ADAM17 Mediates Epidermal Growth Factor Receptor Transactivation and Vascular Smooth Muscle Cell Hypertrophy Induced by Angiotensin II

Haruhiko Ohtsu, Peter J. Dempsey, Gerald D. Frank, Eugen Brailoiu, Sadaharu Higuchi, Hiroyuki Suzuki, Hidekatsu Nakashima, Kunie Eguchi, Satoru Eguchi

Background—Angiotensin II (Ang II) promotes growth of vascular smooth muscle cells (VSMCs) via epidermal growth factor (EGF) receptor (EGFR) transactivation mediated through a metalloprotease-dependent shedding of heparin-binding EGF-like growth factor (HB-EGF). However, the identity of the metalloprotease responsible for this process remains unknown.

Methods and Results—To identify the metalloprotease required for Ang II-induced EGFR transactivation, primary cultured aortic VSMCs were infected with retrovirus encoding dominant negative (dn) mutant of ADAM10 or ADAM17. EGFR transactivation induced by Ang II was inhibited in VSMCs infected with dnADAM17 retrovirus but not with dnADAM10 retrovirus. However, Ang II comparably stimulated intracellular Ca²⁺ elevation and JAK2 tyrosine phosphorylation in these VSMCs. In addition, dnADAM17 inhibited HB-EGF shedding induced by Ang II in A10 VSMCs expressing the AT₁ receptor. Moreover, Ang II enhanced protein synthesis and cell volume in VSMCs infected with control retrovirus, but not in VSMCs infected with dnADAM17 retrovirus.

Conclusion—ADAM17 activated by the AT₁ receptor is responsible for EGFR transactivation and subsequent protein synthesis in VSMCs. These findings demonstrate a previously missing molecular mechanism by which Ang II promotes vascular remodeling. (Arterioscler Thromb Vasc Biol. 2006;26:e133-e137.)

Key Word: AT₁ receptor ■ metalloprotease ■ HB-EGF ■ signal transduction

Angiotensin II (Ang II) and its G protein-coupled receptor (GPCR), Ang II type-I receptor (AT₁), play critical roles in mediating cardiovascular diseases such as hypertension and atherosclerosis. Ang II appears to contribute to vascular remodeling by inducing hypertrophy, hyperplasia, and migration of vascular smooth muscle cells (VSMCs).1–3 It has been shown that Ang II promotes these cellular effects by "trans"-activation of the epidermal growth factor receptor (EGFR) mediated through the AT₁ receptor.2,4 Thus, the EGFR transactivation by Ang II leads to the activation of the downstream growth promoting signal transduction in VSMCs.2,4

Intracellular Ca²⁺ elevation, protein kinase C activation or reactive oxygen species (ROS) production seems to be required for the upstream of EGFR transactivation induced by Ang II through the AT₁ receptor.5–7 Recently, the mechanism of EGFR transactivation via several GPCRs has been shown to involve a proteolytic cleavage ("ecto-domain shedding") of a membrane-anchored EGF ligand precursor to release a biologically active growth factor mediated through ADAM (a disintegrin and metalloprotease) family metalloproteases.8,9 Previously, we and others have shown that heparin-binding EGF (HB-EGF) shedding is essential for EGFR transactivation via the AT₁ receptor.10–12 However, the identity of the ADAM involved in vascular remodeling induced by Ang II remains unknown. By using retrovirus vectors encoding dominant-negative mutants of ADAMs, here, we demonstrate several lines of evidence indicating that ADAM17 is the metalloprotease required for EGFR transactivation and subsequent VSMC hypertrophy induced by Ang II.
Materials and Methods

Reagents

Ang II and EGF were obtained from Sigma and Upstate, respectively. Antibodies selective for Tyr1068-phosphorylated EGFR (EGFR-p), total EGFR, Tyr1007/1008-phosphorylated JAK2 (JAK2-p), ADAM10 and ADAM17 as indicated. Phosphorylation of EGFR and JAK2 were measured by densitometry. B, Intracellular Ca^{2+} concentration in VSMCs stimulated with 100 nmol/L Ang II were measured and the peak and the sustained phase (1 and 2 minutes) stimulations were determined. C, VSMCs expressing control vector, dnADAM17 or dnADAM10 were stimulated with 100 nmol/L Ang II for 2 minutes or 20 ng/mL EGF for 1 minute. Cell lysates were immunoblotted (IB) with antibodies against Tyr1068-phosphorylated EGFR (EGFR-p) and total EGFR. Phosphorylation of EGFR by EGF was measured by densitometry. D, A10 cell expressing control vector or dnADAM17 were cotransfected with vectors encoding AT1, and HB-EGF-ALP. Cells were stimulated with 100 nmol/L Ang II for 60 minutes and ALP activity in the medium was determined. *P<0.05 compared with the basal control. †P<0.05 compared with the stimulated control.

Immunoblotting

Immunoblotting was performed as previously described.5,16

Intracellular Ca^{2+} Measurements

Intracellular Ca^{2+} was measured as described previously by using fura2 as an indicator.15,17

HB-EGF Shedding Assay

Forty-eight hours after transfection of plasmids encoding rat AT1 receptor and HB-EGF-ALP plasmid,15,18 A10 VSMCs were stimulated by Ang II for 60 minutes. The HB-EGF-ALP secreted into the medium was assessed as described previously.19

Protein Assay

Protein assay to estimate protein synthesis was performed as previously described.20

Cell Volume Measurement

Quiescent VSMCs in 6 well plates were stimulated with Ang II (100 nmol/L) for 72 hours. The cells were washed with Hanks balanced salt solution and trypsinized. Cell volume was measured by a coulter counter (Beckman Coulter).
Statistical Analysis

Data were analyzed by using the Student t test. The mean±SEM was determined with a significance level of *P<0.05. In VSMCs, immunoblotting (n=3 to 4) and Ca²⁺ measurement (n=4) were performed in different passages from 2 distinct cell lines. Protein assays (n=4) were performed in separate wells in different passages, and the cell volume measurements (n=3) were performed in separate plates from the same cell lines/passes. In A10 cells, HB-EGF shedding assays were performed with n=3 in separate wells.

Results

To examine the role of ADAMS in mediating EGFR transactivation induced by Ang II in VSMCs, the cells were infected with retrovirus encoding dnADAM10 or dnADAM17. Overexpression of dnADAM10 and dnADAM17 by retroviral transduction and expression of endogenous ADAMS were confirmed by antibodies toward ADAM10 or ADAM17, respectively. Ang II stimulation for 2 minutes resulted in marked phosphorylation of the EGFR at Tyr1068 in control VSMCs, whereas it was completely inhibited by the transduction of dnADAM17, but not by dnADAM10. JAK2 phosphorylation induced by Ang II was not affected by any of these infections (Figure 1A). In addition, Ang II induced comparable intracellular Ca²⁺ elevation, both in the peak and sustained phase, in dnADAM10 and dnADAM17 VSMCs (Figure 1B). Moreover, EGF equally activated EGFR in VSMCs infected with control vector retrovirus, dnADAM17 retrovirus and dnADM10 retrovirus (Figure 1C).

To examine whether ADAM17 is required for HB-EGF shedding induced by Ang II, we performed a reporter assay using HB-EGF-AP chimera vector, an established assay for HB-EGF shedding.¹⁸ Because of the technical difficulty of multiple gene transfection in primary cultured VSMCs, this experiment was done in a cell line, A10 VSMC. A10 VSMCs permanently infected with retrovirus encoding dnADAM17 or its control empty vector were further transfected with plasmids encoding AT₁ receptor and HB-EGF-ALP. Ang II-induced HB-EGF shedding was markedly inhibited in the cells infected with dnADAM17 compared to the control cells (Figure 1D). These data suggest that ADAM17 is the metalloprotease required for EGFR transactivation via HB-EGF shedding induced by Ang II in VSMCs. However, the contribution of ADAM10 to HB-EGF shedding was not evaluated in A10 VSMCs because of the inability of selected cells to sufficiently over-express dnADAM10. The differences in gene transfection and expression observed between A10 cells and primary VSMCs likely reflect, in part, the distinct origins of the 2 cell types.

The EGFR transactivation induced by Ang II has been implicated in VSMC hypertrophy.¹²,¹³ Therefore, we have examined whether ADAM17 activation is required for VSMC protein synthesis induced by Ang II. Ang II increase intracellular protein amount after 72 hours in control VSMCs. However, both basal and Ang II-stimulated protein synthesis was markedly inhibited in VSMCs infected with dnADAM17 retrovirus (Figure 2A). In control VSMCs, Ang II also enhanced cell volume, whereas it was markedly inhibited by dnADAM17 over-expression (Figure 2B). The discrepancy between the basal responses of the cell volume measurement and the protein assay may involve distinct evaluation methodologies or inhibition of basal protein synthesis without decreasing the basal cell volume. In addition, Ang II did not affect VSMC proliferation under these experimental conditions.²⁰ These data suggest that ADAM17 activation is specifically required for hypertrophic responses in VSMCs stimulated by Ang II.

Discussion

In the present study, we have demonstrated an essential role of ADAM17 for EGFR transactivation and subsequent cell hypertrophy induced by Ang II in VSMCs. Although >30 members of ADAMS exist in mammals, only 4 ADAMS, ADAM10, 12, 15 and 17, have thus far been demonstrated to mediate EGFR transactivation by a limited number of GPCRs.⁹ In cardiac myocytes, ADAM12 has been shown to mediate EGFR transactivation induced by phenylphrine. In this same study, a metalloprotease inhibitor blocked EGFR transactivation and subsequent hypertrophic responses by Ang II,¹⁸ but no direct evidence was shown to demonstrate the involvement of ADAM12 in Ang II-induced EGFR transactivation. Recently, the requirement of ADAM17 for EGFR transactivation by Ang II was shown by us and others,²² although these findings were limited to established cell lines. Therefore, to our knowledge this is the first report identifying the ADAM responsible for mediating EGFR transactivation by Ang II in its well recognized target, VSMCs. Our findings are in line with a recent report demonstrating the requirement of ADAM17 in renal tissue remodeling induced by Ang II by using a pharmacological inhibitor.²³
Although there are multiple EGFR ligands, HB-EGF has been intensively reported to be the key EGFR ligand responsible for EGFR transactivation induced by Ang II in various cells including VSMCs. This mechanism is shared in VSMCs stimulated by other GPCR agonists, thrombin and catecholamine. Our data presented here further support the importance of ADAM17 in the HB-EGF shedding in VSMCs. Similarly, ADAM17 appears to be the metalloprotease required for HB-EGF shedding induced by Ang II in COS7 and ACHN cells.

As we previously demonstrated, second messengers such as elevation of intracellular Ca2+ and ROS production seem to be involved in the activation process of ADAM17 by the AT1 receptor. In addition to these second messengers and ADAM17, multiple upstream components have been reported to participate in EGFR transactivation by Ang II. However, the relationships between these distinct signal transduction components still remain unclear. Because ADAM phosphorylation requires the activation of certain ADAM, it is possible that the upstream components may converge on an ADAM kinase that regulates ADAM17 activity. The phosphorylation of ADAM17 may further lead to a particular protein-protein interaction required for the activation. In fact, ADAM interacting adaptor proteins appear to be required for HB-EGF shedding induced by Ang II. Alternatively, ROS could directly activate ADAM17 by oxidizing electrophilic thiols groups critical for ADAM17 activation. In addition, it should be noted that ADAM17 cleaves several distinct substrates including ligand precursors and receptors raising the strong possibility that Ang II further uses ADAM17 to regulate downstream signal transduction beyond the EGFR transactivation thus far recognized. Therefore, future studies need to be conducted on the mechanism for ADAM17 activation by AT1, and the significance of subsequent downstream signaling events.

Both EGFR transactivation and HB-EGF production have been recently implicated in cardiovascular diseases. In addition to Ang II, it is quite likely that other cardiovascular risk factors may mediate their pathologic responses via ADAM family metalloproteases. Therefore, we propose that ADAM17 could be a novel therapeutic target toward vascular remodeling associated with cardiovascular diseases.

Sources of Funding
This work was supported by the National Institutes of Health grants HL076770 (S.E.), DK59778 (P.J.D.), DK63363 (P.J.D.), HL076575 (G.D.F.), Crohn’s and Colitis Foundation of America (P.J.D.), and funds from Tonohata Co, Ltd (S.E.) and Kisyu Hosokawa Co, Ltd (S.E.).

Disclosures
None.

References


ADAM17 Mediates Epidermal Growth Factor Receptor Transactivation and Vascular Smooth Muscle Cell Hypertrophy Induced by Angiotensin II

Haruhiko Ohtsu, Peter J. Dempsey, Gerald D. Frank, Eugen Brailoiu, Sadaharu Higuchi, Hiroyuki Suzuki, Hidekatsu Nakashima, Kunie Eguchi and Satoru Eguchi

Arterioscler Thromb Vasc Biol. 2006;26:e133-e137; originally published online July 13, 2006; doi: 10.1161/01.ATV.0000236203.90331.d0

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/9/e133

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/