ROLE OF EPIDERMAL GROWTH FACTOR RECEPTOR IN THE PATHOGENESIS AND TREATMENT OF ARTERIAL HYPERTENSION

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Epidermal growth factor receptor (EGFR) is a member of receptor tyrosine kinase family. Upon ligand binding, EGFR undergoes autophosphorylation at several tyrosine residues within its intracellular domain and triggers a number of signaling pathways including extracellular signal-regulated kinases, phospholipase Cγ, and phosphoinositide 3-kinase. EGFR regulates cell growth, proliferation and survival, and its overexpression or oncogenic mutations are observed in many human cancers. Therefore, several drugs have been developed to target EGFR for antitumor therapy. In the cardiovascular system, enhanced EGFR signaling is involved in vascular and myocardial hypertrophy. Recent studies suggest that EGFR may contribute to the development of arterial hypertension. Stimulated EGFR induces vasoconstriction and regulates renal tubular Na^+ transport. Apart from its cognate ligand, EGFR is activated by many vasoconstrictors including angiotensin II, norepinephrine and endothelin-1. Enhanced EGFR signaling has been observed in several animal models of hypertension, and synthetic EGFR inhibitors reduce blood pressure in some of these models. Leptin, a peptide hormone which is produced by white adipose tissue and circulates in increased amounts in obese subjects, transactivates EGFR in vascular wall and the kidney, and EGFR inhibitor, AG1478, reduces blood pressure in experimental hyperleptinemia, suggesting that leptin-induced activation of EGFR may be involved in the pathogenesis of hypertension associated with the metabolic syndrome. These data suggest that inhibiting EGFR could be a novel therapeutic strategy for the treatment of hypertension. In addition, some of currently used hypotensive medications, in particular inhibitors of the renin-angiotensin-aldosterone system, modulate EGFR signaling. Biomed Rev 2007; 18: 1-26.

Key words: ErbB receptors, receptor transactivation, extracellular signal-regulated kinases, receptor tyrosine kinase inhibitors, leptin, metabolic syndrome

INTRODUCTION

Epidermal growth factor (EGF), also referred to as epithelial growth factor, is produced in various tissues and exerts stimulatory effects on cell growth, proliferation and survival. Because mutations of EGF receptor (EGFR) are observed in many human cancers, this receptor has been studied mainly as a target for anticancer therapy. Indeed, several EGFR inhibitors are currently available for the treatment of tumors. However, studies performed during the last decade strongly suggest that abnormal EGFR-mediated signaling plays an important role in cardiovascular pathology. EGFR is abundantly expressed

in the vascular wall and myocardium, and is activated not only by EGF itself but also by many vasoconstrictors such as angiotensin II, endothelin-1 and norepinephrine. Enhanced EGFR signaling has been demonstrated in experimental models of arterial hypertension and myocardial hypertrophy. Therefore, targeting EGFR is a promising therapeutic strategy for cardiovascular diseases.

The aim of this article is to provide general overview of EGFR signaling and to discuss role of this receptor in the regulation of vascular tone and renal sodium handling – two major determinants of blood pressure – under physiological conditions and in arterial hypertension. Moreover, potential therapeutic implications are also addressed. Role of EGFR in other aspects of cardiovascular pathology such as congenital heart defects, atherogenesis, myocardial hypertrophy, ischemia-reperfusion injury and hypertension-induced renal damage has been recently described in excellent reviews (1–3).

EGFR AND ITS LIGANDS: AN OVERVIEW

Family of ErbB receptors

EGF receptor was identified as a plasma membrane-bound protein tyrosine kinase in 1980 (4). In 1984, Downward *et al* demonstrated that *v-ErbB* oncogene of avian erythroblastosis virus encodes a truncated form of EGFR (5). Currently, EGF receptor is classified as a first member of ErbB family of receptor tyrosine kinases, and thus is also referred to as ErbB1 or HER1 (from Human Epidermal Growth Factor Receptor 1). In 1984, Schechter *et al* identified *neu* oncogene in ethyl-nitrosourea-induced neuroblastoma, and demonstrated its sequence similarity with *v-ErbB* (6); this oncogene encodes a cell surface protein having a molecular weight of 185 kD (7).

The respective protein encoded by a cellular protooncogene was termed p185[her2/neu] (now referred to as HER2 or ErbB2); oncogenic protein differs from p185[her2/neu] by a single point mutation (valine to glutamic acid substitution at residue 664) (8). Two other members of ErbB family - ErbB3 and ErbB4 - were identified later. Overexpression or oncogenic mutations of ErbB receptors (in particular ErbB1 and ErbB2) are observed in many human tumors. Currently known members of the ErbB family and their ligands are listed in Table 1.

All ErbB receptors are single-chain transmembrane proteins with its N-terminus outside and C-terminus inside the cell (Fig. 1). Upon ligand binding, the receptor dimerizes with the same (homodimerization) or other member of ErbB family (heterodimerization), and intracellular tyrosine kinase (TK) domain is activated to phosphorylate the receptor itself as well as various signaling proteins (see below). No ligand was identified for ErbB2, and recent studies indicate that conformation of this receptor makes it unable to bind any ligand. Indeed, the conformation of extracellular domain of unliganded ErbB2 resembles the conformation of ligand-bound form of other members. Therefore, ErbB2 is able to heterodimerize with other ErbB receptors, and is actually a preferred dimerization partner. When ErB2 forms dimer with other ErbB receptor, it may be phosphorylated by tyrosine kinase domain of its dimeric partner and *vice versa*, TK domain of ErbB2 may phosphorylate the intracellular domain of its partner. Consequently, both components of the dimer are involved in signal transduction, although precise signaling pathways triggered by both partners are not necessarily identical (9).

Apart from ErbB2, ErbB3 is also "atypical" in that it lacks the TK domain. Nevertheless, ligand-bound ErbB3 may non-covalently activate its heterodimeric partner as well as may be

Table 1. *ErbB receptors and their ligands*

Gene	Receptor	Ligands
c-ErbB (ErbB1)	EGFR (ErbB1, HER1)	EGF, TGF-α, HB-EGF, AR, EPR, BTC
neu	p185[her2/neu] (ErbB2, HER2)	-
ErbB3	ErbB3	NRG-1, NRG-2
ErbB4	ErbB4	NRG-1, NRG-2, NRG-3, NRG-4 HB-EGF, EPR, BTC

TGF-α – transforming growth factor-α, HB-EGF – heparin-binding epidermal growth factor, AR – amphiregulin, EPR – epiregulin, BTC – betacellulin, NRG - neuregulins
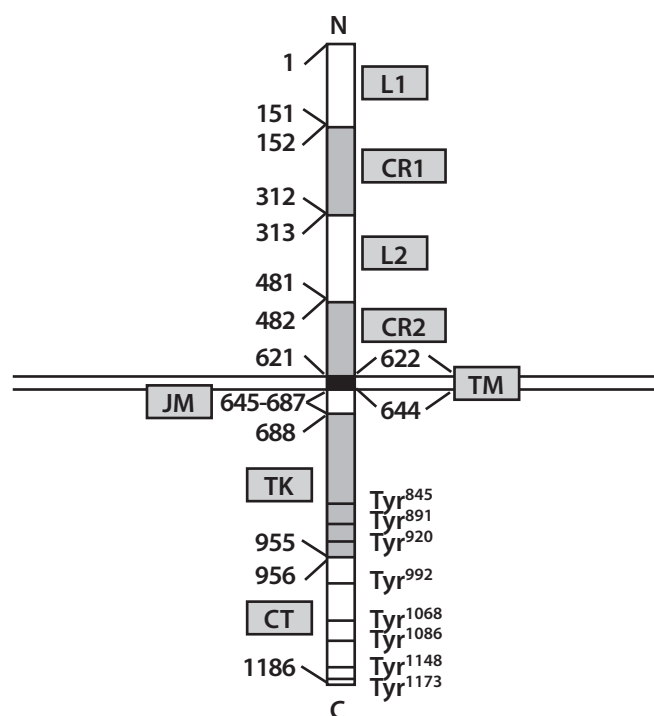
Figure 1. *Structure of human EGF receptor. Numbers of aminoacid residues forming the specific domains are presented on the left (except the transmembrane domain), and tyrosine residues which are phosphorylated on the right. Length of consecutive domains is proportional to number of residues. TM, transmembrane domain, JM, juxtamembrane domain, TK, tyrosine kinase domain, CT, C-terminal domain.*

phosphorylated by its partner to perform signal transduction. Despite "atypical" characteristics of its components, ErbB2/ErbB3 heterodimer is a most potent mitogenic combination among all known ErbB dimmers.

ErbB family is an evolutionary ancient signaling system which, however, was becoming more and more complicated during the evolution. For example, in *Caenorhabditis elegans* only one receptor homologous to mammalian ErbB is known (LET-23), and this receptor has only one ligand (LIN-3). In a fruitfly, *Drosophila melanogaster*, there is one ErbB-like receptor but it may be bound by 5 ligands. Interestingly, one of these ligands is a receptor antagonist, whereas no endogenous antagonists of ErbB receptors were found in mammals.

ErbB receptors and their ligands are involved in the regulation of cell growth, proliferation and survival. Although this signaling system plays an important role during the development and in rapidly proliferating (e.g. epithelial) cells of the adult organism, it was studied mainly in the context

of malignancies. Three types of abnormalities within this system may contribute to uncontrolled cell proliferation: *(i)* oncogenic mutations within the *ErbB* genes, which cause ligand-independent receptor overactivity, *(ii)* overexpression of ErbB receptors, and *(iii)* overexpression of their ligands. Abnormalities of the ErbB system have been observed in many cases of breast, gastric, ovarian, urinary bladder, pancreatic and other cancers (10).

EGF receptor: ligand binding and signaling mechanisms

Human EGFR consists of 1186 aminoacids (Fig. 1) and is a 170 kD protein originating from a 1210-residue precursor (9). Its extracellular domain consists of 4 subdomains which, counting from the N-terminus, are referred to as I, II, III and IV, or L1, CR1, L2 and CR2, respectively. Domains L1 and L2 are involved in ligand binding. Upon interaction with ligand, conformation of the receptor changes such that domain CR1 is exposed to bind CR1 domain of a dimeric partner. With the exception of ErbB2, other ErbB homo- and heterodimers are activated in a "2+2" manner, i.e. each of two receptors in its monomeric form binds one ligand, and then these two ligand-bound monomers dimerize to form an active complex.

Transmembrane domain of EGFR consists of 23 hydrophobic aminoacids. The role of juxtamembrane domain is unknown, however, it is suggested that this region is involved in sorting of newly formed receptors to the basolateral membranes of epithelial cells, as well as in internalization/downregulation of receptors upon ligand binding. The next tyrosine kinase domain (Fig. 1) phosphorylates the receptor itself (autophosphorylation) and intracellular signaling proteins. Finally, C-terminal domain contains 5 autophosphorylation tyrosine residues (Tyr^{992}, Tyr^{1068}, Tyr^{1086}, Tyr^{1148} and Tyr^{1173}), which bind various adaptor proteins containing Src homology-2 (SH2) or phosphotyrosine-binding (PTB) domains (Table 2). Activated EGF receptor triggers many signaling pathways including *(i)* extracellular signal-regulated kinases (ERKs), *(ii)* phosphoinositide 3-kinase (PI3-K) – protein kinase B/Akt, and *(iii)* phospholipase Cγ.

Extracellular signal-regulated kinases-1 and -2 (ERK1/2) are serine/threonine kinases belonging to a large family of mitogen-activated protein kinases (MAPKs), and due to their molecular weights are also referred to as p44 MAPK and p42 MAPK, respectively. Most mitogenic and growth-promoting effects of EGFR are mediated by ERKs. ERKs are activated through the phosphorylation cascade including Ras protein, Raf kinase, and two specific mitogen activated protein kinase

Table 2. *Docking sites for signaling proteins within the intracellular domain of EGFR*

Signaling protein	Docking site
Grb2	Tyr^{1068}, Tyr^{1086}
Shc	Tyr^{1148}, Tyr^{1173}
PLCγ	Tyr^{1173} (for N-terminal SH2 domain of PLCγ) Tyr^{992} (for C-terminal SH2 domain of PLCγ)
p120 Ras GAP	?
PTP-1B	Tyr^{992}, Tyr^{1148}
SHP-1	Tyr^{1173}
c-Src	Tyr^{891}, $Tyr^{920}*$
c-Abl	Tyr^{1086}
p85 of PI3-K	$Tyr^{920}*$
STAT-5	$Tyr^{845}*$

Grb2 – growth hormone receptor-binding protein-2, Shc – Src homology-2 containing adaptor protein, PLCγ – phospholipase C-γ, p120 Ras GAP – GTPase activating protein of 120 kDa, PTP-1B – protein tyrosine phosphatase-1B, SHP-1 – Src homology-containing tyrosine phosphatase-1, c-Src, c-Abl – nonreceptor tyrosine kinases, p85 of PI3-K – regulatory subunit of phosphoinositide 3-kinase, STAT-5 – signal transducer and activator of transcription-5. *residues within the TK domain.

Figure 2. *Mechanisms of EGFR activation and transactivation, and activation of ERKs by EGFR. NOX, NADPH oxidase, MMP, matrix metalloprotease, P, phosphate group. Sites of action of selected inhibitors commonly used in experimental studies are indicated. PD98059 is frequently referred to as "ERK inhibitor", although in fact it inhibits ERK phosphorylation by MEKs, so could be rather referred to as "MEK inhibitor".*

kinases-1 and -2 (MAPKKs or MAP2Ks), also referred to as MAPK/ERK kinase-1 and -2 (MEK1 and MEK2), which finally phosphorylate ERK1 and ERK2, respectively (Fig. 2). Phosphorylated EGFR recruits Growth hormone receptor-binding-2 (Grb2) adaptor protein either directly or through Src homology-containing protein (Shc); in the latter case, Shc first binds to EGFR and then recruits Grb2. Under resting conditions, Grb2 is constitutively bound to "Son of sevenless" (Sos) guanine nucleotide exchange factor. Relocation of Grb2-Sos complex to the plasma membrane allows the interaction of Sos with membrane-bound Ras protein; consequently, Sos catalyzes the exchange of GDP for GTP thus converting Ras from its inactive to active form. GTP-bound Ras activates Raf kinase, which phosphorylates MEK1 and MEK2 (Fig.2). To become active, Ras proteins have to be farnesylated, e.g. bound to the farnesyl group (isoprenoid group produced from mevalonate) by protein farnesyltransferases. Ras possesses an intrinsic GTPase activity; this activity is enhanced by GAP (GTPase-activating proteins). Hydrolysis of Ras-bound GTP
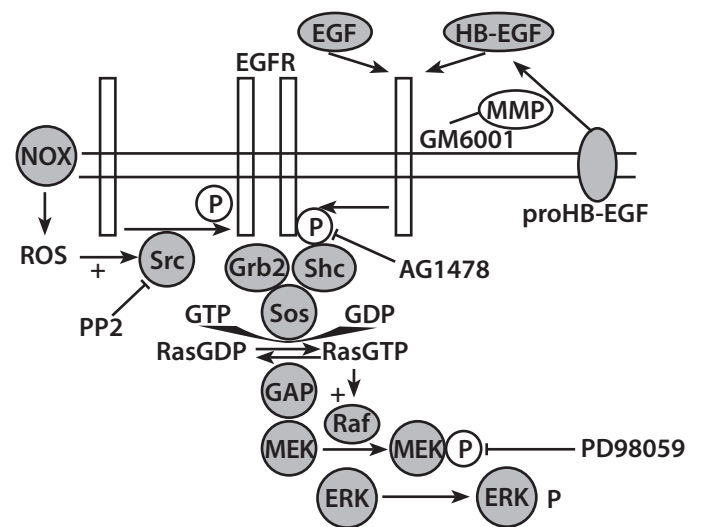
to GDP inactivates Ras and terminates Raf-MEK-ERK signaling.

The second pathway triggered by EGFR is PI3-K. PI3-K phosphorylates phosphoinositides at 3-position of inositol ring thus converting phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-triphosphate, or phosphatidylinositol 4-phosphate to phosphatidylinositol 3,4-bisphosphate. These phosphoinositides activate phosphoinositide-dependent protein kinases-1 and -2 (PDK-1 and PDK-2), which then phosphorylate and activate protein kinase B (Akt). PI3-K-PKB/Akt pathway inhibits apoptosis and plays an important role in the regulation of cell survival. One of the PKB substrates is mammalian target of rapamycin (mTOR), which activates ribosomal p70S6 kinase and thus stimulates protein synthesis. EGFR also activates several phospholipases and enhances breakdown of membrane phospholipids. Phospholipase Cγ (PLCγ) is phosphorylated by TK domain of EGFR and hydrolyzes membrane phosphatidylinositol 4,5-bisphosphate to diacylglycerol (DAG), which activates protein kinase C

(PKC), and inositol 1,4,5-triphosphate (IP_3), which releases Ca^{2+} from intracellular stores. Phospholipase D, which is also activated by EGFR in some experimental systems, hydrolyzes phosphatidylcholine to choline and phosphatidic acid; the latter is an important second messenger. Finally, EGFR may activate phospholipase A_2 which releases arachidonic acid, however, this effect is indirect and results from the stimulation of other signaling pathways. EGFR may also recruit and phosphorylate Signal Transducer and Activator of Transcription-5 (STAT-5) protein, which then translocates to cell nucleus and regulates gene expression. In addition, EGFR may phosphorylate non-receptor tyrosine kinases such as Janus kinase-2 (JAK-2) or c-Src kinase. Finally, EGFR activates protein tyrosine phosphatase SHP-1, which dephosphorylates the receptor itself as well as its substrates thus terminating signaling process. All these pathways are connected by multiple relationships thus forming a very complex signaling network. EGFR-mediated signaling is facilitated by reactive oxygen species. Indeed, EGF stimulates formation of hydrogen peroxide (H_2O_2), which transiently inactivates protein tyrosine phosphatases and thus blocks their inactivating effect on EGFR (11).

ErbB receptor ligands

All ErbB receptor ligands are synthesized as plasma membrane-bound precursors from which mature soluble growth factors are released. Interestingly, both soluble and membrane-bound ligands can bind to their receptors. Membrane-bound ligands bind only receptors on adjacent cells (juxtacrine signaling), whereas soluble ligands signal in a paracrine/autocrine manner. All ligands contain a characteristic EGF-like domain consisting of 40-50 aminoacids. This domain contains 6 spatially conserved cysteine residues arranged in a sequence CX7CX4-5CX10-13CXCX8C (C-cysteine, X-any aminoacid), and linked by three disulfide bonds in a pattern C1-C3, C2-C4 and C5-C6. Ligands of ErbB receptors may be classified into three groups (Table 1): *(i)* epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α) and amphiregulin (AR), which bind only EGFR, *(ii)* heparin-binding epidermal growth factor (HB-EGF), epiregulin (EPR) and betacellulin (BTC), which bind EGFR and ErbB4, and *(iii)* neuregulins (NRG), which bind either ErbB3 and ErbB4 (NRG-1, NRG-2) or only ErbB4 (NRG-3 and NRG-4).

EGF is an archetypical growth factor which was first isolated from mice submandibular gland and identified because of its effect on tooth eruption and eyelid opening in newborn animals (12). Its human equivalent was initially called β-urogastrone, because was isolated from urine and demonstrated to inhibit gastric acid secretion (13). In humans, prepro-EGF gene consists of 24 exons and encodes a 1207-residue precursor containing 9 EGF-like domains in its extracellular portion. The domain closest to the plasma membrane is released as a mature 53-aminoacid EGF, whereas the fate of additional 8 domains is unknown. EGF is detected in blood at a picomolar range, most of it being associated with platelets.

The second EGFR ligand, TGF-α, was isolated from culture media of retrovirally transformed fibroblasts as a "sarcoma growth factor" (14), but is also synthesized in normal tissues such as decidua, brain, keratinocytes, kidneys and macrophages. HB-EGF was identified in conditioned medium of human macrophages as a mitogenic growth factor with high affinity to heparin. A characteristic feature of mature HB-EGF is its heparin-binding domain localized N-terminally to the EGF-like domain. Interaction of this domain with cell surface heparan sulfate facilitates binding of HB-EGF with its receptors. Apart from being a precursor of mature growth factor, membrane-bound proHB-EGF is a receptor for diphtheria toxin (DT) and mediates its entry to the cells. DT binds to aminoacids 106-149 within the EGF-like domain, and therefore cannot bind to truncated HB-EGF which remains in the membrane after cleavage of soluble growth factor. CRM197 is a nontoxic DT analogue with a point mutation in the catalytic domain. CRM197 binds to both membrane-bound and soluble HB-EGF and blocks its interaction with target receptors. Therefore, CRM197 is used in experimental studies to block both paracrine/autocrine and juxtacrine HB-EGF signaling. CRM197 is specific for HB-EGF and does not bind to other ErbB ligands. HB-EGF is widely expressed in various tissues including vascular endothelial and smooth muscle cells (15–17).

Other ligands of ErbB receptors have more restricted tissue distribution and their role is less recognized. Amphiregulin (AR) was identified as an EGFR ligand produced by breast adenocarcinoma cell line; its name derives from the observations that AR may either stimulate or inhibit cell proliferation (reviewed in 18). Betacellulin (BTC) was isolated from culture media of pancreatic β-cell tumor line, however, is expressed also in normal tissues including pancreas, liver, kidney, small intestine, vascular smooth muscle and endothelial cells (for review, see ref. 19). Epiregulin (EPR) was purified from conditioned media of the mouse fibroblast-derived tumor cell line, but is also synthesized by vascular smooth muscle cells (20). Neuregulins (NRG) or heregulins (HRG) create a family of four peptides encoded by separate genes with diverse tissue distribution and regulatory roles (reviewed in 21, 22).

Soluble ErbB receptor ligands are cleaved from their membrane-bound precursors by either soluble matrix metalloproteases (MMPs) or membrane-bound metalloproteases belonging to "A Disintegrin And Metalloprotease" (ADAM) family. The identity of cleaving enzymes was ~~identified~~ established only in some experimental systems and, probably, each ligand precursor may be cleaved by more than one protease depending on the cell type and stimulus. However, it is generally appreciated that ADAMs are most important sheddases. ADAMs are transmembrane proteins consisting of *(i)* N-terminal signal sequence*, (ii)* prodomain, *(iii)* metalloprotease (MP) domain*, (iv)* disintegrin domain, *(v)* cysteine-rich region, *(vi)* EGF-like domain, *(vii)* transmembrane domain, and *(viii)* cytoplasmic domain. The first recognized ADAMs (ADAM1 and ADAM2) were two subunits of a heterodimeric sperm protein, fertillin. Subsequently identified ADAMs are being numbered in an order in which they are discovered. Currently (December 2007), 40 ADAMs are known (the complete list is available online at http://people.virginia.edu/~jw7g/Table_of_the_ADAMs.html). However, only half of ADAMs bear the MP catalytic consensus motif, and catalytic activity was confirmed only for some of them. Gene knockout experiments revealed that tumor necrosis factor-α converting enzyme (TACE or ADAM17) plays an important role in the cleavage of ErbB receptor ligand precursors; other enzymes implicated in this process are ADAM9, ADAM10 and ADAM12 (23).

TRANSACTIVATION OF EGFR BY VASOACTIVE MEDIATORS

In 1996 Daub *et al* (24) observed that several agonists of G-protein coupled receptors (GPCR) such as endothelin-1, lysophosphatidic acid and thrombin, rapidly activated EGFR and ErbB2 in Rat-1 fibroblasts. The phenomenon was too rapid to be mediated by stimulation of ligand synthesis *de novo*, and therefore was named "transactivation", i.e. ligand-independent activation. The term "transactivation" is now commonly used to describe activation of EGFR by factors other than peptide ligands listed in Table 1, although it is evident that in most cases this phenomenon is in fact ligand-mediated. The most common mechanism of EGFR transactivation was first characterized in 1999, when Prenzel *et al* (25) observed that many GPCR ligands activate EGFR by stimulating HB-EGF cleavage from its membrane precursor; the mechanism referred to as "triple membrane spanning signaling" (Fig. 2).

Several other mechanisms of EGFR transactivation have been described. Non-receptor tyrosine kinase, c-Src, phosphorylates EGFR at Tyr[845] within its TK domain and stimulates receptor kinase activity (26). Moreover, growth hormone and prolactin activate EGFR by inducing its phosphorylation at Tyr[1068] by JAK2. Because this residue is a Grb2-docking site, JAK2 may trigger downstream ERK signaling even by mutant EGFR devoid of its TK activity (27). A nonreceptor Ca^{2+}-dependent tyrosine kinase, proline-rich tyrosine kinase (Pyk2) belonging to the focal adhesion kinase (FAK) family, has also been implicated in EGFR activation. Reactive oxygen species (ROS) may activate EGFR through multiple mechanisms. First, ROS inactivate protein tyrosine phosphatases and thus enhance EGFR activity by inhibiting its dephosphorylation. Second, ROS activate intermediate kinases which subsequently phosphorylate EGFR, such as c-Src or JAK-2. Third, ROS activate ADAMs and MMPs and stimulate ligand shedding (28). The relationship between various EGFR-transactivation pathways is complex and cell-specific. In addition, many of these pathways such as c-Src, JAK2 and ROS may also be activated by EGFR.

The interest in role of EGFR in cardiovascular system has emerged when it was realized that this receptor is transactivated in the vascular wall and myocardium by vasoactive mediators such as angiotensin II, endothelin-1 or catecholamines – which not only induce vasoconstriction but also stimulate vascular and myocardial hypertrophy. Below are briefly characterized mechanisms of EGFR transactivation by mediators most relevant for cardiovascular (patho)physiology.

Angiotensin II
Angiotensin II (AII) – the major executor of the renin-angiotensin-aldosterone system – is one of the principal mediators regulating vascular tone and vascular smooth muscle cell hypertrophy and proliferation. Most effects of AII are mediated by AT_1 receptor, a serpentine receptor coupled to G_q protein which stimulates phospholipase C to produce DAG and IP_3 from membrane phosphoinositides. DAG/PKC and IP_3/Ca^{2+} pathways mediate fast contraction of vascular smooth muscle cells in response to angiotensin II. However, AII also activates many other signaling pathways including ERKs, which play an important role in hypertrophic effect of the peptide. Currently available evidence suggest that EGFR is a central link between AT_1 receptors and many protein kinases such as ERK, c-jun N-terminal kinase (JNK), p38 MAPK, c-Src, etc.

Various mechanisms of EGFR activation by angiotensin II have been described in the cardiovascular system depending on cell type and experimental conditions (Table 3). It should be noted that most studies were performed on isolated or cultured cells. Thus, the relevance of these findings to a complex structure of vascular wall is not clear. Moreover, some

Table 3. *Transactivation of EGFR in the vascular wall by angiotensin II.*

Cells	Signaling pathway leading to EGFR activation	Increase in EGFR phosphorylation	EGFR inhibitor-sensitive biological outcome	Ref.
In vitro studies				
Rat cardiac fibroblasts	Ca^{2+}-CM	+	ERK activation, DNA synthesis Increased expression of c-fos	(29)
Rat ventricular myocytes	AT_1-PKC	?	ERK activation, stimulation of sarcolemmal NHE1	(30)
Neonatal rat cardiomyocytes	ADAM12/HB-EGF	+	Cell hypertrophy	(31)
Rat aortic SMC	AT_1-Ca^{2+}-Src	+	ERK activation, protein synthesis	(32, 33)
Rat aortic SMC	AT_1-G_q-IP_3-Ca^{2+}-Pyk2-Src	?	Activation of ERK and PI3-K DNA synthesis	(34, 35)
Rat aortic SMC	AT_1-Ca^{2+}-MMP/ADAM-HB-EGF	+	Activation of ERK and p38 MAPK	(36)
Rat aortic SMC	ROS-c-Src	+ (Y^{1068}, Y^{1173})	?	(37)
Rat aortic SMC	PKC-NOX-ROS-Src	?	PI3-K-NOX-ROS	(38)
Rat aortic SMC	AT_1-Ca^{2+}-H_2O_2-ADAM	+ (Y^{1068})	ERK activation, protein synthesis, cell growth and migration	(39)
Rat aortic SMC	?	+ (Y^{845})	Activation of PKB/Akt and p70S6K, protein synthesis	(40)
Rat aortic SMC	Ca^{2+}-Ca/CMKII-Pyk2/Src	+	ERK stimulation	(41)
Rat aortic SMC	AT_1-ADAM17-HB-EGF	+ (Y^{1068})	Protein synthesis , cell hypertrophy	(42)
Rat mesenteric artery SMC	AT_1-?	?	NOX-ROS-ERK/p38 MAPK	(43)
Rat aortic SMC	MMP/HB-EGF	+ (Y^{1173})	Activation of ERK and PKB/Akt Cell migration and proliferation	(44)
Rat aortic SMC	$cPLA_2$-AA metabolites-p38MAPK-PLD_2-PA	+ (Y^{1068})	Activation of PKB/Akt	(45)
Human coronary artery SMC	G_q protein-independent direct interaction between AT_1 and EGFR	?	ERK stimulation, cell proliferation	(46)
Rat coronary microvascular EC	AT_1-Ca^{2+}-MMP/ADAM-HB-EGF	+	ERK activation, induction of Angpt2 and VEGF, angiogenesis	(47)
Human umbilical vein EC	?	?	Phosphorylation of FAK and paxillin (a FAK substrate), cell migration	(48)
Rat renal afferent arterioles	?	?	Ca^{2+} influx	(49)
In vivo studies				
Rat aorta (AII infusion)	?	+ (acute) – (3-day)	?	(50)
Rat aorta (AII infusion for 26h)	?	+ (Y^{845})	Protein synthesis	(40)
Mice aorta (AII infusion for 24h)	AT_1-ROS-Src-cAbl-caveolin-1	+	?	(51)
Rat mesenteric artery (AII infusion for 26h)	?	?	Protein synthesis	(52)

CM – calmodulin, PKC – protein kinase C, NHE – sodium/proton exchanger, NOX – NADPH oxidase, CMKII – calmodulin-dependent protein kinase II, p70 S6K – ribosomal p70 S6 kinase, $cPLA_2$ – cytosolic phospholipase A_2, PLD_2 – phospholipase D_2, PA – phosphatidic acid, Angpt2 – angiopoietin 2, VEGF – vascular endothelial growth factor, FAK – focal adhesion kinase. "+" – increase in EGFR phosphorylation upon AII treatment was demonstrated, if specific Tyr residues which are phosphorylated were identified, they are presented in parentheses, "?" – EGFR phosphorylation was not examined, and conclusion about receptor activation is based solely on the sensitivity of response to EGFR inhibitors

links in the signaling cascade have sometimes been omitted in the study design, and alternative possible mechanisms have not always been tested. Therefore, the specific signaling mechanisms deciphered in various studies do not necessarily exclude one another. Nevertheless, some general conclusions may be drawn by analyzing the results. There is little doubt that AII transactivates EGFR through the AT_1 receptor. In addition, c-Src-mediated EGFR phosphorylation or metalloprotease-dependent HB-EGF shedding seem to be the two major mechanisms of receptor transactivation, although c-Src activation may be Ca^{2+}-dependent (33), ROS-dependent (37) or may involve Ca^{2+}-sensitive nonreceptor tyrosine kinase, Pyk2 (34). The identity of MMP/ADAMs involved in HB-EGF shedding has been established definitely only in one study (42). It should be noted that although sensitivity of biological outcome to EGFR inhibitors (usually AG1478) was demonstrated in many cases, phosphorylation of this receptor was directly examined only in some studies and if was examined, the identity of Tyr residue(s) which are phosphorylated has not always been established.

Seshiah *et al* (38) described the interesting feed-forward mechanism of the prooxidant effect of AII in smooth muscle cells. It was demonstrated that AII induces a biphasic ROS formation. The early phase (minutes) results from PKC-mediated stimulation of vascular NADPH oxidase (NOX). Then, NOX-derived ROS stimulate c-Src, which phosphorylates EGFR leading to downstream activation of PI3-K. The late phase (hours) of AII-induced ROS formation results from EGFR and PI3-K-mediated NOX activation (38). Thus, ROS may operate both upstream and downstream from EGFR.

Seta *et al* (53) have demonstrated that in COS-7 cells transfected with AT_1 and EGF receptors, AII transactivates EGFR by stimulating AT_1 phosphorylation at Tyr^{319} within its C terminus. Phosphorylated AT_1 activates EGFR by direct interaction between these receptors, independently of any G protein. Substitution of Tyr^{319} by glutamic acid, which mimics phosphorylation by incorporating negatively charged residue, results in constitutive activation of EGFR, although this mutation has no effect on AT_1-G_q-PLC signaling. Miura et al. have demonstrated that G protein-independent mechanism operates also in native human coronary artery smooth muscle cells (46). Interestingly, AII analogue with Tyr^4 and Phe^8 substituted by isoleucines selectively activates this AT_1-EGFR-ERK pathway but fails to stimulate G_q protein-PLC signaling (54). On the contrary, AT_1 Tyr^{319}Phe mutant, which cannot be phosphorylated, is unable to interact with and activate EGFR, but normally stimulates G_q protein. Transgenic mice expressing this mutant fail to develop cardiac hypertrophy and do not exhibit increase in EGFR phosphorylation in response to angiotensin II (55).

High concentration of glucose (27.5 mM) augments AII-induced EGFR transactivation in vascular smooth muscle cells. In contrast, glucose has no effect on EGF-induced EGFR phosphorylation. This effect of glucose is associated with enhanced N-glycosylation of EGFR. It has been demonstrated that 145 kD form of EGFR (predominant under normoglycemic conditions) can be activated by EGF but not transactivated by AII, whereas heavily glycosylated 170 kD EGFR is efficiently stimulated by various agonists of G-protein coupled receptors such as AII, thrombin and sphingosine 1-phosphate (56). These data indicate that diabetes mellitus may augment transactivation of EGFR by AII, and this mechanism probably contributes to abnormalities of vascular tone (see below) and enhanced smooth muscle cell hypertrophy/proliferation in hyperglycemic states.

Although most effects of angiotensin II are mediated by AT_1 receptors, stimulation of less abundant AT_2 receptors opposes some of them. Indeed, AT_2 receptors inhibit vascular smooth muscle cell growth/proliferation and induce vasodilation (57). Stimulation of AT_2R opposes AT_1R-induced EGFR phosphorylation in vascular smooth muscle cells, and this effect is mediated by stimulation of tyrosine phosphatase SHP-1 which dephosphorylates EGFR (47,58).

Stimulation of AT_1 receptors results in their movement to caveolae, which are microdomains of plasma membrane rich in cholesterol and sphingolipids and also contain caveolin-1. A significant fraction of unstimulated EGFR is localized in caveolae under resting conditions (59). Within caveolae, AT_1 interacts with EGFR leading to their phosphorylation by c-Src which is also concentrated therein. In addition, c-Src phosphorylates caveolin-1 thus decreasing its affinity to EGFR. Consequently, AII induces rapid movement of phosphorylated EGFR out of caveolae and its concentration in focal adhesions, where it interacts with vinculin and paxillin. Both depletion of membrane cholesterol (which disrupts caveolae) and disruption of focal adhesions by actin-depolymerizing agents such as cytochalazin D, inhibit AII-induced EGFR transactivation in vascular smooth muscle cells (59). Thus, caveolae and focal adhesions are specialized membrane microdomains which play an important role in AII-induced EGFR transactivation. It is postulated that binding of angiotensin II to AT_1 receptors triggers the following sequence of events *(i)* movement of AT_1 to caveolae, *(ii)* ROS formation and stimulation of c-Src, *(iii)* phosphorylation of EGFR and caveolin-1 by c-Src, and

(iv) movement of phosphorylated EGFR out of caveolae and their concentration in focal adhesions (60). This "localized" signaling allows stimulating EGFR-mediated response without widespread activation of AT_1-stimulated pathways such as ROS and c-Src in the whole cell. Moreover, nonreceptor tyrosine kinase c-Abl may be an intermediate between c-Src and phosphorylation of caveolin. Inhibition of c-Abl impairs angiotensin II-induced traffic of AT_1 to caveolae and subsequent activation of EGFR both *in vitro* and *in vivo* (51).

Catecholamines

Two studies reported transactivation of EGFR by catecholamines in the vascular wall. Phenylephrine stimulates EGFR phosphorylation, ERK activity, and protein synthesis in isolated balloon-injured rat thoracic aorta (61). This effect is abolished by AG1478, CRM197, anti-HB-EGF neutralizing antibody, MMP inhibitors and HB-EGF gene knockout, suggesting that phenylephrine stimulates MMP-dependent proHB-EGF shedding. In addition, Hao et al. (62) have demonstrated that phenylephrine stimulates EGFR phosphorylation at Tyr^{1173} in rat mesenteric arteries in AG1478-sensitive manner.

Endothelin-1

Endothelin-1 (ET-1) is a potent vasoconstrictor, growth factor and vascular cell mitogen. In smooth muscle cells isolated from rat thoracic aorta, ET-1-induced DNA synthesis was dose-dependently inhibited by AG1478 (63). ET-1 stimulated EGFR phosphorylation, its association with Grb2, and ERK phosphorylation in these cells, which was abolished by AG1478 (64). The precise mechanism of ET-1-induced EGFR activation was not established, however, it was demonstrated that ET_A receptor and increase in intracellular Ca^{2+} were involved (64). In contrast, in neonatal rat cardiomyocytes, ET-1 transactivates EGFR in MMP and PKC-dependent but Ca^{2+}, Pyk2 and Src-independent manner (65). In smooth muscle cells isolated from rabbit internal carotid artery, ET-1-induced EGFR phosphorylation was sensitive to deficiency of extracellular Ca^{2+} but not to nifedipine, indicating that Ca^{2+} influx from extracellular space by voltage-independent (nifedipine-insensitive) Ca^{2+} channels mediated this effect (66). Flamant *et al* (67) have shown that ET-1 increases EGFR and ERK phosphorylation level in isolated mice aorta and that these effects are abolished by EGFR inhibitor, PD153035, as well as by endothelin receptor antagonist, bosentan. In smooth muscle cells isolated from rabbit carotid artery, ET-1 increased ERK phosphorylation in AG1478-sensitive manner, suggesting the involvement of EGFR (68).

Aldosterone

Recent studies indicate that aldosterone does not only regulate renal Na^+ handling, but has also many direct effects in the cardiovascular system, including stimulation of ROS formation and inducing myocardial fibrosis (69). Some of injurious effects of aldosterone are mediated by EGFR. Aldosterone exerts two effects on EGFR *(i)* increases receptor density by stimulating its gene expression in mineralocorticoid receptor (MR)-dependent manner, and *(ii)* transactivates EGFR by rapid non-genomic mechanism (70).

Stimulation of EGFR expression by aldosterone was first described in Chinese hamster ovary cells (71). Recently, Grossman *et al* (72) have demonstrated that aldosterone infused by osmotic minipumps increases EGFR expression in aorta and myocardium of adrenalectomized rats, but has no effect on receptor level in adipose tissue and liver. The similar effect of aldosterone was observed *in vitro* in human aortic smooth muscle cells.

Mazak *et al* (73) have demonstrated that aldosterone rapidly augments angiotensin II-induced EGFR transactivation in rat aortic smooth muscle cells, and this effect is abolished by spironolactone, superoxide scavenger, tiron, and reduced glutathione. Similarly, aldosterone augmented mitogenic effect of angiotensin II on rat aortic SMCs by increasing EGFR phosphorylation level (74). In rat aortic endothelial cells, aldosterone increases angiotensin converting enzyme (ACE) expression, the effect partially attenuated by AG1478 (75). The mechanism through which aldosterone transactivates EGF receptor is unclear, but may include ROS, c-Src and MMP-dependent shedding of EGFR ligand(s).

EGFR AND VASCULAR TONE

Many signaling pathways triggered by EGF receptor, such as IP_3-induced increase in intracellular Ca^{2+} and PKC are well-known stimulators of vascular smooth muscle cell contraction. Moreover, ERK and PI3-K have also been implicated in vasoconstrictor response. However, the effect of EGF itself on vascular tone is controversial.

Sumi *et al* (76) demonstrated that intravenously administered human EGF reduced blood pressure by about 8% in anesthetized dog. Consistently with this, EGF infused intraarterially potently relaxed femoral, mesenteric, coronary, carotid and renal vascular beds in this species (77). Similarly, in conscious monkeys BP was decreased by 14-27% following EGF infusion (78). In contrast, human EGF fragment (aminoacids 1-48) infused intravenously tended to increase BP in conscious rat

(although the effect did not reach the level of significance) (78). On the other hand, Gan *et al* (79) observed no effect of EGF administered at a very high dose (30 mg/kg) on regional hemodynamics in anesthetized rat. Taken together, the results of *in vivo* studies are inconsistent and suggest that EGF has a weak and possibly species- and vascular bed-specific effect on vascular tone.

In vitro, EGF constricted rat aortic rings at nanomolar concentrations with maximal constriction approaching 40% of the effect of angiotensin II (80). Moreover, EGF augmented vasoconstrictor effect of angiotensin II on isolated rat pulmonary artery (81). Similarly, EGF itself constricted rabbit aortic rings (82) and augmented constricting effect of bradykinin B1 receptor agonist (83). In contrast, Namiki and Akatsuka (84) observed that EGF dilated isolated rat aortic rings preconstricted with prostaglandin F_2 or norepinephrine. This effect was endothelium-dependent and was abolished by soluble guanylate cyclase inhibitor, suggesting the involvement of endothelium-derived nitric oxide. Similarly, EGF impaired constrictor response of dog coronary artery strips to norepinephrine, although had no effect on vascular tone *per se* (77).

These inconsistent results do not exclude an important role of EGFR signaling in the regulation of vascular tone for several reasons. First, exogenous EGF or other EGFR ligands are rapidly metabolized and may have limited access to their receptors. Second, EGF may have various effects on different vascular beds resulting in little or no net hemodynamic changes. Finally, EGFR "transactivated" by vasoactive mediators may play a more important role than stimulation of this receptor by its cognate ligands.

Despite many studies reporting transactivation of EGFR by vasoactive mediators (see above), only few of them addressed the role of this receptor in the regulation of vascular tone. Hao *et al* (62) have demonstrated that phenylephrine induces sharp and long-lasting contraction of isolated rat mesenteric artery, and that two structurally unrelated EGFR inhibitors, AG1478 and PD153035, abolished the late phase of phenylephrine-induced vasoconstriction. In contrast, these inhibitors had no effect on either basal vascular tone or on KCl-induced vasoconstriction. The effect of EGFR inhibitors was mimicked by ERK but not by p38 MAPK or PI3-K inhibitors, suggesting the major role of ERK in vasoconstriction. In addition, vasoconstriction induced by α_1-adrenergic agonists was abolished by CRM197 and anti HB-EGF antibody, as well as by MMP inhibitor, GM6001 (62). Subsequently, the same group has

demonstrated that EGFR transactivated by phenylephrine increases ROS formation specifically in mitochondria, which is accompanied by hyperpolarization of internal mitochondrial membrane, suggesting enhanced mitochondrial oxidation. Combined pharmacological and genetic approaches have revealed that inhibiting mitochondrial ROS formation attenuates vascular effect of phenylephrine (85). The mechanism through which EGFR stimulates mitochondrial ROS formation, and how mitochondrial ROS increase vascular tone, remains to be established. Flamant *et al* (67) have demonstrated that ET-1-induced contraction of mice aortic rings is abolished by EGFR inhibitor, PD153035. In addition, increase in blood pressure induced by bolus intravenous ET-1 in anesthetized mice is reduced by pretreatment with either PD153035 or AG1478, whereas neither of these inhibitors has any effect on BP in animals not receiving ET-1 (67). Moreover, ET-1-induced increase in blood pressure is markedly impaired in *waved-2* mice, which bear spontaneous mutation in the *egfr* gene. Similarly, BP-elevating effect of ET-1 is attenuated in HB-EGF$^{-/-}$ mice (86). *In* vitro, ET-1 induced contraction of isolated carotid artery segments is attenuated in *waved-2* mice and in HB-EGF$^{-/-}$ mice, whereas these genotypes have no effect on KCl-induced contraction (86). It has been demonstrated that these mutations, as well as pharmacological blockade of EGFR, PI3-K, HB-EGF or MMP in wild-type mice reduced ET-1-induced Ca^{2+} influx to mice and human vascular smooth muscle cells (86). Moreover, AG1478 inhibited ET-1-induced EGFR phosphorylation and contraction of rabbit basilar artery rings in a dose-dependent manner both *in vitro* and *in vivo* (87).

Carmines *et al* (88) have demonstrated that AII-induced constriction of isolated rat renal afferent and efferent arterioles was abolished by AG1478 but not by EGFR-inactive analogue, AG9. In contrast, Escano *et al* have recently demonstrated that AII stimulates ERK in SMCs isolated from rat renal microvessels in EGFR-independent but Src-dependent manner, whereas stimulation of ERK in SMCs isolated from thoracic aorta is EGFR-dependent (89). These data suggest that EGFR is involved in vascular effect of angiotensin II only in large conduit vessels which are not involved in the regulation of peripheral resistance and blood pressure. Consistently with this, AG1478 had no acute effect on blood pressure either in control or in angiotensin II-infused rats (40,89). Finally, Lucchesi et al. (90) have demonstrated that MMP2 and/or MMP9-mediated HB-EGF shedding and EGFR transactivation contribute to the development of myogenic tone (vasoconstriction induced

by increased perfusion pressure) in mouse mesenteric artery. Taken together, these data suggest that EGFR mediates vaso-constriction in response to various humoral and mechanical factors, at least in some vascular beds.

EGFR AND RENAL SODIUM HANDLING

EGF is synthesized in the medullary thick ascending limb of the loop of Henle (mTAL) and in distal convoluted tubule (DCT) of various mammalian species (91,92). ProEGF is detected in the apical membrane of tubular cells, and mature EGF is cleaved to the tubular fluid and is excreted in urine at relatively high levels (10^{-7}-10^{-8} M $versus$ 10^{-11}M in plasma). Removal of salivary glands, a major source of plasma EGF (12), has no effect on EGF excretion in urine, suggesting that the kidney is a predominant source of urinary peptide (93). Interestingly, EGF receptors are localized in the basolateral membranes of tubular cells making it unlikely that locally generated EGF binds to these receptors. However, other EGFR ligands such as TGFα and HB-EGF are also abundantly expressed in the kidney. TGFα is synthesized in medullary and, to a lesser extent, in cortical collecting ducts (94). The highest density of EGF receptors is detected in proximal straight tubule (PST), followed by proximal convoluted tubule (PCT), cortical collecting duct (CCD), inner medullary collecting duct (IMCD), outer medullary collecting duct (OMCD) and distal convoluted tubule (95). It should be noted that EGF receptors are expressed only at low level in DCT and are not expressed at all in mTAL, two major sites of intrarenal EGF production.

EGF is a potent mitogen for renal tubular and mesangial cells and, therefore, renal EGFR signaling has been studied mainly in the context of hyperproliferative kidney diseases such as diabetic nephropathy. EGFR plays an important role in renal regeneration after acute toxic or ischemic insults. In addition, abnormal EGFR sorting to the apical membranes of tubular cells may contribute to the development of polycystic kidney diseases. Much less is known about the role of EGF and its receptor in the regulation of renal sodium handling. The results of $in vivo$ studies examining the effect of EGF on renal function are controversial. Infusion of EGF to the renal artery of anesthetized rat induces reversible decrease in glomerular filtration rate (GFR) due to constriction of pre- and postglomerular vessels (reduced renal blood flow), and mesangial cell contraction (decrease in filtration fraction). These effects result in the decrease in sodium and potassium excretion, however, urine volume does not change, which suggests increased free water clearance (96). In contrast, infusion of

betacellulin by osmotic minipumps for 7 days increased urine output by almost 100% in the rat (97). Intravenously infused EGF increased urine output and Na$^+$ excretion but had no effect on K$^+$ excretion in conscious sheep, suggesting that renal effect of EGF may be species-dependent (98,99). These variable results may result, at least in part, from opposite effects of EGF on transport in various tubular segments and on other processes affecting renal Na$^+$ handling, such as renal vascular tone or renin secretion. Indeed, EGF and TGF-α reduce renin secretion by rat renal cortical slices (100).

If effect of EGF on tubular sodium transport is considered, both stimulation and inhibition have been observed depending on species, tubule segment and experimental conditions. Thus, EGF inhibited Na$^+$ reabsorption by isolated rabbit CCD by 44-59% (101), most likely by inhibiting amiloride-sensitive epithelial sodium channels (ENaC) (102). Incubation of immortalized murine collecting duct cell line, mCT1, with EGF or TGF-α for 24 hours resulted in ERK-dependent inhibition of amiloride-sensitive Na$^+$ current and reduction of mRNA^{ENaC} level, suggesting long-term regulation of ENaC by EGF (103). Interestingly, aldosterone increases EGFR expression in Madin-Darby Canine Kidney (MDCK) cells, which results in AG1478-sensitive and ERK-dependent inhibition of ENaC (104). It is suggested that stimulation of EGFR by aldosterone is a negative feedback mechanism aimed to limit the stimulatory effect of this mineralocorticoid on sodium transport. Moreover, AG1478 and U0126 which inhibit EGFR and ERK, respectively, reduce basal Na$^+$ transport in MDCK cells, suggesting that EGFR-dependent inhibition of ENaC operates even without stimulation with exogenous EGF or aldosterone, possibly due to autocrine signaling of membrane-bound EGFR agonist(s) (104). Tong and Stockand have demonstrated that EGF reduces ENaC open probability without affecting the number of channels in the plasma membrane (105). This effect of EGF results from PLCγ-mediated depletion of plasma membrane phosphatidylinositol 4,5-bisphosphate, an allosteric activator of ENaC, but is not associated with channel phosphorylation.

However, stimulatory effect of EGF on Na$^+$ transport has also been observed. EGF stimulates Na$^+$-dependent phosphate reabsorption in $in vitro$ microperfused rabbit proximal tubule (106) and in cultured porcine PCT-derived LLC-PK1 cells (107). Moreover, EGF stimulated HCO$_3$$^-$- dependent Na$^+$ uptake by rabbit proximal tubular cells (108). Gekle et al. (109) have demonstrated that EGF stimulates Na$^+$/H$^+$-exchanger (NHE) in MDCK cells; NHE is a major pathway of apical

Na$^+$ influx in the proximal tubule. Interestingly, this effect of EGF is reproduced by aldosterone, which transactivates the EGF receptor in these cells (109, 110). Although MDCK cells are a model of collecting duct principal cells, aldosterone increases NHE-3 expression and activity in EGFR-dependent manner also in primary culture of human proximal tubule cells (111).

Sodium reabsorption by tubular cells is a two-step process. First, Na$^+$ passively enters the cell through the apical membrane, and then in actively extruded across the basolateral membrane by Na$^+$,K$^+$-ATPase. Although apical Na$^+$ carriers are different in various tubular segment, Na$^+$,K$^+$-ATPase is a common transporting mechanism along the nephron. In addition, Na$^+$,K$^+$-ATPase maintains low intracellular Na$^+$ concentration allowing its passive transport from the tubular fluid across the apical membrane. Thus, Na$^+$,K$^+$-ATPase plays a pivotal role in active Na$^+$ reabsorption and is regulated by most, if not all, mediators involved in control of Na$^+$ balance (112). Abnormalities of renal Na$^+$,K$^+$-ATPase regulation result in aberrant renal Na$^+$ handling and contribute to the development of hypertension in various experimental models (113). Ten years ago, it was demonstrated that EGF increased Na$^+$,K$^+$-ATPase activity measured as ouabain-sensitive Rb$^+$ uptake in rat proximal tubular cells at rate-limiting Na$^+$ concentration but not under V$_{max}$ conditions, which suggests that EGF increases enzyme/s affinity for sodium but does not change its catalytic rate (114). Recently, we have demonstrated that EGF infused into the renal artery under general anesthesia stimulates, in ERK-dependent manner, catalytic activity of renal Na$^+$,K$^+$-ATPase measured under V$_{max}$ conditions (115). Despite various experimental protocols, these two studies (114, 115) suggest that EGF and possibly other intrarenally generated EGFR ligands stimulate sodium pump in tubular cells.

TRANSACTIVATION OF EGF RECEPTOR IN TUBULAR CELLS AND ITS ROLE IN ROS-DEPENDENT ALTERATIONS OF NA$^+$ TRANSPORT

Renal Na$^+$ reabsorption is stimulated by mediators which reduce natriuresis and increase blood pressure such as norepinephrine and angiotensin II, and inhibited by natriuretic factors such as dopamine, nitric oxide and atrial natriuretic peptide (112). Little is known about the effect of these mediators on EGFR phosphorylation level in tubular cells. However, angiotensin II transactivates EGF receptor in rabbit and mouse proximal tubular cells (116–118). Moreover, H$_2$O$_2$ transactivates EGFR in opossum kidney cells (119), rabbit proximal tubular cells (116) and LLC-PK1 cells (120). In rabbit PCT

cells, transactivation of EGFR by H$_2$O$_2$ is mediated by c-Src and p38 MAPK (121, 122). In rat cultured tubular cell line, NRK-52E, endothelin transactivates EGFR by reactive oxygen species-dependent mechanism (123). It was demonstrated that endothelin stimulates NOX, and NOX-derived ROS transiently inactivate Src homology-2 containing protein tyrosine phosphatase-1 (SHP-1) thus inhibiting EGFR dephosphorylation and increasing its activity. Moreover, α_{2B} adrenergic receptor agonists (124), glucose (125), albumin (126) and cyclic stretch (127) transactivate EGFR in various tubular segments, partially via ROS-dependent mechanism.

It is possible that a part of antinatriuretic effect of mediators such as angiotensin II, norepinephrine and endothelin-1 is mediated by EGFR. In addition, ROS stimulate tubular Na$^+$ transport and therefore, enhanced intrarenal oxidative stress may contribute to the development of arterial hypertension (128). This antinatriuretic effect of ROS is usually attributed to scavenging of nitric oxide, which is generated in the kidney in relatively high amounts and exerts a tonic inhibitory effect on Na$^+$ transport (129). However, ROS may stimulate Na$^+$ reabsorption also in NO-independent manner (130). Indeed, hydrogen peroxide stimulates Na$^+$ transport in amphibian A6 tubular cell monolayers by activating EGFR (131).

Taking into account these observations, we examined the effect of H$_2$O$_2$ infused to the renal artery under general anesthesia on renal Na$^+$,K$^+$-ATPase activity in the rat. We observed that H$_2$O$_2$ increased renal cortical and medullary Na$^+$,K$^+$-ATPase activity and this effect was abolished by ERK inhibitor, PD98059 (132). Previously, it has been shown that H$_2$O$_2$ infused into the renal medullary interstitium reduces natriuresis and increases blood pressure in the long run (133,134). Thus, renal Na$^+$,K$^+$-ATPase may mediate antinatriuretic and blood pressure-elevating effect of H$_2$O$_2$. These findings may explain a "H$_2$O$_2$ paradox", i.e. the contradictive observations that plasma H$_2$O$_2$ level is increased in hypertensive animals and humans and that blood pressure is reduced in catalase-overexpressing mice (which suggests prohypertensive effect of H$_2$O$_2$), despite the fact that H$_2$O$_2$ dilates blood vessels and may operate as an endothelium-dependent hyperpolarizing factor (132). Subsequently, we have demonstrated that H$_2$O$_2$ increases c-Src phosphorylation level at Tyr^{418}, ERK 1/2 phosphorylation, and EGFR phosphorylation at Tyr^{845} and, to a lesser extent, at Tyr^{1068} in the renal cortex and medulla (135,136). The effect of H$_2$O$_2$ on c-Src phosphorylation was abolished by Src kinase inhibitor, PP2, but not by AG1478 or PD98059, its effect on EGFR phosphorylation was abolished by PP2 and AG1478, and effect on ERK phosphorylation

and Na^+,K^+-ATPase activity by both these inhibitors as well as by PD98059. These data indicate that H_2O_2 stimulates renal sodium pump by the mechanism involving sequential activation of c-Src, EGF receptor and ERK (115). Moreover, GM6001, CRM197 and anti-HB-EGF neutralizing antibody had no effect on H_2O_2-induced Src-EGFR-ERK pathway at doses at which these inhibitors partially attenuated the effect of angiotensin II, suggesting that metalloprotease-dependent shedding of HB-EGF precursor plays only minor role in the stimulation of renal EGFR by H_2O_2. These results indicate that EGFR and ERK-dependent stimulation of Na^+,K^+-ATPase mediates, at least in part, antinatriuretic and prohypertensive effects of intrarenal oxidative stress.

EGFR IN EXPERIMENTAL HYPERTENSION

EGFR signaling is enhanced in various animal models of hypertension. Spontaneously hypertensive rat (SHR) is a model of essential hypertension which develops independently of salt intake. In contrast, Dahl salt-sensitive rats (DSSR) are a model of essential hypertension which develops only if dietary sodium intake is high. In 1993, *Saltis et al* (137) have first noted augmented growth response to EGF of vascular smooth muscle cells isolated from SHR in comparison to control cells obtained from normotensive Wistar-Kyoto (WKY) rats. However, EGF receptor density and $mRNA^{EGFR}$ levels were equal in both strains, suggesting a postreceptor enhancement of EGFR signaling in SHR. Subsequently, Fujino et al. reported that the level of HB-EGF in the left ventricle is transiently enhanced in SHR during the development of myocardial hypertrophy. In addition, the level of $mRNA^{EGFR}$ is permanently higher in SHR than in WKY rats (138). Hao *et al* (62) observed that activity of MMP-7 was enhanced in mesenteric arteries of SHR, which was accompanied by accelerated HB-EGF processing. Interestingly, this was observed at the onset of hypertension (at the age of 5 weeks), suggesting that HB-EGF/EGFR signaling contributes to the development of hypertension rather than is secondary to blood pressure elevation. Moreover, the effect of angiotensin II on EGFR and ERK phosphorylation was enhanced in aortic smooth muscle cells isolated from SHR (139). Consistently with these observations, EGF constricted endothelium-denuded thoracic aorta isolated from SHR but not from WKY (140).

Swaminathan and Sambhi (141) observed a 3-fold higher density of EGF receptors in the kidney and 2-fold higher receptor density in aorta of Dahl salt-sensitive rats kept on high salt diet in comparison to Dahl salt-resistant animals. Moreover,

affinity of EGFR to its ligand was greater in aorta (but not in the kidney) of salt-sensitive rats kept on high-salt diet. EGFR mRNA and protein levels are greater in the renal cortex of DSSR than in salt-resistant rats even before the development of hypertension, suggesting that enhanced EGFR signaling is not secondary to blood pressure elevation. Interestingly, high-salt diet had no effect on EGF receptor abundance in the kidney of normotensive Sprague-Dawley rats, but further increased receptor density in the kidney of Dahl salt-sensitive rats (142). Moreover, EGF-stimulated phosphorylation of EGFR at multiple sites (Tyr^{845}, Tyr^{992}, Tyr^{1068}) in vascular smooth muscle cells is greater in Dahl salt-sensitive rats than in either Dahl salt-resistant or Sprague-Dawley rats, which is accompanied by prolonged ERK and PKB/Akt activation (142). Finally, increased EGF receptor density was observed in the kidney and aorta of Lyon hypertensive rats compared to Lyon normotensive rats (143).

Controversies exist as to whether EGFR signaling is altered in stroke-prone SHR (SHRSP), a substrain of SHR especially prone to develop ischemic cerebral stroke. Kim *et al* (144) observed no difference in EGFR expression and phosphorylation level in aorta between SHRSP and control WKY rats. In addition, EGFR phosphorylation in SHRSP was similar before (5 week-old animals) and after (10-20 week-old animals) the development of hypertension. In contrast, Dorrance *et al* (145) found increased proEGF and EGFR mRNA levels in aorta and cerebral arteries of adult SHRSP. In addition, EGFR mRNA (but not $mRNA^{proEGF}$) level was higher in young prehypertensive animals.

Controversies also exist about role of EGFR in angiotensin II-induced hypertension. Kim *et al* (50) observed that although acute infusion of angiotensin II increased EGFR phosphorylation level in the rat aorta, AII infusion for 3 days had no effect on EGFR phosphorylation but stimulated phosphorylation of platelet-derived growth factor receptor. In the other study, hypertension induced in the rat by 4-week infusion of AII was associated with increased $mRNA^{EGFR}$ and protein level in aorta, but phosphorylation of the receptor was not examined (146). Lautrette *et al* (147) studied the role of renal EGFR in the development of angiotensin II-induced hypertension and renal damage. It was demonstrated that infusion of angiotensin II for 2 months increased blood pressure by about 40 mmHg in mice, which was associated with higher renal TGFα protein (but not mRNA) level, greater ADAM17/TACE activity, and enhanced EGFR phosphorylation (but not absolute EGFR level) in the kidney. However, expression of a dominant-nega-

tive EGFR specifically in the proximal tubule, TGFα gene knockout, or treatment with ADAM17/TACE inhibitor did not reduce BP in this model, although these treatments normalized EGFR phosphorylation and prevented renal damage. Although these data suggest that renal EGFR is not involved in AII-induced hypertension, it should be noted that neither of these approaches completely eliminated EGFR signaling. Dominant-negative EGFR was expressed only in the proximal tubules, TGFα$^{-/-}$ genotype did not eliminate other EGFR ligands, and TACE inhibitor did not block ligand processing by other MMP/ADAMs. Thus, although AII is a well-established EGFR transactivator (Table 3), role of EGFR in AII-induced hypertension is, paradoxically, controversial.

Several studies examined the role of EGFR in arterial hypertension induced by deoxycorticosterone acetate (DOCA) and high-salt diet. Florian *et al* (148) observed that EGF and TGF-α constricted aortic rings isolated from DOCA-salt rats but had no effect in normotensive animals. The similar vasoconstrictor effect was observed in 1-kidney 1-clip hypertension model. Constrictor response to EGF was observed after 14 days of DOCA-salt treatment when blood pressure is already elevated. These findings suggest that EGFR does not drive the development of hypertension in this model, but may contribute to the maintenance of high blood pressure. Increased vasoconstrictor potency of EGF results most likely from excess of mineralocorticoids rather than from blood pressure elevation, since DOCA-salt treatment induces a similar phenomenon in Wistar-Furth rats in which blood pressure only marginally increases. Although EGFR mRNA level is increased in aorta of DOCA-salt rats, the level of receptor itself is unchanged, suggesting that enhanced constricting effect of EGF is a postreceptor phenomenon (149). Enhanced constrictor response to EGF in DOCA-salt rats is mediated by ERK, PI3-K, and partially by PKC and Rho-dependent kinase (150).

Chronic administration of synthetic nitric oxide synthase inhibitors such as Nω-nitro-L-arginine (L-NNA) or Nω-nitro-L-arginine methyl ester (L-NAME) is an established model of hypertension. It has been shown that EGF contracts thoracic aorta of L-NNA-treated hypertensive rats to the greater extent than aorta of control animals (140).

EGFR IN LEPTIN-INDUCED AND OBESITY-ASSOCIATED HYPERTENSION

Metabolic syndrome (MS) is a commonly observed cluster of related abnormalities which directly or indirectly result from central obesity. The most important components of MS are insulin resistance, hyperinsulinemia, dyslipidemia (reduced HDL and increased triglycerides), elevated blood pressure, and impaired glucose tolerance or overt type 2 diabetes. Metabolic syndrome is a major cause of arterial hypertension. Indeed, it is estimated that in developed countries about 2/3 cases of arterial hypertension are directly associated with excess body weight (151).

Little is known about role of EGFR in arterial hypertension associated with the MS. Several studies have demonstrated increased EGFR phosphorylation level in mesenteric (152), coronary (153) and renal arteries (154) of rats with experimental streptozotocin-induced diabetes. In addition, AG1478 corrects abnormal vascular reactivity (e.g. reduces enhanced reactivity to vasoconstrictors and improves impaired reactivity to vasodilators) in diabetic but not in control animals. However, streptozotocin-induced diabetes is a model of type 1 diabetes, which is not a component of MS and is not associated with hypertension until nephropathy develops. Recently, increase in absolute and phosphorylated EGFR levels was observed in the kidney of Goto-Kakizaki rats, a model of type 2 diabetes and hypertension (155). However, this model is characterized by lower body weight, hypoinsulinemia and hypotriglyceridemia, and thus does not reproduce abnormalities associated with the MS.

Although the mechanism of MS-related hypertension is incompletely understood, recent studies suggest an important role of adipose tissue-derived signaling proteins designated adipokines (156). In particular, leptin, a product of the *ob* gene, is secreted by adipose tissue and regulates energy balance by inhibiting food intake and stimulating energy expenditure. Several lines of evidence suggest the role of leptin in obesity-associated hypertension *(i)* plasma leptin concentration is increased in obese subjects reflecting greater amount of adipose tissue and resistance to anorectic effect of this hormone, *(ii)* chronic leptin administration or transgenic overexpression increase BP in experimental animals, and *(iii)* high leptin correlates with increased BP in humans independently of body weight (157–159). Previously, we and others have examined the model of hypertension induced in healthy rats by central or peripheral administration of exogenous leptin. Although this model does not reproduce all abnormalities associated with obesity, it allows studying specific consequences of leptin excess (158,160). Although the role of EGFR in this model, as well as in other models of obesity-associated hypertension, was not previously investigated, some observations suggest that leptin may be associated with EGFR signaling. First, it was

observed 20 years ago that EGFR expression in the liver was reduced in three models of obesity associated with deficient leptin signaling, leptin-deficient *ob/ob* mice, leptin resistant *db/db* mice, and leptin resistant Zucker fa/fa rat (161). Second, EGF and other EGFR ligands such as HB-EGF are produced in adipocytes and stimulate adipogenesis, which suggests that their production may be enhanced in obesity (162). Indeed, plasma HB-EGF positively correlates with body mass index (163). Third, unsaturated fatty acids, which circulate in increased amounts in obese subjects, activate EGFR through the unknown mechanism (164). Finally, leptin has been shown to transactivate the EGF receptor in several cancer cell lines (165,166). Nevertheless, until recently, no study examined the effect of leptin on EGF receptor in nontransformed cells.

Previously, we have demonstrated that leptin infused into the renal artery stimulates renal Na^+,K^+-ATPase in H_2O_2 and ERK-dependent manner (132). The observation that leptin activates ERK in H_2O_2-dependent manner also in other cell types including endothelial cells (167) led us to hypothesize that EGFR may be involved in renal effect of leptin. Indeed, we demonstrated that stimulation of renal ERK and Na^+,K^+-ATPase by leptin is abolished by EGFR inhibitors, AG1478 and PD158780, as well as by Src inhibitor, PP2. Moreover, the effect of leptin was unaffected by GM6001. Taken together, these data suggest that leptin stimulates renal sodium pump by transactivating the EGF receptor in H_2O_2 and c-Src dependent but MMP-independent manner (115).

Likewise, we have recently demonstrated that experimental hyperleptinemia induced in normal rats by 7-day administration of exogenous hormone induces blood pressure elevation and impairs Na^+ excretion by stimulating renal Na^+,K^+-ATPase (160,168). Moreover, these effects were prevented by NOX inhibitor, apocynin, suggesting the involvement of oxidative stress (169,170). Therefore, in subsequent studies we aimed to elucidate if EGFR-ERK pathway is activated when leptin is administered systemically for prolonged period of time and, if so, whether this pathway contributes to BP elevation in our hyperleptinemic model.

We demonstrated that leptin infused into the abdominal aorta increases EGFR phosphorylation level at Tyr[845] and, to a lesser extent, Tyr[1068], not only in the kidney but also in the aortic wall (135). Moreover, leptin stimulated c-Src and ERK1/2 phosphorylation in the aortic wall. Although aorta is a large conduit artery and does not contribute to the regulation of vascular tone, these data suggest that leptin transactivates EGFR also in the vascular wall. In addition, we observed that

if leptin is administered systemically at a dose of 0.5 mg/kg/day which raises its plasma concentration about fourfold, ERKs are transiently stimulated, i.e. ERK1/2 phosphorylation level in the kidney and aortic wall is markedly elevated after 4 days but only moderately elevated after 8 days of leptin administration (136). Moreover, ERK inhibitor, PD98059, reduced BP in the early phase (3-5 days) but not in the later phase (6-8 days) of leptin administration (136). Indeed, later phase of leptin-induced hypertension is mainly mediated by O_2^- dependent scavenging of NO (171). Finally, we demonstrated that systemically administered leptin increases EGFR phosphorylation at Tyr[845] and Tyr[1068] in the aortic wall and the kidney, and that administration of AG1478 as well as PP2 reduces BP in leptin-treated but not in control rats (135). In contrast, MMP/ADAM inhibitors, GM6001 or doxycycline, had no effect on BP in leptin-treated rats. Recently, Chao *et al* (172) have reported that leptin transactivates EGFR in rat vascular smooth muscle cells, which is consistent with our previous findings made in intact aortic and renal tissue (115). Taken together, these data indicate that transactivation of EGFR and subsequent stimulation of ERK contribute to the development of leptin-induced hypertension by stimulating renal Na^+,K^+-ATPase and, possibly, by inducing vasoconstriction. However, if this mechanism operates in hyperleptinemic obese animals and humans, remains to be established.

EGFR AS A TARGET FOR ANTIHYPERTENSIVE THERAPY

Drugs targeting ErbB receptors currently available in clinical practice are classified into two groups: *(i)* antibodies specific for extracellular receptor domain, and *(ii)* receptor tyrosine kinase inhibitors. Recombinant humanized antibody recognizing the extracellular domain of ErbB2 (trastuzumab, Herceptin) is approved for the treatment of metastatic breast cancer. Antibodies specific for EGFR, cetuximab (Erbitux) and panitumumab (Vectibix), are used in patients with advanced metastatic colorectal cancer. Receptor tyrosine kinase inhibitors are categorized as reversible or irreversible. Reversible inhibitors compete with the ATP-binding site within the kinase domain, whereas irreversible inhibitors alkylate Cys[773] within the ATP binding pocket and permanently inactivate the kinase. Unlike antibodies, most kinase inhibitors have broad specificity toward various ErbB receptors. Currently available reversible inhibitors are quinazoline derivatives. Gefitinib (Iressa) and erlotinib (Tarceva) are orally available derivatives of AG1478 selective for EGFR tyrosine kinase, whereas lapatinib targets both EGFR and ErbB2 with com-

parable activity. Irreversible inhibitors inactivate all members of the ErbB family and are effective toward some tumor cells resistant to reversible inhibitors. Currently, no irreversible inhibitor is approved for therapy but two compounds from this group, HKI-272 and BIBW, are in phase II clinical trials for the treatment of advanced breast cancer and other solid tumors (10). Until now, very little is known about effect of these drugs on blood pressure in humans.

Studies in which attempts have been undertaken to inhibit EGFR in experimental hypertension are listed in Table 4. These attempts may be divided into two groups. The first group includes "specific" therapies such as EGFR kinase inhibitors, as well as inhibitors of transactivation pathways (e.g. Src or MMP/ADAM inhibitors). As can be seen, the effect of these treatments on BP is controversial. EGFR-specific antisense oligodeoxynucleotides were used in two studies but only partially reduced blood pressure (173,174). However, antisense oligonucleotides only partially reduced total EGFR expression, and thus could not completely block its activity. EGFR kinase inhibitors were tested in two studies; AG1478 normalized blood pressure in leptin-induced hypertension (136), whereas gefitinib had no effect on BP in L-NAME induced hypertension (175). It should be noted that blood pressure is much higher in the L-NAME model (mean BP about 170 mmHg) than in our study (SBP about 150 mmHg), and L-NAME was administered for 4 weeks (175) vs. 1-week leptin treatment in our study. It is possible that EGFR blockade is more effective in reducing BP in moderate than in severe hypertension. Among MMP/ADAM inhibitors, doxycycline reduced BP in SHR (62), but two more selective inhibitors had no effect on BP in angiotensin II-induced or phenylephrine-induced hypertension (31,147). It should be noted that doxycycline is a broad-range MMP/ADAM inhibitor, whereas compounds used in the remaining studies were selective toward specific ADAMs and therefore could be less effective.

Despite these inconsistencies, EGFR is potentially a very attractive target for antihypertensive therapy. First, EGFR is a "final common pathway" for various vasoconstrictors and thus its inhibition may be more effective than specific therapies aimed to block only one of these mediators. Second, inhibiting EGFR may reduce target organ damage such as glomerulopathy or left ventricular hypertrophy even independently of lowering BP. Third, some vessel-dilating hypotensive drugs such as Ca^{2+} antagonists or α_1-adrenergic receptor antagonists may impair renal perfusion and induce fluid retention. If EGFR mediates enhanced tubular Na^+ reabsorption as suggested by some

studies, inhibiting it may simultaneously normalize vascular tone and renal Na^+ handling. Finally, hyperleptinemia, which accompanies the MS, may contribute to the development of malignancies (176,177), possibly via transactivating the EGFR (165,166); thus, inhibiting EGFR could simultaneously reduce the risk of cancer in hyperleptinemic states.

EGFR signaling is also affected by less specific antihypertensive therapies such as RAAS inhibitors, ET-1 receptor antagonists and antioxidants (Table 4), some of them are well established antihypertensive drugs or are known to reduce blood pressure in experimental models. The effect of these treatments on EGFR was, however, addressed only in few studies. Angiotensin-converting enzyme inhibitor, imidapril, AT_1 receptor antagonist, losartan, and aldosterone antagonist, spironolactone, have been shown to reduce EGFR phosphorylation level in some, but not all, studies (Table 4). However, each of these drugs has EGFR-independent effects, so the causal relationship between inhibiting EGFR and lowering BP remains unclear. ERK inhibitor, PD98059, has been consistently demonstrated to reduce BP in several models of hypertension (136,179,180), but ERKs are activated by factors other than EGFR, so ERK inhibitors may be more effective than EGFR inhibitors. Finally, NOX inhibitor, apocynin, reduces blood pressure in many experimental models, but its effect on EGFR phosphorylation *in vivo* is controversial (136,178). In theory, other strategies may also be undertaken to inhibit EGFR in hypertension, such as cholesterol depletion in caveolae by drugs like statins, AT_1 receptor antagonists blocking selectively its G-protein independent interaction with EGFR, farnesyltransferase inhibitors which inhibit farnesylation of Ras proteins, or c-Abl inhibitors such as imatinib (Glyvec) currently used in the treatment of chronic myelogenous leukemia.

CONCLUSION AND PERSPECTIVES

Data presented above strongly suggest that EGFR is involved in the pathogenesis of arterial hypertension, at least in some animal models. EGFR is abundantly expressed in the vascular wall and renal tubules, and this receptor – either activated by its peptide ligand(s) or transactivated by factors such as ROS, angiotensin II or leptin – may elicit vasoconstriction and renal Na^+ retention. Enhanced EGFR signaling has been observed in many animal models of hypertension, and inactivation of this receptor by genetic or pharmacological approaches reduces blood pressure in some of these models.

Nevertheless, many aspects remain to be clarified. It is unknown if EGFR plays any role in human hypertension. It

Table 4. *Targeting EGFR in experimental hypertension*

Experimental model	Species	Treatment	Effect of treatment on BP	Other outcomes	Ref.
Specific therapies					
Angiotensin II infusion for 14 days	rat	EGFR-AS (2 mg/kg total dose) in continuous i.v. infusion	↓*	↓ total and phosphorylated EGFR in the left ventricle	(173)
SHR	rat	EGFR-AS (1.5 mg/kg once a week for 2 months)	↓**	↓ total and phosphorylated EGFR in the left ventricle	(174)
L-NAME induced	rat	gefitinib	No change	↓ EGFR phosphorylation in the kidney ↓ renal lesions	(175)
Leptin-induced hypertension	rat	AG1478 PP2	↓	↓ EGFR phosphorylation in aorta and kidney	(136)
Angiotensin II infusion for 2 months	mice	WTACE2 (ADAM17/TACE inhibitor)	No change	Amelioration of renal lesions and EGFR phosphorylation in the kidney	(147)
Phenylephrine or Angiotensin II infusion for 7 days	mice	KB-R7785 (ADAM12 inhibitor)	No change	Amelioration of left ventricular hypertrophy	(31)
SHR	rat	Doxycycline (MMP inhibitor) for 5 weeks	↓	↓ HB-EGF shedding in mesenteric artery	(62)
Currently used hypotensive drugs					
3/4 nephrectomy	mice	Losartan (AT$_1$ antagonist)	↓	Reduction of renal EGFR phosphorylation, TACE activity and TGFα level	(147)
Aldosterone + 1% NaCl in drinking water	rat	Imidapril (ACE inhibitor)	No change	↓ EGFR phosphorylation in the left ventricle	(178)
SHR	rat	enalapril +atenolol	↓	↓ HB-EGF in the left ventricle, no effect on total EGFR***	(138)
Aldosterone + 1% NaCl in drinking water	rat	spironolactone	No change	↓ EGFR phosphorylation in the left ventricle	(178)
SHRSP	rat	spironolactone	↓	↓ EGFR mRNA in aorta	(145)
T2DM without obesity	rat	ABT-627 (ET$_A$ receptor antagonist)	↓	↓ EGFR phosphorylation in the kidney	(155)
Aldosterone + 1% NaCl in drinking water	rat	apocynin (NOX inhibitor)	No change	No change in EGFR phosphorylation in the left ventricle	(178)
Leptin-induced hypertension	rat	apocynin (NOX inhibitor)	↓	↓ EGFR phosphorylation in aorta and kidney	(136)
AII-induced	rat	PD98059	↓	↓ ERK phosphorylation in vasculature	(179)
DOCA-salt induced	rat	PD98059	↓	↓ ERK phosphorylation in vasculature	(180)
Leptin-induced hypertension	rat	PD98059	↓	↓ EGFR phosphorylation in aorta and kidney	(136)

*moderate decrease in comparison to AII-treated group, but not complete normalization
** reduction of SBP by 10% only in young but not in adult animals.
***no change in absolute level of EGFR mRNA was observed; EGFR phosphorylation was not studied

is also unclear if other ErbB receptors are transactivated by mechanisms similar to EGFR and, if so, whether these receptors are involved in the regulation of vascular tone and renal Na$^+$ transport. Currently, it is much too early to recommend EGFR inhibitors for the treatment of hypertension. The effect of these drugs on BP in humans must first be examined, possibly initially in a subset of patients with hypertension and cancers for which these drugs are currently indicated. However, even if positive response is observed, the concern of possible side effects will remain, especially if it is realized that EGFR kinase inhibitors may also target other ErbB receptors, particularly *in vivo* when compound concentration in all compartments cannot be strictly controlled. In this context, it should be noted that trastuzumab induces cardiomyopathy in a subset of patients (181).

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REFERENCES

1. Dreux AC, Lamb DJ, Modjtahedi H, Ferns GA. The epidermal growth factor receptors and their family of ligands: their putative role in atherogenesis. *Atherosclerosis* 2006; 186: 38-53.

2. Chan HW, Smith NJ, Hannan RD, Thomas WG. Tackling the EGFR in pathological tissue remodelling. *Pulm Pharmacol Ther* 2006; 19: 74-78.

3. Downey JM, Davis AM, Cohen MV. Signaling pathways in ischemic preconditioning. *Heart Fail Rev* 2007; 12: 181-188.

4. Ushiro H, Cohen S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. *J Biol Chem* 1980; 255: 8363-8365.

5. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, *et al*. Close similarity of epidermal growth factor receptor and *v-erb-B* oncogene protein sequences. *Nature* 1984; 307: 521-527.

6. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, *et al*. The *neu* oncogene: an *erb-B*-related gene encoding a 185,000-Mr tumour antigen. *Nature* 1984; 312: 513-516.

7. Drebin JA, Stern DF, Link VC, Weinberg RA, Greene MI. Monoclonal antibodies identify a cell-surface antigen associated with an activated cellular oncogene. *Nature* 1984; 312: 545-548.

8. Bargmann CI, Weinberg RA. Oncogenic activation of the *neu*-encoded receptor protein by point mutation and deletion. *EMBO J* 1988; **7**: 2043-2052.

9. Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, *et al*. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. *Mol Cell* 2003; 12: 541-552.

10. Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, *et al*. ErbB receptors: from oncogenes to targeted cancer therapies. *J Clin Invest* 2007; 117: 2051-2058.

11. Lee SR, Kwon KS, Kim SR, Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J Biol Chem* 1998; 273: 15366-15372.

12. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J Biol Chem* 1962; 237: 1555-1562.

13. Gregory H. Isolation and structure of urogastrone and its relationship to epidermal growth factor. *Nature* 1975; 257: 325-327.

14. de Larco JE, Todaro GJ. Growth factors from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci U S A* 1978; 75: 4001-4005.

15. Raab G, Klagsbrun M. Heparin-binding EGF-like growth factor. *Biochim Biophys Acta* 1997; 1333: F179-F199.

16. Iwamoto R, Mekada E. Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev* 2000; 11: 335-344.

17. Nishi E, Klagsbrun M. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a mediator of multiple physiological and pathological pathways. *Growth Factors* 2004; 22: 253-260.

18. Berasain C, Castillo J, Perugorría MJ, Prieto J, Avila MA. Amphiregulin: a new growth factor in hepatocarcinogenesis. *Cancer Lett* 2007; 254: 30-41.

19. Dunbar AJ, Goddard C. Structure-function and biological role of betacellulin. *Int J Biochem Cell Biol* 2000; 32: 805-815.

20. Takahashi M, Hayashi K, Yoshida K, Ohkawa Y, Komurasaki T, Kitabatake A, *et al*. Epiregulin as a major autocrine/paracrine factor released from ERK- and p38MAPK-activated vascular smooth muscle cells. *Circulation* 2003; 108: 2524-2529.

21. Garratt AN. "To erb-B or not to erb-B..." Neuregulin-1/ErbB signaling in heart development and function. *J*

Mol Cell Cardiol 2006; 41: 215-218.

22. Breuleux M. Role of heregulin in human cancer. *Cell Mol Life Sci* 2007; 64: 2358-2377.

23. Ohtsu H, Dempsey PJ, Eguchi S. ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am J Physiol Cell Physiol* 2006; 291: C1-C10.

24. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 1996; 379: 557-560.

25. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, *et al*. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 1999; 402: 884-888.

26. Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr[845] and Tyr[1101] is associated with modulation of receptor function. *J Biol Chem* 1999; 274: 8335-8343.

27. Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, *et al*. Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. *Nature* 1997; 390: 91-96.

28. Frank GD, Mifune M, Inagami T, Ohba M, Sasaki T, Higashiyama S, *et al*. Distinct mechanisms of receptor and nonreceptor tyrosine kinase activation by reactive oxygen species in vascular smooth muscle cells: role of metalloprotease and protein kinase C-δ. *Mol Cell Biol* 2003; 23: 1581-1589.

29. Murasawa S, Mori Y, Nozawa Y, Gotoh N, Shibuya M, Masaki H, *et al*. Angiotensin II type 1 receptor-induced extracellular signal-regulated protein kinase activation is mediated by Ca^{2+}/calmodulin-dependent transactivation of epidermal growth factor receptor. *Circ Res* 1998; 82: 1338-1348.

30. Gunasegaram S, Haworth RS, Hearse DJ, Avkiran M. Regulation of sarcolemmal Na^+/H^+ exchanger activity by angiotensin II in adult rat ventricular myocytes: opposing actions via AT_1 versus AT_2 receptors. *Circ Res* 1999; 85: 919-930.

31. Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, *et al*. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med* 2002; 8: 35-40.

32. Eguchi S, Numaguchi K, Iwasaki H, Matsumoto T, Yamakawa T, Utsunomiya H, *et al*. Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *J Biol Chem* 1998; 273: 8890-8896.

33. Eguchi S, Iwasaki H, Hirata Y, Frank GD, Motley ED, Yamakawa T, *et al*. Epidermal growth factor receptor is indispensable for c-Fos expression and protein synthesis by angiotensin II. *Eur J Pharmacol* 1999; 376: 203-206.

34. Eguchi S, Iwasaki H, Inagami T, Numaguchi K, Yamakawa T, Motley ED, *et al*. Involvement of PYK2 in angiotensin II signaling of vascular smooth muscle cells. *Hypertension* 1999; 33: 201-206.

35. Bokemeyer D, Schmitz U, Kramer HJ. Angiotensin II-induced growth of vascular smooth muscle cells requires an Src-dependent activation of the epidermal growth factor receptor. *Kidney Int* 2000; 58: 549-558.

36. Eguchi S, Dempsey PJ, Frank GD, Motley ED, Inagami T. 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. *J Biol Chem* 2001; 276: 7957-7962.

37. Ushio-Fukai M, Griendling KK, Becker PL, Hilenski L, Halleran S, Alexander RW. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2001; 21: 489-495.

38. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 2002; 91: 406-413.

39. Saito S, Frank GD, Motley ED, Dempsey PJ, Utsunomiya H, Inagami T, et al. Metalloprotease inhibitor blocks angiotensin II-induced migration through inhibition of epidermal growth factor receptor transactivation. *Biochem Biophys Res Commun* 2002; 294: 1023-1029.

40. Voisin L, Foisy S, Giasson E, Lambert C, Moreau P, Meloche S. EGF receptor transactivation is obligatory for protein synthesis stimulation by G protein-coupled receptors. *Am J Physiol Cell Physiol* 2002; 283: C446-C455.

41. Ginnan R, Singer HA. CaM kinase II-dependent activation of tyrosine kinases and ERK1/2 in vascular smooth muscle. *Am J Physiol Cell Physiol* 2002; 282: C754-C761.

42. Ohtsu H, Dempsey PJ, Frank GD, Brailoiu E, Higuchi S, Suzuki H, *et al*. ADAM17 mediates epidermal growth factor receptor transactivation and vascular smooth muscle cell hypertrophy induced by angiotensin II. *Arterioscler Thromb Vasc Biol* 2006; 26: e133-e137.

43. Touyz RM, Cruzado M, Tabet F, Yao G, Salomon S, Schiffrin EL. Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. *Can J Physiol Pharmacol* 2003; 81: 159-167.

44. Yang X, Zhu MJ, Sreejayan N, Ren J, Du M. Angiotensin II promotes smooth muscle cell proliferation and migration through release of heparin-binding epidermal growth factor and activation of EGF-receptor pathway. *Mol Cells* 2005; 20: 263-270.

45. Li F, Malik KU. Angiotensin II-induced Akt activation through the epidermal growth factor receptor in vascular smooth muscle cells is mediated by phospholipid metabolites derived by activation of phospholipase D. *J Pharmacol Exp Ther* 2005; 312: 1043-1054.

46. Miura S, Zhang J, Matsuo Y, Saku K, Karnik SS. Activation of extracellular signal-activated kinase by angiotensin II-induced Gq-independent epidermal growth factor receptor transactivation. *Hypertens Res* 2004; 27: 765-770.

47. Fujiyama S, Matsubara H, Nozawa Y, Maruyama K, Mori Y, Tsutsumi Y, *et al*. Angiotensin AT_1 and AT_2 receptors differentially regulate angiopoietin-2 and vascular endothelial growth factor expression and angiogenesis by modulating heparin binding-epidermal growth factor (EGF)-mediated EGF receptor transactivation. *Circ Res* 2001; 88: 22-29.

48. Montiel M, de la Blanca EP, Jiménez E. Angiotensin II induces focal adhesion kinase/paxillin phosphorylation and cell migration in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 2005; 327: 971-978.

49. Che Q, Carmines PK. Angiotensin II triggers EGFR tyrosine kinase-dependent Ca^{2+} influx in afferent arterioles. *Hypertension* 2002; 40: 700-706.

50. Kim S, Zhan Y, Izumi Y, Yasumoto H, Yano M, Iwao H. In vivo activation of rat aortic platelet-derived growth factor and epidermal growth factor receptors by angiotensin II and hypertension. *Arterioscler Thromb Vasc Biol* 2000; 20: 2539-2545.

51. Ushio-Fukai M, Zuo L, Ikeda S, Tojo T, Patrushev NA, Alexander RW. cAbl Tyrosine kinase mediates reactive oxygen species- and caveolin-dependent AT1 receptor signaling in vascular smooth muscle: role in vascular hypertrophy. *Circ Res*, 2005; 97: 829-836.

52. Beaucage P, Moreau P. EGF receptor transactivation in angiotensin II and endothelin control of vascular protein synthesis in vivo. *J Cardiovasc Pharmacol* 2004; 44: S20-S23.

53. Seta K, Sadoshima J. Phosphorylation of tyrosine 319 of the angiotensin II type 1 receptor mediates angiotensin II-induced trans-activation of the epidermal growth factor receptor. *J Biol Chem* 2003; 278: 9019-9026.

54. Yee DK, Suzuki A, Luo L, Fluharty SJ. Identification of structural determinants for G protein-independent activation of mitogen-activated protein kinases in the seventh transmembrane domain of the angiotensin II type 1 receptor. *Mol Endocrinol* 2006; 20: 1924-1934.

55. Zhai P, Galeotti J, Liu J, Holle E, Yu X, Wagner T, *et al*. An angiotensin II type 1 receptor mutant lacking epidermal growth factor receptor transactivation does not induce angiotensin II-mediated cardiac hypertrophy. *Circ Res* 2006; 99: 528-536.

56. Konishi A, Berk BC. Epidermal growth factor receptor transactivation is regulated by glucose in vascular smooth muscle cells. *J Biol Chem* 2003; 278: 35049-35056.

57. Carey RM. Update on the role of the AT_2 receptor. *Curr Opin Nephrol Hypertens* 2005; 14: 67-71.

58. Shibasaki Y, Matsubara H, Nozawa Y, Mori Y, Masaki H, Kosaki A, *et al*. Angiotensin II type 2 receptor inhibits epidermal growth factor receptor transactivation by increasing association of SHP-1 tyrosine phosphatase. *Hypertension* 2001; 38: 367-372.

59. Ushio-Fukai M, Hilenski L, Santanam N, Becker PL, Ma Y, Griendling KK, et al. Cholesterol depletion inhibits epidermal growth factor receptor transactivation by angiotensin II in vascular smooth muscle cells: role of cholesterol-rich microdomains and focal adhesions in angiotensin II signaling. *J Biol Chem* 2001; 276: 48269-48275.

60. Zuo L, Ushio-Fukai M, Ikeda S, Hilenski L, Patrushev N, Alexander RW. Caveolin-1 is essential for activation of Rac1 and NAD(P)H oxidase after angiotensin II type 1 receptor stimulation in vascular smooth muscle cells: role in redox signaling and vascular hypertrophy. *Arterioscler Thromb Vasc Biol* 2005; 25: 1824-1830.

61. Zhang H, Chalothorn D, Jackson LF, Lee DC, Faber JE.

Transactivation of epidermal growth factor receptor mediates catecholamine-induced growth of vascular smooth muscle. *Circ Res* 2004; 95: 989-997.

62. Hao L, Du M, Lopez-Campistrous A, Fernandez-Patron C. Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ Res* 2004; 94: 68-76.

63. Iwasaki H, Eguchi S, Marumo F, Hirata Y. Endothelin-1 stimulates DNA synthesis of vascular smooth-muscle cells through transactivation of epidermal growth factor receptor. *J Cardiovasc Pharmacol* 1998; 31: S182-S184.

64. Iwasaki H, Eguchi S, Ueno H, Marumo F, Hirata Y. Endothelin-mediated vascular growth requires p42/p44 mitogen-activated protein kinase and p70 S6 kinase cascades via transactivation of epidermal growth factor receptor. *Endocrinology* 1999; 140: 4659-4668.

65. Kodama H, Fukuda K, Takahashi T, Sano M, Kato T, Tahara S, *et al*. Role of EGF receptor and Pyk2 in endothelin-1-induced ERK activation in rat cardiomyocytes. 2002; 34: 139-150.

66. Kawanabe Y, Hashimoto N, Masaki T. Characterization of Ca^{2+} channels involved in ET-1-induced transactivation of EGF receptors. *Am J Physiol Heart Circ Physiol* 2002; 283: H2671-H2675.

67. Flamant M, Tharaux PL, Placier S, Henrion D, Coffman T, Chatziantoniou C, et al. Epidermal growth factor receptor trans-activation mediates the tonic and fibrogenic effects of endothelin in the aortic wall of transgenic mice. *FASEB J* 2003; 17: 327-329.

68. Kawanabe Y, Nozaki K, Hashimoto N, Masaki T. Involvement of extracellular Ca^{2+} influx and epidermal growth factor receptor tyrosine kinase transactivation in endothelin-1-induced arachidonic acid release. *Br J Pharmacol* 2003; 139: 1516-1522.

69. Kalidindi SR, Tang WH, Francis GS. Drug insight: aldosterone-receptor antagonists in heart failure – the journey continues. *Nat Clin Pract Cardiovasc Med* 2007; 4: 368-378.

70. Grossmann C, Gekle M. Non-classical actions of the mineralocorticoid receptor: misuse of EGF receptors? *Mol Cell Endocrinol* 2007; 277: 6-12.

71. Krug AW, Grossmann C, Schuster C, Freudinger R, Mildenberger S, Govindan MV, et al. Aldosterone stimulates epidermal growth factor receptor expression. *J Biol Chem* 2003; 278: 43060-43066.

72. Grossmann C, Krug AW, Freudinger R, Mildenberger S, Voelker K, Gekle M. Aldosterone-induced EGFR expression: interaction between the human mineralocorticoid receptor and the human EGFR promoter. *Am J Physiol Endocrinol Metab* 2007; 292: E1790-E1800.

73. Mazak I, Fiebeler A, Muller DN, Park JK, Shagdarsuren E, Lindschau C, *et al*. Aldosterone potentiates angiotensin II-induced signaling in vascular smooth muscle cells. *Circulation* 2004; 109: 2792-2800.

74. Min LJ, Mogi M, Li JM, Iwanami J, Iwai M, Horiuchi M. Aldosterone and angiotensin II synergistically induce mitogenic response in vascular smooth muscle cells. *Circ Res* 2005; 97: 434-442.

75. Sugiyama T, Yoshimoto T, Tsuchiya K, Gochou N, Hirono Y, Tateno T, *et al*. Aldosterone induces angiotensin converting enzyme gene expression via a JAK2-dependent pathway in rat endothelial cells. *Endocrinology* 2005; 146: 3900-3906.

76. Sumi S, Inoue K, Hosotani R, Kogire M, Doi R, Yun M, *et al*. Effect of human epidermal growth factor (hEGF) on splanchnic circulation in dogs. *Life Sci* 1990; 47: 1115-1119.

77. Gan BS, MacCannell KL, Hollenberg MD. Epidermal growth factor-urogastrone causes vasodilatation in the anesthetized dog. *J Clin Invest* 1987; 80: 199-206.

78. Keiser JA, Ryan MJ. Hemodynamic effects of epidermal growth factor in conscious rats and monkeys. *Proc Natl Acad Sci U S A* 1996; 93: 4957-4961.

79. Gan BS, Bolt GR, Hollenberg MD, MacCannell KL. Species variation in the vascular effects of epidermal growth factor-urogastrone. *Proc West Pharmacol Soc* 1986; 29: 483-484.

80. Berk BC, Brock TA, Webb RC, Taubman MB, Atkinson WJ, Gimbrone MA, et al. Epidermal growth factor, a vascular smooth muscle mitogen, induces rat aortic contraction. *J Clin Invest* 1985; 75: 1083-1086.

81. Rippetoe PE, Olson JW, Altiere RJ, Pauly TH, Gillespie MN. Epidermal growth factor augments reactivity to angiotensin II in the rat pulmonary circulation. *Am Rev Respir Dis* 1989; 140: 821-824.

82. Merkel LA, Rivera LM, Colussi DJ, Perrone MH. Inhibition of EGF-induced vasoconstriction in isolated rabbit aortic rings with the tyrosine kinase inhibitor RG50864. *Biochem Biophys Res Commun* 1993; 192: 1319-1326.

83. deBlois D, Drapeau G, Petitclerc E, Marceau F. Syner-

gism between the contractile effect of epidermal growth factor and that of des-Arg9-bradykinin or of alpha-thrombin in rabbit aortic rings. *Br J Pharmacol* 1992; 105: 959-967.

84. Namiki A, Akatsuka N. Vascular smooth muscle relaxation induced by epidermal growth factor is endothelium-dependent. *Eur J Pharmacol* 1990; 180: 247-254.

85. Hao L, Nishimura T, Wo H, Fernandez-Patron C. Vascular responses to α1-adrenergic receptors in small rat mesenteric arteries depend on mitochondrial reactive oxygen species. *Arterioscler Thromb Vasc Biol* 2006; 26: 819-825.

86. Chansel D, Ciroldi M, Vandermeersch S, Jackson LF, Gomez AM, Henrion D, *et al*. Heparin binding EGF is necessary for vasospastic response to endothelin. *FASEB J* 2006; 20: 1936-1938.

87. Kawanabe Y, Masaki T, Hashimoto N. Involvement of epidermal growth factor receptor-protein tyrosine kinase transactivation in endothelin-1-induced vascular contraction. *J Neurosurg* 2004; 100: 1066-1071.

88. Carmines PK, Fallet RW, Che Q, Fujiwara K. Tyrosine kinase involvement in renal arteriolar constrictor responses to angiotensin II. *Hypertension* 2001; 37: 569-573.

89. Escano CS, Keever LB, Gutweiler AA, Andresen BT. Angiotensin II activates ERK independently of receptor tyrosine kinases in renal smooth muscle cells: implications for blood pressure regulation. *J Pharmacol Exp Ther* 2008; 324: 34-42.

90. Lucchesi PA, Sabri A, Belmadani S, Matrougui K. Involvement of metalloproteinases 2/9 in epidermal growth factor receptor transactivation in pressure-induced myogenic tone in mouse mesenteric resistance arteries. *Circulation* 2004; 110: 3587-93.

91. Salido E, Barajas L, Lechago J, Laborde N, Fisher D. Immunocytochemical localization of epidermal growth factor in mouse kidney. *J Histochem Cytochem* 1986; 34: 1155-1160.

92. Salido EC, Yen PH, Shapiro LJ, Fisher DA, Barajas L. In situ hybridization of prepro-epidermal growth factor mRNA in the mouse kidney. *Am J Physiol* 1989; 256: F632-F638.

93. Skov Olsen P, Nexø E, Poulsen SS, Hansen HF, Kirkegaard P. Renal origin of rat urinary epidermal growth factor. *Regul Pept* 1984; 10: 37-45.

94. Liu JY, Lei WH, Brody AR, Hoyle GW. Investigation of tissue preparation conditions for non-radioactive in situ hybridization: localization of transforming growth factor-α message in rat kidney. *Histochem J* 1998; 30: 793-798.

95. Breyer MD, Redha R, Breyer JA. Segmental distribution of epidermal growth factor binding sites in rabbit nephron. *Am J Physiol* 1990; 259: F553-F558.

96. Harris RC, Hoover RL, Jacobson HR, Badr KF. Evidence for glomerular actions of epidermal growth factor in the rat. *J Clin Invest* 1988; 82**:** 1028-1039.

97. Howarth GS, Bastian SE, Dunbar AJ, Goddard C. Beta-cellulin promotes growth of the gastrointestinal organs and effects a diuresis in normal rats. *Growth Factors* 2003; 21: 79-86.

98. Gow CB, Phillips PA, Chandler KD, Moore GP. Hormonal, fluid, and electrolyte responses of sheep during chronic intravenous infusion of epidermal growth factor. *Am J Physiol* 1993; 265: R203-R210.

99. Gow CB, Grant SL, Phillips PA. Effects of indomethacin on epidermal growth factor-induced renal responses in sheep. *Clin Exp Pharmacol Physiol* 1997; 24: 680-685.

100. Antonipillai I. Epidermal growth factor is a potent inhibitor of renin secretion. *Hypertension* 1993; 21: 654-659.

101. Vehaskari VM, Hering-Smith KS, Moskowitz DW, Weiner ID, Hamm LL. Effect of epidermal growth factor on sodium transport in the cortical collecting tubule. *Am J Physiol* 1989; 256: F803-F809.

102. Muto S, Furuya H, Tabei K, Asano Y. Site and mechanism of action of epidermal growth factor in rabbit cortical collecting duct. *Am J Physiol* 1991; 260: F163-F169.

103. Shen JP, Cotton CU. Epidermal growth factor inhibits amiloride-sensitive sodium absorption in renal collecting duct cells. *Am J Physiol Renal Physiol* 2003; 284: F57-F64.

104. Grossmann C, Freudinger R, Mildenberger S, Krug AW, Gekle M. Evidence for epidermal growth factor receptor as negative-feedback control in aldosterone-induced Na^+ reabsorption. *Am J Physiol Renal Physiol* 2004; 286: F1226-F1231.

105. Tong Q, Stockand JD. Receptor tyrosine kinases mediate epithelial Na^+ channel inhibition by epidermal growth factor. *Am J Physiol Renal Physiol* 2005; 288: F150-F161.

106. Quigley R, Baum M. Effects of epidermal growth factor and transforming growth factor-α on rabbit proximal tubule solute transport. *Am J Physiol* 1994; 266: F459-F465.

107. Goodyer PR, Kachra Z, Bell C, Rozen R. Renal tubular cells are potential targets for epidermal growth factor. *Am J Physiol* 1988; 255: F1191-F1196.

108. Ruiz OS, Qiu YY, Cardoso LR, Arruda JA. Regulation of the renal Na-HCO$_3$ cotransporter: IX. Modulation by insulin, epidermal growth factor and carbachol. *Regul Pept* 1998; 77: 155-161.

109. Gekle M, Freudinger R, Mildenberger S, Silbernagl S. Aldosterone interaction with epidermal growth factor receptor signaling in MDCK cells. *Am J Physiol Renal Physiol* 2002; 282: F669-F679.

110. Gekle M, Freudinger R, Mildenberger S, Silbernagl S. Rapid actions of aldosterone on cells from renal epithelium: the possible role of EGF-receptor signaling. *Steroids* 2002; 67: 499-504.

111. Drumm K, Kress TR, Gassner B, Krug AW, Gekle M. Aldosterone stimulates activity and surface expression of NHE3 in human primary proximal tubule epithelial cells (RPTEC). *Cell Physiol Biochem* 2006; 17: 21-28.

112. Féraille E, Doucet A. Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control. *Physiol Rev* 2001; 81: 345-418.

113. Doris PA. Renal proximal tubule sodium transport and genetic mechanisms of essential hypertension. *J Hypertens* 2000; 18: 509-519.

114. Féraille E, Carranza ML, Rousselot M, Favre H. Modulation of Na$^+$,K$^+$-ATPase activity by a tyrosine phosphorylation process in rat proximal convoluted tubule. *J Physiol* 1997; 498: 99-108.

115. Bełtowski J, Wójcicka G, Trzeciak J, Marciniak A. H$_2$O$_2$ and Src-dependent transactivation of the EGF receptor mediates the stimulatory effect of leptin on renal ERK and Na$^+$,K$^+$-ATPase. *Peptides* 2006; 27: 3234-3244.

116. Cui XL, Ding Y, Alexander LD, Bao C, Al-Khalili OK, Simonson M, *et al*. Oxidative signaling in renal epithelium: Critical role of cytosolic phospholipase A2 and p38(SAPK). *Free Radic Biol Med* 2006; 41: 213-221.

117. Chen J, Chen JK, Neilson EG, Harris RC. Role of EGF receptor activation in angiotensin II-induced renal epithelial cell hypertrophy. *J Am Soc Nephrol* 2006; 17: 1615-1623.

118. Alexander LD, Ding Y, Alagarsamy S, Cui XL, Douglas JG. Arachidonic acid induces ERK activation via Src SH2 domain association with the epidermal growth factor receptor. *Kidney Int* 2006; 69: 1823-1832.

119. Lee JS, Kim SY, Kwon CH, Kim YK. EGFR-dependent ERK activation triggers hydrogen peroxide-induced apoptosis in OK renal epithelial cells. *Arch Toxicol* 2006; 80: 337-346.

120. Dong J, Ramachandiran S, Tikoo K, Jia Z, Lau SS, Monks TJ. EGFR-independent activation of p38 MAPK and EGFR-dependent activation of ERK1/2 are required for ROS-induced renal cell death. *Am J Physiol Renal Physiol* 2004; 287: F1049-F1058.

121. Zhuang S, Schnellmann RG. H$_2$O$_2$-induced transactivation of EGF receptor requires Src and mediates ERK1/2, but not Akt, activation in renal cells. *Am J Physiol Renal Physiol* 2004; 286: F858-F865.

122. Zhuang S, Yan Y, Han J, Schnellmann RG. p38 kinase-mediated transactivation of the epidermal growth factor receptor is required for dedifferentiation of renal epithelial cells after oxidant injury. *J Biol Chem* 2005; 280: 21036-21042.

123. Cheng-Hsien C, Yung-Ho H, Yuh-Mou S, Chun-Cheng H, Horng-Mo L, Huei-Mei H, *et al*. Src homology 2-containing phosphotyrosine phosphatase regulates endothelin-1-induced epidermal growth factor receptor transactivation in rat renal tubular cell NRK-52E. *Pflugers Arch* 2006; 452: 16-24.

124. Cussac D, Schaak S, Denis C, Paris H. α$_{2B}$-Adrenergic receptor activates MAPK via a pathway involving arachidonic acid metabolism, matrix metalloproteinases, and epidermal growth factor receptor transactivation. *J Biol Chem* 2002; 277: 19882-19888.

125. Saad S, Stevens VA, Wassef L, Poronnik P, Kelly DJ, Gilbert RE, *et al*. High glucose transactivates the EGF receptor and up-regulates serum glucocorticoid kinase in the proximal tubule. *Kidney Int* 2005; 68: 985-997.

126. Reich H, Tritchler D, Herzenberg AM, Kassiri Z, Zhou X, Gao W, *et al*. Albumin activates ERK via EGF receptor in human renal epithelial cells. *J Am Soc Nephrol* 2005; 16: 1266-1278.

127. Alexander LD, Alagarsamy S, Douglas JG. Cyclic stretch-induced cPLA2 mediates ERK 1/2 signaling in rabbit proximal tubule cells. *Kidney Int* 2004; 65: 551-563.

128. Garvin JL, Ortiz PA. The role of reactive oxygen species in the regulation of tubular function. *Acta Physiol Scand* 2003; 179: 225-232.

129. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? *Am J Physiol Regul Integr Comp Physiol* 2005; 289: R913-R935.

130. Silva GB, Ortiz PA, Hong NJ, Garvin JL. Superoxide

stimulates NaCl absorption in the thick ascending limb via activation of protein kinase C. *Hypertension* 2006; 48: 467-472.

131. Markadieu N, Crutzen R, Blero D, Erneux C, Beauwens R. Hydrogen peroxide and epidermal growth factor activate phosphatidylinositol 3-kinase and increase sodium transport in A6 cell monolayers. *Am J Physiol Renal Physiol* 2005; 288: F1201-F1212.

132. Marciniak A, Jamroz-Wiśniewska A, Borkowska E, Bełtowski J. Time-dependent effect of leptin on renal Na^+,K^+-ATPase activity. *Acta Biochim Pol* 2005; 52: 803-809.

133. Makino A, Skelton MM, Zou AP, Cowley AW. Increased renal medullary H_2O_2 leads to hypertension. *Hypertension* 2003; 42: 25-30.

134. Chen YF, Cowley AW, Zou AP. Increased H_2O_2 counteracts the vasodilator and natriuretic effects of superoxide dismutation by tempol in renal medulla. *Am J Physiol Regul Integr Comp Physiol* 2003; 285: R827-R833.

135. Jamroz-Wiśniewska A, Wójcicka G, Łowicka E, Książek M, Bełtowski J. Transactivation of epidermal growth factor receptor in vascular and renal systems in rats with experimental hyperleptinemia: Role in leptin-induced hypertension. *Biochem Pharmacol* 2008; In press.

136. Wójcicka G, Jamroz-Wiśniewska A, Widomska S, Książek M, Bełtowski J. Role of extracellular signal-regulated kinases (ERK) in leptin-induced hypertension. *Life Sci* 2008; 82: In press.

137. Saltis J, Agrotis A, Bobik A. Differences in growth characteristics of vascular smooth muscle from spontaneously hypertensive and Wistar-Kyoto rats are growth factor dependent. *J Hypertens* 1993; 11: 629-637.

138. Fujino T, Hasebe N, Fujita M, Takeuchi K, Kawabe J, Tobise K, *et al*. Enhanced expression of heparin-binding EGF-like growth factor and its receptor in hypertrophied left ventricle of spontaneously hypertensive rats. *Cardiovasc Res* 1998; 38: 365-374.

139. Touyz RM, Wu XH, He G, Salomon S, Schiffrin EL. Increased angiotensin II-mediated Src signaling via epidermal growth factor receptor transactivation is associated with decreased C-terminal Src kinase activity in vascular smooth muscle cells from spontaneously hypertensive rats. *Hypertension* 2002; 39: 479-485.

140. Florian JA, Dorrance A, Webb RC, Watts SW. Mineralocorticoids upregulate arterial contraction to epidermal growth factor. *Am J Physiol Regul Integr Comp Physiol*

2001; 281: R878-R886.

141. Swaminathan N, Sambhi MP. Induction of high affinity epidermal growth factor binding in the aorta of Dahl hypertensive rats fed with high salt diet. *Hypertens Res* 1996; 19: 65-68.

142. Ying WZ, Sanders PW. Enhanced expression of EGF receptor in a model of salt-sensitive hypertension. *Am J Physiol Renal Physiol* 2005; 289: F314-F321.

143. Swaminathan N, Vincent M, Sassard J, Sambhi MP. Elevated epidermal growth factor receptor levels in hypertensive Lyon rat kidney and aorta. *Clin Exp Pharmacol Physiol* 1996; 23: 793-796.

144. Kim S, Zhan Y, Izumi Y, Iwao H. Cardiovascular effects of combination of perindopril, candesartan, and amlodipine in hypertensive rats. *Hypertension* 2000; 35: 769-774.

145. Dorrance AM, Osborn HL, Grekin R, Webb RC. Spironolactone reduces cerebral infarct size and EGF-receptor mRNA in stroke-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2001; 281: R944-R950.

146. Sambhi MP, Swaminathan N, Wang H, Rong H. Increased EGF binding and EGFR mRNA expression in rat aorta with chronic administration of pressor angiotensin II. *Biochem Med Metab Biol* 1992; 48: 8-18.

147. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, *et al*. Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med* 2005; 11: 867-874.

148. Florian JA, Watts SW. Epidermal growth factor: a potent vasoconstrictor in experimental hypertension. *Am J Physiol* 1999; 276: H976-H983.

149. Northcott C, Florian JA, Dorrance A, Watts SW. Arterial epidermal growth factor receptor expression in deoxycorticosterone acetate-salt hypertension. *Hypertension* 2001; 38: 1337-1341.

150. Kim J, Lee CK, Park HJ, Kim HJ, So HH, Lee KS, *et al*. Epidermal growth factor induces vasoconstriction through the phosphatidylinositol 3-kinase-mediated mitogen-activated protein kinase pathway in hypertensive rats. *J Pharmacol Sci* 2006; 101: 135-143.

151. Chaldakov GN, Fiore M, Tonchev AB, Dimitrov D, Pancheva R, Rancic G, *et al*. Homo obesus: a metabotrophin-deficient species. Pharmacology and nutrition insight. *Curr Pharm Des* 2007; 13: 2176-2179.

152. Benter IF, Yousif MH, Griffiths SM, Benboubetra M, Akhtar S. Epidermal growth factor receptor tyrosine kinase-mediated signalling contributes to diabetes-in-

duced vascular dysfunction in the mesenteric bed. *Br J Pharmacol* 2005; 145: 829-836.

153. Yousif MH, Benter IF, Akhtar S. The role of tyrosine kinase-mediated pathways in diabetes-induced alterations in responsiveness of rat carotid artery. *Auton Autacoid Pharmacol* 2005; 25: 69-78.

154. Benter IF, Yousif MH, Hollins AJ, Griffiths SM, Akhtar S. Diabetes-induced renal vascular dysfunction is normalized by inhibition of epidermal growth factor receptor tyrosine kinase. *J Vasc Res* 2005; 42: 284-291.

155. Portik-Dobos V, Harris AK, Song W, Hutchinson J, Johnson MH, Imig JD, et al. Endothelin antagonism prevents early EGFR transactivation but not increased matrix metalloproteinase activity in diabetes. *Am J Physiol Regul Integr Comp Physiol* 2006; 290: R435-R441.

156. Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Curr Pharm Des* 2003; 9: 1023-1031.

157. Bełtowski J. Role of leptin in blood pressure regulation and arterial hypertension. *J Hypertens* 2006; 24: 789-801.

158. Tümer N, Erdös B, Matheny M, Cudykier I, Scarpace PJ. Leptin antagonist reverses hypertension caused by leptin overexpression, but fails to normalize obesity-related hypertension. *J Hypertens* 2007; 25: 2471-2478.

159. Bełtowski J. Central vs. peripheral leptin excess in the pathogenesis of obesity- associated hypertension. *J Hypertens* 2008; 26: In press.

160. Bełtowski J, Wójcicka G, Marciniak A, Jamroz A. Oxidative stress, nitric oxide production, and renal sodium handling in leptin-induced hypertension. *Life Sci* 2004; 74: 2987-3000.

161. Blackshear PJ, Stumpo DJ, Kennington EA, Tuttle JS, Orth DN, Thompson KL, et al. Decreased levels of hepatic epidermal growth factor receptors in obese hyperglycemic rodents. *J Biol Chem* 1987; 262: 12356-12364.

162. Adachi H, Kurachi H, Homma H, Adachi K, Imai T, Morishige K, et al. Epidermal growth factor promotes adipogenesis of 3T3-L1 cell in vitro. *Endocrinology* 1994; 135: 1824-1830.

163. Matsumoto S, Kishida K, Shimomura I, Maeda N, Nagaretani H, Matsuda M, et al. Increased plasma HB-EGF associated with obesity and coronary artery disease. *Biochem Biophys Res Commun* 2002; 292: 781-786.

164. Vacaresse N, Lajoie-Mazenc I, Augé N, Suc I, Frisach MF, Salvayre R, et al. Activation of epithelial growth factor receptor pathway by unsaturated fatty acids. *Circ Res* 1999; 85: 892-899.

165. Shida D, Kitayama J, Mori K, Watanabe T, Nagawa H. Transactivation of epidermal growth factor receptor is involved in leptin-induced activation of janus-activated kinase 2 and extracellular signal-regulated kinase 1/2 in human gastric cancer cells. *Cancer Res* 2005; 65: 9159-9163.

166. Ogunwobi O, Mutungi G, Beales IL. Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH_2-terminal kinase activation. *Endocrinology* 2006; 147: 4505-4516.

167. Singh P, Hoffmann M, Wolk R, Shamsuzzaman AS, Somers VK. Leptin induces C-reactive protein expression in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2007; 27: e302-e307.

168. Bełtowski J, Jamroz-Wiśniewska A, Borkowska E, Wójcicka G. Up-regulation of renal Na^+, K^+-ATPase: the possible novel mechanism of leptin-induced hypertension. *Pol J Pharmacol* 2004; 56: 213-222.

169. Bełtowski J, Wójcicka G, Jamroz-Wiśniewska A, Borkowska E, Marciniak A. Antioxidant treatment normalizes nitric oxide production, renal sodium handling and blood pressure in experimental hyperleptinemia. *Life Sci* 2005; 77: 1855-1868.

170. Bełtowski J, Jamroz-Wiśniewska A, Borkowska E, Nazar J, Marciniak A. Antioxidant treatment normalizes renal Na^+,K^+-ATPase activity in leptin-treated rats. *Pharmacol Rep* 2005; 57: 219-228.

171. Marciniak A, Borkowska E, Kędra A, Rychlik M, Bełtowski J. Time-dependent transition from H_2O_2-extracellular signal-regulated kinase- to O_2^--nitric oxide-dependent mechanisms in the stimulatory effect of leptin on renal Na^+,K^+,-ATPase in the rat. *Clin Exp Pharmacol Physiol* 2006; 33: 1216-1224.

172. Chao HH, Hong HJ, Liu JC, Lin JW, Chen YL, Chiu WT, et al. Leptin stimulates endothelin-1 expression via extracellular signal-regulated kinase by epidermal growth factor receptor transactivation in rat aortic smooth muscle cells. *Eur J Pharmacol* 2007; 573: 49-54.

173. Kagiyama S, Eguchi S, Frank GD, Inagami T, Zhang YC, Phillips MI. Angiotensin II-induced cardiac hypertrophy and hypertension are attenuated by epidermal growth fac-

tor receptor antisense. *Circulation* 2002; 106: 909-912.

174. Kagiyama S, Qian K, Kagiyama T, Phillips MI. Antisense to epidermal growth factor receptor prevents the development of left ventricular hypertrophy. *Hypertension* 2003; 41: 824-829.

175. François H, Placier S, Flamant M, Tharaux PL, Chansel D, Dussaule JC, *et al*. Prevention of renal vascular and glomerular fibrosis by epidermal growth factor receptor inhibition. *FASEB J* 2004; 18: 926-928.

176. Garofalo C, Surmacz E. Leptin and cancer. *J Cell Physiol* 2006; 207: 12-22.

177. Schäffler A, Schölmerich J, Buechler C. Mechanisms of disease: adipokines and breast cancer – endocrine and paracrine mechanisms that connect adiposity and breast cancer. *Nat Clin Pract Endocrinol Metab*. 2007; 3: 345-354.

178. Nakano S, Kobayashi N, Yoshida K, Ohno T, Matsuoka H. Cardioprotective mechanisms of spironolactone associated with the angiotensin-converting enzyme/epidermal growth factor receptor/extracellular signal-regulated kinases, NAD(P)H oxidase/lectin-like oxidized low-density lipoprotein receptor-1, and Rho-kinase pathways in aldosterone/salt-induced hypertensive rats. *Hypertens Res* 2005; 28: 925-936.

179. Muthalif MM, Karzoun NA, Gaber L, Khandekar Z, Benter IF, Saeed AE, *et al*. Angiotensin II-induced hypertension: contribution of Ras GTPase/Mitogen-activated protein kinase and cytochrome P450 metabolites. *Hypertension* 2000; 36: 604-609.

180. Muthalif MM, Benter IF, Khandekar Z, Gaber L, Estes A, Malik S, *et al*. Contribution of Ras GTPase/MAP kinase and cytochrome P450 metabolites to deoxycorticosterone-salt-induced hypertension. *Hypertension* 2000; 35: 457-463.

181. Lemmens K, Doggen K, De Keulenaer GW. Role of neuregulin-1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure. *Circulation* 2007; 116: 954-960.