Activation of MAPKs by Angiotensin II in Vascular Smooth Muscle Cells

METALLOPROTEASE-DEPENDENT EGF RECEPTOR ACTIVATION IS REQUIRED FOR ACTIVATION OF ERK AND p38 MAPK BUT NOT FOR JNK

Satoru Eguchi‡§, Peter J. Dempsey¶¶, Gerald D. Frank‡, Evangeline D. Motley¶¶‡, and Tadashi Inagami¶¶

From the Departments of ***Biochemistry, ¶¶Cell Biology, and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, the ¶¶Department of Anatomy and Physiology, Meharry Medical College, Nashville, Tennessee 37208, and the **Pacific Northwest Research Institute, Seattle, Washington 98122

In cultured vascular smooth muscle cells (VSMC), the vasculotrophic factor, angiotensin II (AngII) activates three major MAPKs via the G_{q} coupled AT_{1} receptor. Extracellular signal-regulated kinase (ERK) activation by AngII requires Ca^{2+}-dependent "transactivation" of the EGF receptor that may involve a metalloprotease to stimulate processing of an EGF receptor ligand from its precursor. Whether EGF receptor transactivation also contributes to activation of other members of MAPKs such as p38MAPK and c-Jun N-terminal kinase (JNK) by AngII remains unclear. In the present study, we have examined the effects of a synthetic metalloprotease inhibitor BB2116, and the EGF receptor kinase inhibitor AG1478 on AngII-induced activation of MAPKs in cultured VSMC. BB2116 markedly inhibited ERK activation induced by AngII or the Ca^{2+} ionophore A23187 without affecting the activation by EGF or PDGF. BB2116 as well as HB-EGF neutralizing antibody inhibited the EGF receptor transactivation by AngII, suggesting a critical role of HB-EGF in the metalloprotease-dependent EGF receptor transactivation. In addition to the ERK activation, activation of p38MAPK and JNK by AngII was inhibited by an AT_{1} receptor antagonist, RNH6270. A23187 and EGF markedly activate p38MAPK, whereas A23187 but not EGF markedly activates JNK, indicating the possible contribution of the EGF receptor transactivation to the p38MAPK activation. The findings that both BB2116 and AG1478 specifically inhibited activation of p38MAPK but not JNK by AngII support this hypothesis. From these data, we conclude that ERK and p38MAPK activation by AngII requires the metalloprotease-dependent EGF receptor transactivation, whereas the JNK activation is regulated without involvement of EGF receptor transactivation.

Angiotensin II (AngII), * the major bioactive peptide of the renin-angiotensin system, plays a fundamental role, not only in controlling cardiovascular and renal homeostasis but also contributing to various cardiovascular diseases such as hypertension, atherosclerosis, and heart failure. In addition to the anti-hypertensive effects, both AngII-converting enzyme inhibitors and AngII type-1 (AT_{1}) receptor antagonists appear to exert tissue-protective effects against these diseases. Therefore, the growth-stimulating activity of the AT_{1} receptor likely contributes to the progression of cardiovascular remodeling (1–3). In vascular smooth muscle cells (VSMC), AngII is believed to transmit its growth-promoting signal through activation of tyrosine kinases (4–7) that may involve PYK2 (8–10), c-Src (11), JAK2 (12), platelet-derived growth factor (PDGF)-β receptor (13), and the epidermal growth factor (EGF) receptor (14).

We have reported that Ca^{2+}-dependent transactivation of the EGF receptor (EGFR) through the AT_{1} receptor is essential for the activation of downstream Ser/Thr kinases (ERK, Akt/protein kinase B, and p70 S6 kinase), and subsequent c-Fos induction and protein synthesis by AngII in cultured rat VSMC (14–16). Thus, the EGFR transactivation could play a central role in AngII-mediated vascular remodeling. This new concept of a G protein-coupled receptor (GPCR) signaling, originally reported in Rat1 fibroblasts (17), is now supported by a similar transactivation of the EGFR by a variety of GPCRs in many cells (18–20). Several mechanisms involving an upstream tyrosine kinase, reactive oxygen species, and metalloproteases have been proposed for the transactivation of EGFR by GPCRs (7, 18–22). Although it has proven difficult to detect GPCR-induced release of endogenous EGFR ligands (14, 22, 23), the metalloprotease-dependent shedding of heparin-binding EGF-like growth factor (HB-EGF) (22) is an attractive mechanism of the EGFR transactivation in VSMC. This is because HB-EGF is a major EGF-like growth factor synthesized in VSMC, is a potent mitogenic and chemotactic factor for VSMC, and is implicated in the pathogenesis of atherosclerosis and restenosis following balloon injury (24).

The p38 mitogen-activated protein kinase (p38MAPK) and c-Jun N-terminal kinase (JNK) are members of MAPKs that are preferentially stimulated by environmental stresses and inflammatory cytokines. Like ERK, these stress-activated MAPKs phosphorylate specific subsets of transcriptional factors, thereby regulating cellular processes of inflammation, receptor; GPCR, G protein-coupled receptor; EGF, epidermal growth factor; EGF, EGF receptor; HB-EGF, heparin-binding EGF-like growth factor; PDGF, platelet-derived growth factor; VSMC, vascular smooth muscle cells; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; ADAM, a disintegrin and metalloprotease.
proliferation, differentiation, apoptosis, and/or survival (25–27). They also are likely to play critical roles in cardiovascular disease (27, 28). Indeed, AngII has recently been shown to activate both p38MAPK (29) and JNK (30) in cultured VSMC. The p38MAPK may positively regulate VSMC growth induced by AngII (29), whereas JNK was activated in a balloon-injured artery that could be inhibited by an AT1 antagonist (31). Thus, there is considerable interest in defining the signal transduction pathways by which AngII activates these stress-activated MAPKs. In the present study, we have examined the hypothesis that a metalloprotease-dependent EGFR transactivation regulates p38MAPK and/or JNK activation by AngII in VSMC. Here we report several lines of evidence indicating that, in addition to ERK, AngII-induced p38MAPK activation requires the metalloprotease-dependent EGFR transactivation, which may involve HB-EGF processing, whereas JNK activation is not mediated by the metalloprotease-dependent system.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies—**AngII and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Chemical Co. AG1478, PD169316, and anisamycin were obtained from Calbiochem. The metalloprotease inhibitors BB2116 and BB94 were kindly provided by Dr. Helen Mills (British Biotech). RHI-6270 (32), the active form of a prodrug type AT1, antagonist CS-866, was a gift from Sankyo Co., Ltd. EGF, PDGF-BB, and antibodies to phosphotyrosine and JAK2 were obtained from Upstate Biotechnology. The antibody directed to Thr180/Tyr204-EGF, PDGF-BB, and antibodies to phosphotyrosine and JAK2 were obtained from Calbiochem.

**Cell Culture—**VSMC were prepared from the thoracic aorta of Hartmann Sprague-Dawley rats by the explant method and cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum as previously described (33). In all experiments, subcultured VSMC from passages 3–10 were used and showed >99% immunostaining of smooth muscle α-actin antibody. The expression of AT1, but not AT2, was confirmed as previously described (34). Cultured human aortic VSMC were obtained from Clonetics, and subcultured according to the manufacturer's manual. Cells at ~80% confluence in culture wells were made quiescent by serum deprivation for 3 days prior to treatment.

**Immunoprecipitation—**After stimulation, cells were lysed with ice-cold immunoprecipitation buffer (50 mM HEPES, pH 7.5, 50 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10 mM sodium pyrophosphate, 1 mM Na3VO4, 30 mM 2-(p-nitrophenyl) phosphate, 100 mM NaF, 10% glycerol, 1.5 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 10 μM/ml aprotin). Lysates were centrifuged at 14,000 × g for 5 min, and the supernatant was immunoprecipitated with the antibody and protein A/G-agarose for 16 h at 4 °C as described previously (14).

**Immunoblotting—**Cells lysates or immunocomplex lysates were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted using an ECL detection kit (Amersham Pharmacia Biotech) as described previously (14).

**Measurement of Intracellular Ca2+—**Fura-2 was used to monitor changes in intracellular Ca2+ concentration using a previously described procedure (35). After incubation in serum-free medium for 48 h, cells were trypsinized, incubated with 4 μM fura-2-acetoxymethylester for 30 min at 37 °C in Krebs-Ringer HEPES solution (20 mM HEPES, pH 7.4, 130 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1.5 mM CaCl2, 10 mM glucose, 0.01% bovine serum albumin), and resuspended to 2 × 106 cells/ml. Measurements of fluorescence were made at 37 °C using a SPEX dual wavelength fluorometer (excitation at 340 and 380 nm; emission at 510 nm).

**Measurement of Soluble HB-EGF Generation—**After cell-surface proteins of human VSMC were biotinylated by 1 mg/ml Sulfo-NHS-LC-biotin (Pierce) for 30 min at 4 °C in Hanks' balanced salt solution, VSMC were rinsed and incubated in the cultured medium for 30 min at 37 °C and stimulated with or without AngII. Culture medium was collected and precipitated with immobilized streptavidin-agarose (Pierce) and immunoblotted with anti-human-HB-EGF antibody.

**Reproducibility of the Results—**Unless stated otherwise, results are representative of at least three experiments giving similar results.

**RESULTS**

Specific Inhibition of AngII-induced ERK Activation by BB2116, a Metalloprotease Inhibitor—A recent study indicates that the transactivation of EGFR by several GPCR agonists requires pro-HB-EGF processing that can be blocked by a synthetic metalloprotease inhibitor, BB94 (22). We have shown that AngII mainly activates ERK through the EGFR transactivation in VSMC (14). To determine whether this cascade involves metalloprotease activation, the effect of two hydroxyamic acid-based metalloprotease inhibitors BB2116 and BB94 on AngII-induced ERK activation was examined in cultured rat VSMC. As shown in Fig. 1 (A and B), BB2116 markedly and concentration-dependently inhibited AngII-induced ERK1/2 activation as assessed by their phosphorylation. By contrast, BB2116 had no effect on ERK activation induced by EGF or PDGF-BB (Fig. 1B). BB94 also inhibited AngII-induced ERK activation in VSMC to a similar extent as BB2116 (Fig. 1C).

The EGFR transactivation and subsequent ERK activation by AngII require intracellular Ca2+ elevation through the Gq-coupled AT1 receptor but not protein kinase C (PKC) (14, 34). In agreement with our previous observations (14, 34), ERK activation by the Ca2+ ionophore A23187 was markedly inhibited by BB2116, whereas PMA-induced ERK activation was minimally affected (Fig. 2A). BB2116 did not alter AngII-induced intracellular Ca2+ elevation (Fig. 2B), indicating that the metalloprotease inhibitor had no effect on AngII binding to
Angiotensin II Signals through Metalloprotease

7959

FIG. 2. Involvement of metalloprotease in the Ca{
2+}-dependent ERK activation but not in the AngII-induced nonreceptor tyrosine kinase activation. A, VSMC were pretreated with or without BB2116 (10 μM) for 30 min and stimulated with AngII (100 nM) for 3 min. B, cell lysates were analyzed by immunoblotting with antibodies as indicated. C, VSMC were pretreated with or without BB2116 (10 μM) for 30 min and stimulated with 100 nM AngII for 5 min (left panel) or 10 min (right panel). Cell lysates were immunoprecipitated with antibodies as indicated (left panel). Cell lysates were analyzed by immunoblotting with antibodies as indicated without prior immunoprecipitation (right panel).

where a GPCR agonist, AngII, can activate three major MAPKs (ERK, JNK, and p38MAPK) (29, 30, 34). We measured the activation of these MAPKs by immunoblotting with their phospho-specific antibodies. This method allows us to assess activation of these different MAPKs on the same membrane. AngII activated these MAPKs with comparable time courses of their activation by AngII with those by PMA, PDGF and EGF, but not by EGF (Fig. 5A). These differences may be due to different effects of PDGF and EGFR on intracellular Ca{
2+} concentration, because PDGF, but not EGF, elevated Ca{
2+} concentration in VSMC (Fig. 5C). These data suggest that the Ca{
2+}-dependent EGFR transactivation by AngII could also signal to p38MAPK as well as ERK. In contrast, JNK activation by AngII may require a distinct Ca{
2+} effector other than the EGFR.

Metalloprotease Inhibitor and EGFR Kinase Inhibitor Suppressed p38MAPK Activation but Not JNK Activation by AngII—To define the signaling relay leading to p38MAPK and JNK activation by AngII, we compared the respective time courses of their activation by AngII with those by PMA, A23187, PDGF, or EGF. PMA preferentially activated ERK, whereas A23187 markedly activated all three MAPKs (Fig. 5A). PDGF and EGF markedly activated p38MAPK and ERK, whereas JNK was markedly activated by PDGF but not by EGF (Fig. 5B). The differences may be due to different effects of PDGF and EGFR on intracellular Ca{
2+} concentration, because PDGF, but not EGF, elevated Ca{
2+} concentration in VSMC. In contrast, JNK activation by AngII may require a distinct Ca{
2+} effector other than the EGFR.

Metalloprotease Inhibitor and EGFR Kinase Inhibitor Suppressed p38MAPK Activation but Not JNK Activation by AngII—To define whether the metalloprotease-dependent EGFR transactivation participates in AngII-induced p38MAPK activation, effects of BB2116, BB94, and the selective EGFR kinase inhibitor AG1478 on AngII-induced activation of p38MAPK and JNK were studied. As shown in Fig. 6 (A and B), BB2116 markedly inhibited AngII-induced p38MAPK phosphorylation, whereas it had no effect on the JNK phosphorylation. AngII-induced p38MAPK activation was similarly inhibited by pretreatment with BB94 (Fig. 6C). The BB-EFG neutralizing antibody also markedly inhibited AngII-induced p38MAPK activation in cultured human VSMC (Fig. 6D). Moreover, two different EGFR kinase inhibitors, AG1478 as well as PD158780, markedly inhibited both AngII- and EGF-induced
p38MAPK phosphorylation, whereas they had no significant effect on the activation by the well-known p38MAPK agonist, anisomycin (Fig. 7 A). Consistent with our previous publications (14), AngII-induced ERK activation was also markedly inhibited by AG1478. By contrast, AngII-induced JNK activation was not affected by the EGFR inhibitor (Fig. 7 B). These data strongly indicate that, in addition to ERK activation, the metalloprotease-dependent EGFR transactivation involving HB-EGF shedding mediates the p38MAPK activation by AngII in VSMC.

DISCUSSION

The major finding of the present study is that AngII-stimulated ERK and p38MAPK activation requires metalloprotease and HB-EGF. A (pp38MAPK) by AngII in VSMC. A and B, VSMC were pretreated with 10 μM BB2116 or its solvent dimethyl sulfoxide (0.1%) for 30 min, and then stimulated with AngII (100 nM) for 10 min (A) or 30 min (B). C, VSMC were pretreated with or without 10 μM BB96 for 30 min and then stimulated with AngII (100 nM) for 10 min. D, human VSMC were pretreated with or without HB-EGF neutralizing antibody (40 μg/ml) for 1 h and then stimulated with AngII (100 nM) for 10 min. Cell lysates were analyzed by immunoblotting with antibodies as indicated by repeated reprobing.

in inhibited by AG1478. By contrast, AngII-induced JNK activation was not affected by the EGFR inhibitor (Fig. 7B). These data strongly indicate that, in addition to ERK activation, the metalloprotease-dependent EGFR transactivation involving HB-EGF shedding mediates the p38MAPK activation by AngII in VSMC.

The major finding of the present study is that AngII-stimulated ERK and p38MAPK activation requires metalloprotease-dependent EGFR transactivation in cultured VSMC, whereas JNK activation does not. In addition, studies using a neutralizing HB-EGF antibody suggest that processing of HB-EGF by the AngII-induced metalloprotease activation is involved in the EGFR transactivation. These data will provide new insight into the signaling mechanism by which AngII orchestrates several transcriptional events that contribute to vascular remodeling as illustrated in Fig. 8.

The transactivation of the EGFR by GPCRs is an important mechanism that may explain cell growth promotion by GPCRs (7, 18–20). Recently, the metalloprotease-dependent generation of HB-EGF has been reported to mediate the EGFR transactivation (22) and ERK activation (38) by certain GPCRs. In COS-7 cells, insulin-like growth factor 1 also induces HB-EGF-dependent EGFR transactivation through a metalloprotease activation (29). In the present study, we have shown that a synthetic metalloprotease inhibitor BB2116 as well as the HB-EGF neutralizing antibody attenuated EGFR transactivation by AngII. The metalloprotease inhibitor BB2116 as well as BB94 also inhibited ERK activation by AngII. The specificity of BB2116 as a synthetic hydroxamate metalloprotease inhibitor that can inhibit processing of membrane-anchored proteins (40–42), including EGFR ligand precursors (43, 44), has been

FIG. 4. AT1 receptor antagonist RNH6270 blocked activation of MAPks (ERK, JNK, and p38MAPK) by AngII in VSMC. A, VSMC were stimulated with AngII (100 nM) for indicated durations. B and C, VSMC were pretreated with or without RNH6270, the active form of a prodrug type AT1, antagonist, at 1 μM for 30 min, and stimulated with AngII (100 nM) for 10 min (B) or 30 min (C). Cell lysates were analyzed by immunoblotting with antibodies as indicated by repeated reprobing.

FIG. 5. Distinct effect of PMA, A23187, PDGF, and EGF on MAPks phosphorylation. A, VSMC were stimulated with PMA (100 nM) or A23187 (10 μM) for indicated durations. B, VSMC were stimulated with PDGF-BB or EGF (100 ng/ml) for indicated durations. Cell lysates were analyzed by immunoblotting with antibodies as indicated by repeated reprobing. C, VSMC were stimulated with PDGF-BB or EGF (100 ng/ml), and intracellular Ca²⁺ concentration was determined.

FIG. 6. AngII-induced p38MAPK activation but not JNK activation requires metalloprotease and HB-EGF. A and B, VSMC were pretreated with 10 μM BB2116 or its solvent dimethyl sulfoxide (0.1%) for 30 min, and then stimulated with AngII (100 nM) for 10 min (A) or 30 min (B). C, VSMC were pretreated with or without 10 μM BB96 for 30 min and then stimulated with AngII (100 nM) for 10 min. D, human VSMC were pretreated with or without HB-EGF neutralizing antibody (40 μg/ml) for 1 h and then stimulated with AngII (100 nM) for 10 min. Cell lysates were analyzed by immunoblotting with antibodies as indicated by repeated reprobing.
ERK and p38MAPK by AngII require Ca\textsuperscript{2+} activation by AngII in VSMC. According to this model, activation of MAPKs by AngII is Ca\textsuperscript{2+}-dependent. VSMC were pretreated with or without AG1478 (250 nM) or PD158780 (100 nM) for 30 min, and stimulated with AngII (100 nM), EGF (100 ng/ml), or anisomycin (20 ng/ml) for 10 min. VSMC were pretreated with AG1478 (250 nM) or its solvent dimethyl sulfoxide (DMSO, 0.1%) for 30 min and stimulated with AngII (100 nM) for indicated durations. Cell lysates were analyzed by immunoblotting with antibodies as indicated by repeated probing.

The activation mechanism and identity of the metalloprotease mediating the cleavage of pro-HB-EGF leading to the transactivation of EGFR by AngII remains uncertain. The ADAM (a disintegrin and metalloprotease) family of metalloproteases is believed to mediate proteolysis of the EGFR ligands precursors (45, 46). Among the family, ADAM17/TACE and ADAM9/MDC9 can be inhibited by hydroxamic acid-based metalloprotease inhibitors (47–49). ADAM17/TACE has been shown to cleave pro-transforming growth factor \beta (50). Interestingly, ADAM17/TACE knockout mice have a phenotype very similar to that observed in EGFR knockout mice, indicating its possible role in the processing of other EGFR ligands (50). Recently, ADAM9/MDC9 was shown to mediate PKC-dependent cleavage of pro-HB-EGF (51). However, the PKC-dependent metalloprotease does not contribute to the EGFR transactivation by GPCRs (22), and our present data showed that BB2116 preferentially inhibited Ca\textsuperscript{2+}-induced but not PMA-stimulated ERK activation. Additionally, Ca\textsuperscript{2+} influx was observed to stimulate HB-EGF release independent from PKC that was specifically inhibited by the metalloprotease inhibitors (52). These observations are consistent with our previous observation and by others that AngII-induced EGFR transactivation is independent of PKC (14, 53) and requires intracellular Ca\textsuperscript{2+} elevation (14). The identification and characterization of the putative Ca\textsuperscript{2+}-sensitive metalloprotease that is indispensable for the EGFR transactivation by AngII must await further investigation.

Recently, much progress has been accomplished in defining the signal transduction pathway(s) by which GPCRs activate ERK. However, little is known regarding the mechanism of activation of other MAPKs by GPCRs (19, 54, 55). The present results strongly indicate that the metalloprotease-dependent EGFR activation through HB-EGF generation also contributes to p38MAPK activation in the G\textsubscript{q} -coupled AT\textsubscript{1} receptor cascade in VSMC. A recent publication implicated an Src family tyrosine kinase in p38MAPK activation by G\textsubscript{q} (56). Additionally, p38MAPK activation by AngII in VSMC was shown to involve generation of reactive oxygen species (29). Because we have previously shown the inducible association of EGFR and c-Src by AngII (14) and that c-Src is activated by reactive oxygen species (57), c-Src could be a possible link that connects the transactivated EGFR to p38MAPK in response to AngII in VSMC.

Although we showed that PDGF activates JNK, and AngII has been shown to transactivate PDGF receptor (13), the interplay of these pathways in the context of AngII-induced MAPKs requires further investigation.
volvement of PDGF receptor in AngII-induced JNK activation is unlikely, because AngII failed to induce tyrosine phosphorylation of PDGF receptor in our VSMC (14). How does AngII activate JNK in VSMC? A recent study indicated that a Ca^{2+}-sensitive tyrosine kinase mediates the JNK activation by AngII in cultured VSMC (30), and the present study showed that intracellular Ca^{2+} elevation by AngII is sufficient to activate JNK. Thus, a Ca^{2+}-sensitive tyrosine kinase other than the EGFR (8, 10) is a candidate involved in JNK activation by AngII.

HB-EGF has long been implicated in vascular diseases (24). Interestingly, the metalloprotease inhibitor BB94 inhibited VSMC migration and DNA synthesis after arterial injury (58). We and others recently reported that not only AngII (14) but also endothelin (59), thrombin (38), oxidized low density lipoproteins (60), and mechanical stretch (61) induce the trans-activation of EGFR in cultured VSMC, suggesting the pathogenic role of the transactivation in vascular remodeling. Our data presented here connect the extracellular signaling through metalloprotease-dependt processing of EGFR ligands such as HB-EGF and the transactivation of EGFR by which various vascular pathogens including AngII exert their function.

Acknowledgments—We thank Kunie Eguchi and Trinita Fitzgerald for their excellent technical assistance.

REFERENCES
1. Goodfriend, T. L., Elliott, M. E., and Catt, K. J. (1996) N Engl. J Med. 334, 1649–1654.
2. Dzau, V. J. (1998) Eur Heart J. 19, 32–36.
3. Dostal, D. E., and Baker, K. M. (1999) Circ. Res. 85, 643–650.
4. Friend, K., Kishi, T., Liaseque, B., and Alexander, R. W. (1997) Hypertension 29, 366–373.
5. Berk, B. C. (1999) J. Am. Soc. Nephrol. 10, 562–568.
6. Inagami, T., Eguchi, S., Numaguchi, K., Motley, E. D., Taz, H., Matsutomo, T., and Yamakawa, T. (1999) J. Am. Soc. Nephrol. 10, 557–561.
7. Eguchi, S., and Inagami, T. (2000) Regul. Pept. 91, 13–20.
8. Eguchi, S., Iwaki, H., Inagami, T., Numaguchi, K., Yamakawa, T., Motley, E. D., Owada, K. M., Marumo, F., and Hirata, Y. (1999) Hypertension 33, 201–206.
9. Ishida, M., Ishida, T., Thomas, S. M., and Berk, B. C. (1998) Circ. Res. 82, 7–12.
10. Marver, M. H., Schieffer, B., Paxton, W. G., Heerdt, L., Berk, B. C., Delafontaine, P., and Bernstein, K. E. (1995) Nature 375, 247–250.
11. Linsenm, A. D., Benjamin, C. W., and Jones, D. A. (1995) J. Biol. Chem. 270, 12563–12568.
12. Eguchi, S., Kusuguchi, K., Iwaki, H., Matsutomo, T., Yamakawa, T., Utsunomiya, H., Motley, E. D., Owada, K. M., Marumo, F., and Hirata, Y. (1999) J. Biol. Chem. 274, 36843–36852.
13. Eguchi, S., Iwaki, H., Motley, E. D., Kuskow, K., Owada, K. M., Marumo, F., and Hirata, Y. (1999) J. Biol. Chem. 274, 557–560.
14. Zwick, E., Hackel, P. O., Prendzel, N., and Ulrich, A. (1999) Trends Pharmacol. Sci. 20, 408–412.
15. Luttrel, L. M., Daek, Y., and Lefkowitz, R. J. (1999) Cur. Opin. Cell Biol. 11, 177–183.
16. Carpenter, G. (1999) J. Cell Biol. 146, 697–702.
17. Cunnick, J. M., Dorsey, J. F., Standley, T., Turkson, J., Kraker, A. J., Fry, D. W., Jove, R., and Wu, J. (1998) J. Biol. Chem. 273, 14468–14475.
18. Prendzel, N., Zwick, E., Daek, H., Leeser, M., Abraham, R., Wallasch, C., and Ullrich, A. (1999) Nature 402, 884–888.
19. Tsai, W., Morrell, A. D., and Peralta, E. G. (1999) EMBO J. 15, 4597–4605.
20. Raab, G., and Klagesbrun, M. (1997) Biochim. Biophys. Acta 1333, F179–F199.
21. Widmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Physiol. Rev. 79, 143–180.
22. Ip, T. Y., and Davis, R. J. (1998)Curr. Opin. Cell Biol. 10, 205–219.
23. New, L., and Han, J. (1998) Trends. Cardiovasc. Med. 8, 220–228.
24. Force, T., Pombo, C. M., Arruven, A. J., Bonventre, J. V., and Kyriakis, J. M. (1999) Circ. Res. 84, 1324–1332.
25. Ushio, F. M., Alexander, R. W., Akers, M., and Griendling, K. K. (1998) J. Biol. Chem. 273, 15022–15029.
26. Schmitz, U., Ishida, T., Ishida, M., Surapisitchat, J., Hasham, M. I., Pelech, S., and Berk, B. C. (1998) Circ. Res. 82, 1272–1278.
27. Kim, S., Irimi, Y., Yano, M., Hamaguchi, A., Miura, K., Yamakawa, S., Miyazaki, H., and Iwas, H. (1998) Circulation 97, 1731–1737.
28. Mareno, M., Tate, H., Fukuda, M., Miyamoto, N., Yamagishi, A., and Koike, H. (1995) Eur. J. Pharmacol. 285, 181–188.
29. Schmitz, U., Ishida, T., Ishida, M., Surapisitchat, J., Hasham, M. I., Pelech, S., and Berk, B. C. (1998) Circ. Res. 82, 1272–1278.
30. Kim, S., Irimi, Y., Yano, M., Hamaguchi, A., Miura, K., Yamakawa, S., Miyazaki, H., and Iwao, H. (1998) Circulation 97, 1731–1737.
31. Mareno, M., Tate, H., Fukuda, M., Miyamoto, N., Yamagishi, A., and Koike, H. (1995) Eur. J. Pharmacol. 285, 181–188.