Cellular Signaling by Endothelin Peptides: Pathways to the Nucleus

Michael S. Simonson, Yizheng Wang, and Michael J. Dunn

ABSTRACT

Endothelins (ET) are potent regulatory peptides that evoke diverse responses in glomerular mesangial cells. These include short-term actions, such as contraction and secretion, and long-term, adaptive responses, such as cell growth. Although much attention has been focused on the second messenger cascades, which govern short-term effects, the pathways of cytosolic and nuclear signaling, which effect long-term changes, remain unclear. Several distal signaling events by ET receptors have been characterized in rat mesangial cells. These include activation of a cytosolic protein kinase, mitogen-activated protein kinase and an inducible transcription factor, activator protein-1 (AP-1). This review focuses on the activation of mitogen-activated protein kinase and activator protein-1 by ET and discusses the potential role of these third and fourth messengers in controlling long-term cellular adaptations. Characterization of these and other cytosolic and nuclear signals should provide important insights into the pleiotropic actions of ET peptides.

Key Words: Endothelin, peptide hormones, signal transduction, mitogen-activated protein kinase, activator protein-1, gene expression

Cell signaling by peptide hormones—such as angiotensin II, arginine vasopressin, atrial natriuretic peptide, and bradykinin—plays a central role in maintaining renal homeostasis (1). It has long been known that diverse renal processes such as hemodynamics, glomerular filtration, and tubular reabsorption and secretion are tightly controlled by regulatory peptides. In addition, the kidney is a major site of action for the endothelins (ET) (2), recently described vasoconstrictor peptides (see references 3–7 for reviews). ET are a family of 21-amino-acid peptide hormones found in at least three isoforms. Each isoform derives from distinct preproETs encoded by separate genes (Figure 1) (2,8–11). Although mouse/rat ET-2 was originally proposed as a novel ET isoform (vasoactive intestinal contractor (12)), genomic Southern blotting of rat DNA reveals that the vasoactive intestinal contractor is the mouse/rat analog of human ET-2 (11). ET peptides are closely homologous to sarafotoxin peptides isolated from the burrowing asp, Atractaspis engaddensis, and it seems likely that the two gene families share common origins in evolution (13). ET is abundantly secreted from the vascular endothelium of both large vessels and microvessels, but ET is also secreted at numerous vascular and nonvascular sites in the central nervous system, lung, kidney, gut, pituitary and hypothalamus, eye, and amnion (3,4,6). Although it is still too early to define a precise physiological role for ET, ET peptides regulate a surprisingly diverse array of biological actions. These actions include contraction of vascular and nonvascular smooth muscle, neuromodulation and neurotransmission, secretion of biologically active compounds such as prostaglandins and steroids, and perhaps control of cell growth. The potential importance of ET as a mitogen is underscored by the fact that ET augments anchorage-independent growth in semisolid agarose (14), a marker of the transformed phenotype. Table 1 summarizes the short-term and long-term actions in which ET has been hypothesized to play a role in the kidney (6,7,15).

Glomerular mesangial cells provide a useful in vitro model for studying the biological actions and pathways of cell signaling evoked by ET in the renal glomerulus (see reference 16 for a review). In the glomerulus, ET acts principally in a paracrine mode, secreted from the closely apposed glomerular endothelial cells (17). Thus, the regulation of ET secretion from these capillary endothelial cells is likely to control local concentrations of ET. However, there is also some evidence that ET is secreted from mesangial cells themselves (18–20), thereby suggesting an autocrine mode of ET action as well. In addition, in glomerular inflammation, macrophages (21) and polymorphonuclear leukocytes (22) are also likely sources of local ET release. Whatever the cellular source of ET secretion, ET binds to abundant, cog-
Figure 1. Sequence homology and proposed structure of ET isopeptides and sarafotoxin S6b. All ET peptides and sarafotoxins are thought to share a similar hairpin loop structure as a result of two intrachain disulfide bonds. These peptides also have a hydrophobic COOH terminus. Homologous regions between the different isopeptides are enclosed in the box.

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and emphasize recent studies that we believe illustrate fruitful areas for future research. Figure 2 summarizes a working model of the signal transduction pathways activated by ET in mesangial cells.

**SIGNALS GENERATED BY ET AT THE PLASMA MEMBRANE**

**ET Receptors**

In rat mesangial cells, ET binds to both high-affinity and low-affinity receptors, which can be differentiated by equilibrium binding studies; however, the reported $K_d$ values are divergent, perhaps because of differences in cell passage and strains (31–33). The existence of two ET receptor subtypes in mesangial cells is also supported by cross-linking followed by fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (33,34). Only ET-1 and ET-2 appear to bind with high affinity to a 73-kd receptor, whereas all ET isopeptides bind to a lower-molecular-size 60-kd receptor (33). Although ET receptor subtypes have not been cloned in the kidney, molecular cloning reveals that ET receptors in other tissues belong to the G protein-coupled family of receptors (35–38). On the basis of sequence homology and differential affinity of the expressed receptors, these clones appear to encode two different subtypes: (I) a high-affinity receptor designated $ET_\alpha$, which is specific for ET-1 (35) and (II) a lower-affinity, non-selective $ET_\beta$ receptor, which recognizes ET-1, ET-2, and ET-3 (36–38). Steady-state mRNA levels for both
TABLE 1. Summary of proposed renal actions for ET peptides

<table>
<thead>
<tr>
<th>Renal Hemodynamics</th>
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<tr>
<td>Potent vasoconstrictor of the renal vasculature, thereby decreasing RBF</td>
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<tr>
<td>Decreases GFR by contraction of glomerular arterioles</td>
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<td>Reduces the ultrafiltration coefficient, Kf, probably as the result of the contraction of mesangial cells</td>
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<th>Regulation of Na+ Excretion</th>
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<td>Multiple, often contrasting actions. Net result probably depends on whether ET is a local or circulating hormone in the kidney</td>
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<td>Natriuretic Actions of ET</td>
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<tr>
<td>Increase of atrial natriuretic peptide secretion; direct action of ET at atrial myocytes</td>
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<tr>
<td>Inhibition of renin secretion; local effect on juxtaglomerular cells</td>
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<td>Inhibition of Na+-K+-ATPase in medullary collecting ducts</td>
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| Antinatriuretic Actions of ET |
| Reduction in filtered load of Na+ due to decrease in GFR |
| Increase in plasma aldosterone; direct effect of ET on adrenal glomerulosa or from renal ischemia-induced renin |
| Reduction of postglomerular (peritubular) capillary hydrostatic pressure |

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<th>Water Regulation</th>
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<tr>
<td>Postulated to regulate water permeability in luminal membrane of collecting ducts; ET antagonizes arginine vasopressin-dependent cAMP accumulation in collecting ducts in vitro</td>
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<th>Other Actions</th>
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<tr>
<td>Growth factor for mesangial cells in culture; also stimulates secretion of platelet-derived growth factor</td>
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<td>Stimulation of prostaglandin synthesis in numerous renal cell types</td>
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the ETα and ETβ receptors have been observed in the kidney (35,36), although their expression in mesangial cells has not been investigated. If ET receptors follow the paradigm for other G protein-coupled receptors, then it is likely that more than two receptor subtypes exist. Therefore, further analysis of the structure and expression of ET receptors in the mesangium must await the isolation and characterization of ET receptor clones from mesangial cell cDNA libraries.

Effectors Linked to ET Receptors

As with most G protein-coupled receptors, activated ET receptors couple to multiple effector systems to produce a complicated network of second messengers at the plasma membrane. The combined actions of these diverse second messenger systems determine the magnitude and specificity of the biological response to ET. These pathways have been extensively characterized (see references 3 and 4 for reviews) and will only be briefly discussed here. The activation of a phosphatidylinositol-specific phospholipase C is a rapid transmembrane event following activation of ET receptors in mesangial cells (26,27) and in virtually all other cells studied (3). In rat mesangial cells, pertussis toxin reduces ET-stimulated phospholipase C activity by about one half (39), suggesting that different classes of G proteins couple ET receptors to phospholipase C. The stimulation of inositol phosphate (InsP) turnover by ET-1 is rapid and dose-dependent (Figure 3). ET-1 (0.1 μM) was more potent than 1.0 μM arginine vasopressin (27), a potent phosphobipase C-linked ligand in mesangial cells. Also shown in Figure 3 is an analysis of the InsP isomers produced by ET-1. Both Ins(1,4,5)P3 and Ins(1,3,4,5)P4 are elevated by ET-1; Ins(1,4)P2 and Ins(1,3,4)P2 probably result from the 5-phosphomonoesterase-catalyzed dephosphorylation of Ins(1,4,5)P3 and Ins(1,3,4,5)P4, respectively (27). The activation of phospholipase C produces at least two second messengers: (1) the neutral diacylglycerol, which remains within the plane of the plasma membrane and activates protein kinase C, and (2) the water-soluble Ins(1,4,5)P3, which diffuses and binds to a receptor-gated Ca2+ channel to release Ca2+ from specialized stores within the endoplasmic reticulum. From the dose-response curve in Figure 3, it is also
apparent that only the low-affinity, ET_{B}-like receptor stimulates phospholipase C in mesangial cells. This is an important observation because it demonstrates that ET receptor subtypes activate different transmembrane signaling cascades and that the differential activation of signaling pathways is presumably reflected in different biological responses.

**Ca^{2+} Signaling by ET Receptors: A Nearly Universal Signal**

In nearly all systems tested thus far, ET evokes a complicated pattern of Ca^{2+} signaling, which is responsible for many of the short-term actions of ET including contraction and secretion. In rat mesangial cells, the patterns of Ca^{2+} signaling appear to depend on what specific receptor subtype is activated. Stimulation of the high-affinity (ETA-like) receptor causes a monophasic increase of cytosolic free [Ca^{2+}] due to the activation of a ligand-gated Ca^{2+} channel(s) (27) (Figure 4). Gating of ET-sensitive Ca^{2+} entry is not affected by dihydropyridine or phenylalkylamine Ca^{2+} channel blockers and is sustained for at least 10 min, although the absolute increase in cytosolic free [Ca^{2+}] is relatively modest (24,27). It is not clear whether gating of this channel by ET receptors occurs directly via G proteins or indirectly via diffusible second messengers or protein phosphorylation. In contrast, stimulation of the low-affinity (ET_{B}-like) receptor in mesangial cells causes a dose-dependent, biphasic increase in cytosolic [Ca^{2+}] consisting of a rapid spike increase followed by a lesser but sustained phase (24,27) (Figure 4). The duration of this sustained phase is especially pronounced with ET-1 and ET-2 (24). We have shown that two distinct but interdependent mechanisms work in concert to produce an integrated Ca^{2+} signal. ET stimulates the release of intracellular Ca^{2+}, presumably by the action of Ins(1,4,5)P_{3} to gate a Ca^{2+} channel in specialized Ca^{2+} stores in the endoplasmic reticulum. In addition, the low-affinity receptor also increases Ca^{2+} influx through a ligand-sensitive Ca^{2+} channel that appears to be distinct from classical voltage-gated, dihydropyridine-sensitive L-type channels. The spike increase in Ca^{2+} results from both the intracellular
release of Ca2+ and the extracellular Ca2+ influx, whereas the sustained phase of Ca2+ depends on the extracellular Ca2+ influx. Although Ca2+ entry in mesangial cells occurs via voltage-independent channels, it is important to note that, in vascular smooth muscle cells, ET augments voltage-sensitive Ca2+ entry by an indirect pathway as well as through ligand-gated channels (40–42).

What are the biological consequences of ET-induced Ca2+ signaling? First, the observation that ET-induced mesangial contraction correlates with activation of a phospholipase C-based Ca2+ signaling system suggests that contraction occurs via pharmacomechanical coupling (23). The ability of ET to augment voltage-sensitive Ca2+ entry in smooth muscle cells suggests a second pathway by which ET might amplify contraction in response to an initial depolarizing stimulus. Second, the multiplicity of Ca2+ signaling pathways activated by ET receptors suggests that classical dihydropyridine Ca2+ channel blockers will be only partially effective in antagonizing the biological effects of ET in the kidney.

Other Effectors Linked to ET Receptors

Signal transduction by ET receptors can be viewed as a lateral network of interacting pathways, and the interactions of these systems determine the final biological response to ET in mesangial cells (Figure 2). Thus, studies aimed at identifying other effectors linked to ET receptors are proceeding rapidly. For example, it is well established that ET activates phospholipase A2 to produce prostaglandin E2 and other cyclooxygenase metabolites (23). Prostaglandin E2 might constitute an important negative feedback signal to dampen ET-induced contraction. ET is also a potent stimulus for phospholipase D activity to produce phosphatidic acid and diacylglycerol (43), although the biological consequences of this pathway are unclear. In an especially interesting development, ET-1 had been shown to enhance tyrosine phosphorylation of at least five distinct target proteins in mesangial cells (44). The addition of epidermal growth factor (EGF), a classical ligand for receptor protein tyrosine kinase (PTK), also induces tyrosine phosphorylation of the same proteins (44). Epidermal growth factor fails to activate phospholipase C in mesangial cells. Thus, it appears that ET and epidermal growth factor, which bind to G protein-coupled receptors and receptor PTK, respectively, activate a common signaling pathway with possible significance for the mitogenic actions of ET. Taken together, the experiments described above suggest that we have only begun to unravel the complicated pathways of transmembrane signaling by ET receptors. Moreover, the fact that ET stimulates multiple effectors suggests that signal transduction to the nucleus does not occur in a linear fashion but rather involves multiple, perhaps interacting networks between different signal transduction pathways.

**PROTEIN KINASE CASCADES IN THE CYTOSOL**

Reversible phosphorylation/dephosphorylation of proteins plays a critical role in amplifying and disseminating signals throughout the cell. Ligands like EGF and platelet-derived growth factor, which bind to receptor PTK, stimulate activity of the receptor’s intrinsic tyrosine kinase. For ligands like ET, however, which bind to G protein-coupled receptors, the receptor is not thought to possess intrinsic protein kinase activity, and thus, the activation of a PTK cascade is indirect (44). In addition, we mentioned earlier that ET stimulates protein kinase C, a family of Ca2+ and phospholipid-dependent protein serine kinases that are activated by the binding of diacylglycerol. ET elevates diacylglycerol as a consequence of phosphatidylinositol (PtdIns) hydrolysis by phospholipase C. When activated by ET, protein kinase C is associated with the inner leaflet of the plasma membrane where it phosphorylates proteins involved in the signal transduction pathway. In vascular smooth muscle cells, ET-1 stimulates S6 kinase (45), which phosphorylates the sixth protein on the small ribosomal subunit and is postulated to contribute to mitogenic signaling. Clearly, activated ET receptors increase the activity of diverse protein kinases.

We have recently focused on the ability of ET to activate another protein kinase, the 42- and 44-kd MAP kinase (46,47) (p42/p44; also known as mitotubule-associated protein kinase and extracellular signal-regulated kinase). Diverse growth factors such as insulin, insulin-like growth factor-1, EGF, and platelet-derived growth factor activate MAP kinases through the phosphorylation of tyrosine and threonine. Activated MAP kinase, in turn, functions as a serine/threonine kinase, and this family of kinases has been called “switch kinases” because they switch a signal linked to tyrosine phosphorylation into eventual serine or threonine phosphorylation (47,48). Other examples of this type of switch kinase include Kemptide insulin-stimulated kinase (49) and raf-1 kinase (50). Target proteins that are phosphorylated and potentially regulated by MAP kinases include ribosomal protein S6 kinase, raf-1 kinase, microtubule-associated protein, and the nuclear protooncogene c-jun (47,51). In human vascular smooth muscle cells, ET-1 stimulates S6 kinase, perhaps through prior activation of MAP kinase (45). Thrombin is the first agonist linked to G protein-coupled receptors that has been reported to phosphorylate MAP kinase (52).

Accordingly, we evaluated the effects of ET-1 and ET-3 on MAP kinase in rat cultured glomerular mes-
angial cells. We used myelin basic protein (MBP) as a substrate to assess MAP kinase activity because MBP is rapidly phosphorylated by MAP kinase (47,52). ET-1 (0.1 μM), but not ET-3, rapidly stimulated MAP kinase activity at 2 min with peak stimulation at 5 to 10 min (Figure 5). MAP kinase activation after the addition of ET-1 returned to baseline after 20 min. Protein kinase C down-regulation by phorbol ester reduced, but did not eliminate, ET-1 activation of MAP kinase. Dose-response relationships reveal that the increase of MAP kinase activity closely correlates with the occupancy of the low-affinity receptor, with threshold stimulation at 1 nM ET-1. In contrast, concentrations of ET-1 that activate the high-affinity receptor (1 to 20 pM) failed to increase MAP kinase activity. Presently, we do not know whether ET-1 stimulates all forms of MAP kinase (p42, p44, p54) or whether there is selective activation of a single kinase. As discussed above, ET-1 enhances the tyrosine phosphorylation of several target proteins and it is possible that these ET-1-dependent pathways are also responsible for MAP kinase activation, which requires both tyrosine and threonine phosphorylation. To better understand the role of MAP kinase in signaling pathways important for ET-1-induced cellular responses, it will be essential to delineate how ET triggers MAP kinase, which target proteins are phosphorylated, and what physiological actions (i.e., proliferation, secretion, contraction) are regulated by this kinase.

NUCLEAR SIGNALING BY ET RECEPTORS

For ET peptides to cause phenotypic adaptations in target cells, these peptides must somehow differentially regulate gene expression. Changes in gene expression by ET can, in principle, be regulated at multiple levels including transcriptional control, RNA processing, mRNA stability, and posttranslational modification of preexisting proteins. Because we have previously shown that ET-1 markedly induces the expression of c-fos mRNA in mesangial cells (27), our initial focus has been on AP-1 transcription factors. Moreover, AP-1 complexes are attractive models for study because they are regulated by many of the same signal transduction pathways evoked by ET (i.e., protein kinase C and Ca2+ signaling). AP-1 complexes are prototypes for a group of inducible transcription factors that couple receptor-generated second messengers to changes in gene expression (53–55). Such changes in gene expression often underlie the long-term, phenotypic adaptations of cells in response to extracellular ligands. AP-1 is not a single protein but rather a mixture of homodimers and heterodimers composed of various fos and jun gene products. Fos family genes include c-fos, fos B, Δfos B (a possible dominant negative mutant), fra-1, and fra-2; jun family genes consist of c-jun, jun B, and jun D. AP-1 dimers bind to cis-acting elements (5′-TGAC/GTCA-3′) in the promoter and enhancer regions of numerous cellular and viral genes to regulate transcription initiation (56,57). AP-1 proteins are pleiotropic regulators of cell physiology. For example, in some tissues (i.e., in the bone and skin), AP-1 proteins are abundantly expressed in postmitotic cells and are thought to contribute to the maintenance of the differentiated phenotype (58). In other cell types, including the mesangial cell, AP-1 proteins are rapidly induced by mitogenic stimuli, and the increase in fos and jun transcription occurs independent of new protein synthesis. Thus, AP-1 complexes belong to a group of transcription factors (e.g., egr-1, nur-77, c-myc, etc.) that are induced as part of the cellular immediate-early gene response (59). The induction of these proteins is postulated to initiate a cascade of gene regulatory events that directs expression of the delayed gene set and results in a specific biological response and change in cell phenotype. Thus, the activation of AP-1 might direct the changes in gene expression induced by ET. Evidence is presented below that ET isopeptides regulate AP-1 activity by multiple mechanisms.

One mechanism by which ET regulates AP-1 activity is by inducing the expression of fos and jun genes (30). The induction of fos and jun genes not only increases the amount of AP-1 available for DNA binding but also controls the mixture of AP-1 homodimers and heterodimers. The "mixing and matching" of AP-1 dimers has important consequences for AP-1 activity because it is likely that different dimers vary in...
DNA:protein or protein:protein interactions and in transcriptional activity. Thus, we first asked whether \textit{fos} and \textit{jun} genes in mesangial cells are induced by ET and whether ligand-specific patterns of induction are evident. Concentrations of ET-1 that activate the low-affinity (ET\textsubscript{A}-like) receptor rapidly increase steady-state mRNA levels for a subset of \textit{fos}/\textit{jun} genes, including \textit{c-fos}, \textit{fra-1}, \textit{c-jun}, and \textit{jun B} (30) (Figure 6). In contrast, mRNA levels for \textit{fos} B and \textit{jun D} were unchanged by ET-1 or by 17% serum (data not shown). Although serum induces \textit{fos} B mRNA in NIH 3T3 fibroblasts (60), we were unable to document an increase in \textit{fos} B after the activation of ET receptors, which suggests cell-specific patterns of AP-1 induction. Consistent with our data, previous experiments have shown that \textit{jun D} is not induced by serum or phorbol esters in fibroblasts (61). The induction of \textit{c-fos} and \textit{c-jun} by ET-1 was transient, whereas the induction of \textit{fra-1} and \textit{jun B} was sustained. It is also important to note that the induction of \textit{fos} and \textit{jun} genes was insensitive to cycloheximide, demonstrating that ET-1 induces these genes as part of the cellular immediate-early response (30).

In contrast to the activation of the low-affinity ET receptor by ET-1, the activation of the high-affinity (ET\textsubscript{A}-like) receptor results in a modest induction of \textit{c-fos} and \textit{fra-1} transcripts only (Figure 6) (30). \textit{c-jun} and \textit{jun B} are not induced by the high-affinity receptor. We conclude, therefore, that the activation of different ET receptor subtypes leads to differential expression of \textit{fos} and \textit{jun} genes. Differential induction of these genes might be reflected in the biological responses produced by these ET receptor subtypes. It is also important to note that the gene for preproET-1 contains a novel AP-1 cis-element upstream of TATA that regulates the transcription of this gene (62). Thus, this AP-1 cis-element could provide a mechanism by which ET up-regulates its own expression via activation of AP-1 complexes.

How might activation of ET receptor subtypes result in differential induction of \textit{fos} and \textit{jun} genes? As shown in Figure 7, the \textit{c-fos} gene contains at least four upstream regulatory elements that control transcription initiation. First, the SRE (serum response element) at -310, which binds the serum response factor (58,63) and other accessory proteins, responds to protein kinase C-dependent and protein kinase C-independent signals and mediates induction by serum and a variety of growth factors. Deletions within the SRE severely impair growth factor and serum stimulation of \textit{c-fos} transcription. Another cis-element at -60 mediates the transcriptional response to both \textit{Ca}\textsuperscript{2+} and cAMP and is thus characterized as the \textit{Ca}\textsuperscript{2+}/cAMP response element (CaCRE) (63). The role of CaCRE in growth factor-stimulated \textit{c-fos} transcription remains unclear. Yet another response element has been characterized at -345 (sis-inducible factor response element [SIFRE]); it mediates the response to the sis/platelet-derived growth factor but not to the serum or phorbol esters. Finally, an AP-1/CaCRE-like sequence at -295 is believed to bind members of the AP-1 and CRE/ATF family. It is important to note, however, that the AP-1/CaCRE and SIFRE elements require an intact SRE to direct a
strong growth factor response (63). In contrast to the c-fos promoter, the c-jun promoter contains a single variant AP-1 site upstream of TATA that is the key element for induction by phorbol esters and growth factors (64) (Figure 7). This modified AP-1 element contains an extra A residue (5'-GTGACATCAT-3') and appears to be conserved in evolution, which suggests that it has functional significance for the regulation of c-jun transcription (53). In contrast to the consensus AP-1 cis-element, which recognizes AP-1 complexes that contain c-fos, preliminary experiments with HeLa cell nuclear extracts suggest that the variant AP-1 element fails to bind complexes containing c-fos or fos-related antigens (53). The c-jun promoter was also one of the first examples of a promoter subject to positive autoregulation by its own gene product (65).

A working model for the differential induction of fos and jun genes in mesangial cells by distinct ET receptor subtypes is shown in Figure 7. As discussed above, the low-affinity ET receptor couples to phospholipase C and numerous other effectors. Thus, it is tempting to speculate that the induction of c-fos is mediated by the SRE via the action of protein kinase C or other protein kinase cascades, such as MAP or raf-1 kinase. Given the ability of other elements to synergistically amplify a transcriptional response at the SRE, it is likely that elements responding to Ca2+ (CaCRE) or other signals (perhaps a PTK at SIFRE) amplify transactivation at the SRE. The induction of c-jun transcription might occur at the variant AP-1 site responding to protein kinase C. The c-jun promoter is not known to have a Ca2+-sensitive element; therefore, Ca2+ signaling in response to the low-affinity receptor is not likely to contribute. In contrast to the low-affinity receptor, activation of the high-affinity receptor by ET-1 leads to gating of a ligand-sensitive Ca2+ channel without an increase in phospholipase C activity. Thus, it seems likely that the CaCRE is responsible for the induction of c-fos by this receptor. The inability of the high-affinity receptor to induce c-jun might result from the inability to activate protein kinase C and the absence of Ca2+-sensitive cis-element in the c-jun promoter. In this model, the activation of phospholipase C by the low-affinity receptor would serve as the key element in the differential regulation of fos and jun genes by ET receptor subtypes. Further experiments are underway to test these hypotheses.

It is also important to note that ET probably regulates AP-1 activity by posttranslational mechanisms, such as reversible phosphorylation/dephosphorylation of Fos or Jun proteins. The phosphorylation of Fos and Jun proteins has been shown to regulate both the DNA-binding and transactivation properties of AP-1 complexes (53,58). In this context, it is interesting to speculate that fos or jun might be substrates for MAP kinase. As mentioned above, it was recently shown that MAP kinase phosphorylates two serine residues in the NH2-terminal A1 transactivation domain of c-jun, which increases transcriptional activation by c-jun (51). These serine residues are phosphorylated in response to mitogens, phorbol esters, and proto-oncogene Ha-ras as part of a mitogenic signaling cascade (66). It is also possible that MAP kinase phosphorylates transcription factors such as serum response factor to increase c-fos transcription.

FUTURE DIRECTIONS

The ability of mesangial cells to respond to ET peptides is tightly regulated. Although much progress has been made in deciphering the cellular signals governing the mesangial response to ET, many challenges remain. For example, our knowledge of second messenger cascades stimulated by ET is incomplete. It will also be important to identify other protein kinase cascades and inducible transcription factors activated by ET receptors. Similarly, it remains unknown how protein kinase cascades initiated by ET in the cytosol regulate transcription factor activity in the nucleus. Finally, it will be essential to link these cytosolic and nuclear signals to biological actions by identifying the downstream protein and gene targets of MAP kinase and AP-1. It is certain that many other protein kinases and inducible transcription factors contribute to nuclear signaling by ET peptides. However, the study of MAP kinase and AP-1 transcription factors should provide useful insights into the complex signal transduction pathways responsible for the pleiotropic physiological actions of ET peptides in the mesangium.

ACKNOWLEDGMENT

The work was supported by grant HL-22563 from the NIH.

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