expression require PKC α activation in HPAE cells. Transfection of a PKC α -specific siRNA decreased PKC α expression in HPAE cells (Fig. 5A), whereas PKD2 expression was not affected by the transfection. PKD2 activation upon AII stimulation in HPAE cells was inhibited by the siRNA transfection (Fig. 5B), which correlated with decreased P-selectin surface expression (Fig. 5C). Thus, AII-induced PKD2 activation and P-selectin surface expression in HPAE cells are mediated by the PKC α signaling pathway.

Fig. 3. Knockdown of PKD2 expression inhibits AII-induced P-selectin surface expression. HPAE cells were transfected with 10 nM scrambled control (sicon), PKD2-specific siRNAs (siPKD2), or mock-transfected (Con). A: Cell lysates collected 2 days after the transfection were resolved by SDS-PAGE and immunoblotted with anti-PKD2, anti-PKCα, or anti-actin antibodies. The blots are representative of three separate experiments. B: HPAE cells were treated with AII (100 nM, 15 min). P-selectin surface expression was assessed 2 days after the transfection. Data are presented as means ± S.E.M. * indicates a value significantly different from that of cells transfected with the control siRNA (sicon) and challenged with AII, $P < 0.05$; # indicates a value significantly different from that of cells transfected with the control siRNA (sicon), $P < 0.05$.

AII induces P-selectin-dependent monocyte adhesion to endothelial cells

To evaluate the role of P-selectin surface expression in AII-induced endothelial adhesiveness, we examined whether AII-induced P-selectin expression promotes monocyte adhesion to endothelial cells. HPAE cells were treated with AII (100 nM, 15 min). Adhesion assays were then performed. Very few of THP-1 monocytes were observed to adhere to un-stimulated HPAE cells. The number of THP-1 monocytes bound to AII-stimulated HPAE cells was significantly higher than that bound to the control endothelial monolayer. Pre-treatment of HPAE cells for 30 min with P-selectin-blocking monoclonal antibody (1:200) markedly reduced AII-induced monocyte adhesion (Fig. 6). This study demonstrated a functional correlation of rapid P-selectin surface expression and monocyte adhesion induced by AII.

Fig. 4. Blockade of AT1 receptor inhibited AII-induced PKD2 activation and P-selectin surface expression. HPAE cells were not pre-treated or pre-treated with either the AT1-receptor antagonist (R1x, 1 μM telmisartan) or AT2-receptor antagonist (R2x, 1 μM PD 123,319) for 30 min prior to AII (100 nM, 15 min) treatment. HPAE cells without any treatment were used as the control (Con). A: cell lysates were resolved by SDS-PAGE, transferred onto polyvinylidene difluoride membranes, and immunoblotted with anti-PKD (pSer744/748) antibody. The same membrane was stripped and blotted with anti-PKD2 antibody. Representative blots and bar graphs show the change of PKD2 phosphorylation. Results shown are representative of three independent experiments. B: cell surface P-selectin expression in HPAE cells was assessed as described in Materials and Methods. Data are presented as means ± S.E.M. * indicates a value significantly different from that of cells treated with AII alone (AII), $P < 0.05$; # indicates a value significantly different from that of cells without any treatment (Con), $P < 0.05$.

PKCα and PKD2 siRNAs inhibit AII-induced monocyte adhesion to endothelial cells

To confirm the functional role of PKCα and PKD2 in AII-induced monocyte/endothelial cell adhesion, we used the siRNAs against PKCα and PKD2 to knock down their expression in HPAE cells. The effects of the siRNAs on THP-1 monocyte adhesion to HPAEs were examined. Knock-down of PKCα or PKD2 expression caused a significant reduction of AII-induced monocyte adhesion to HPAEs (Fig. 7). Our results indicate that PKCα and PKD2 play important roles in AII-induced monocyte adhesion to endothelial cells.

Fig. 5. Requirement for PKC α in signaling AII-induced PKD2 phosphorylation and P-selectin surface expression. HPAE cells were transfected with 10 nM scrambled control (sicon), PKC α -specific siRNAs (siPKC α), or mock-transfected (con). The cells were challenged with AII (100 nM, 15 min) 2 days later. A: Cell lysates were resolved by SDS-PAGE, transferred on PVDF membranes, and immunoblotted with anti-PKC α , anti-PKD2, or anti-actin antibody. B: Representative blots and bar graphs show the change of PKD2 phosphorylation. Results shown are representative of three independent experiments. C: Cell surface P-selectin expression in HPAE cells was assessed as described in Materials and Methods. Data are presented as means $\pm$ S.E.M. * indicates a value significantly different from that of control cells (sicon) treated with AII, $P < 0.05$; # indicates a value significantly different from that of cells transfected with the control siRNA (sicon), $P < 0.05$.

Fig. 6. Inhibition of AII-induced monocyte adhesion to endothelial cells by a P-selectin function-blocking antibody. HPAE cells were stimulated with AII (100 nM, 15 min) or pretreated with P-selectin-blocking antibody (1:200) for 30 min before AII challenge. A: Representative images of THP-1 monocytes bound to an endothelial monolayer without AII treatment (Con), endothelial monolayer treated with AII alone (AII), and endothelial monolayer pretreated with P-selectin-blocking antibody and then treated with AII (AII + AB). B: THP-1 monocytes bound to HPAE cells were assessed as described in Materials and Methods. Data are presented as means $\pm$ S.E.M. * indicates a value significantly different from that of cells treated with AII alone, $P < 0.05$; # indicates a value significantly different from that of cells without any treatment (Con), $P < 0.05$.

Discussion

It is now well accepted that AII possesses pro-inflammatory activity and is involved in various inflammatory processes that may be crucial for the progression of cardiovascular diseases (2–4, 38). The leukocyte-endothelium interaction induced by AII may contribute to the initiation of vascular inflammation (7, 8). P-selectin, when expressed at the plasma membrane of endothelial cells, can mediate the initial capture of

leukocytes (5, 6). Several studies have indicated that AII may trigger P-selectin surface expression to initiate leukocyte adhesion to endothelial cells (8, 39, 40). Our results suggest that AII-induced PKD2 activation promotes the surface exposure of P-selectin and monocyte adhesion to endothelial cells.

AII has also been shown to induce platelet-endothelial cell interactions in cerebral venules that included rolling and adhesion of platelets on the endothelial cells (41). Importantly, AII-induced platelet/endothelial cell inter-

Fig. 7. Knockdown of PKC α or PKD2 expression inhibits AII-induced monocyte adhesion to endothelial cells. HPAE cells were transfected with 10 nM scrambled control (sicon), PKC α -specific siRNA (siPKC α), or PKD2-specific siRNAs (siPKD2). Two days later, the HPAE cells were treated with AII (100 nM, 15 min). THP-1 monocyte adhesion was assessed as described in Materials and Methods. Data are presented as means $\pm$ S.E.M. * indicates a value significantly different from that of cells transfected with the control siRNA (sicon) and challenged with AII, $P < 0.05$; # indicates a value significantly different from that of cells transfected with the control siRNA (sicon), $P < 0.05$.

actions were attenuated by treatment with P-selectin antibody, suggesting a critical role of P-selectin in their interactions. Thus, our finding that AII can directly trigger P-selectin surface expression via PKD2 activation may provide a potential mechanism of AII-induced platelet/endothelial cell interactions.

AII signaling is mediated by AT1 and AT2 receptors, which may have antagonistic effects against each other (42). AT1-receptor activation and the resultant induction of local vascular inflammation have been suggested to play a central role in atherosclerosis (43). Several signaling pathways have been reported to mediate the pro-inflammatory effects of AT1 receptors (38), including the activation of NADPH oxidase, protein kinases, and phospholipases, which can mediate the up-regulation of NF- κ B. NF- κ B activation then leads to increased expression of cytokines, chemokines, and adhesion molecules. Our present study suggests that PKD2 activation is a new pro-inflammatory signaling mechanism of the AT1 receptor. The transient and rapid PKD2 activation by AT1-receptor signaling appears to induce the early endothelial adhesiveness by stimulating endothelial exocytosis, which may play an important role in the initiation of vascular inflammation. Even though the acute effect of AII on P-selectin surface expression is independent of *de novo* protein synthesis, we cannot exclude the possibility that PKD2 may also regulate the transcription of pro-inflammatory molecules such as ICAM-1 and VCAM-1. Indeed, PKD has been reported

to mediate NF- κ B activation (44, 45). A recent study demonstrated that PKD2-mediated NF- κ B activation is required for LPA-induced IL-8 production in epithelial cells (44). Thus, PKD2 may not only mediate the acute effects of AII on the early onset of endothelial adhesiveness by regulating membrane trafficking, it may also modulate the persistent and chronic inflammatory responses of AII by stimulating the transcription of pro-inflammatory mediators. Overall, our study indicates that PKD2 activation is a pro-inflammatory signal induced by the AT1 receptor in endothelial cells.

PKC activation is a key signaling mechanism in AII-induced cellular functions (31–36). Stimulation of AII type 1 receptor can activate phospholipase C (PLC) followed by production of 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃) (46). Binding of DAG to PKC leads to activation and translocation of PKC to the plasma membrane. The role of PKC in regulated membrane trafficking is well established (47). We have shown that PKC can regulate the exocytotic machinery in endothelial cells (28). In our present study, we demonstrated that AII-induced P-selectin surface expression required PKC α activation. Since the localization and activation of PKD are regulated by its binding to DAG and PKC (21), PKD may serve to integrate the signals from PKC and DAG. Several recent studies indicate that PKD is highly mobile and can shuttle between different subcellular compartments, and the subcellular localization of PKD can be regulated by extracellular stimuli (48–50). Thus, locally produced DAG by AII may recruit PKC and PKD to the plasma membrane, thereby triggering endothelial exocytosis and the resultant P-selectin surface expression.

Further studies are needed to determine the downstream targets of PKD2 that mediate AII-induced P-selectin surface expression. Recently (18), the C-terminus of PKD (both PKD1 and PKD2) was identified to contain a postsynaptic density-95/discs large/zonula occludens-1 (PDZ)-binding motif, which are short peptide sequences that can bind to PDZ proteins containing 70–90 amino acid PDZ domains. PDZ proteins are known to control the transport and function of membrane proteins (51, 52). It has been suggested that PKD may regulate the translocation of proteins to the plasma membrane by its interaction with PDZ proteins (18). It will be interesting to know whether PDZ proteins are involved in AII stimulated P-selectin surface expression.

In summary, our study suggests that AII can directly trigger endothelial exocytosis via PKC α -dependent PKD2 activation. The resultant P-selectin surface expression could be an important mechanism in endothelial inflammation triggered by AII signaling.

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