Differential Trafficking and Desensitization of Human ET_A and ET_B Receptors Expressed in HEK 293 Cells

XIAOLING DAI[1] AND JAMES J. GALLIGAN

Department of Pharmacology and Toxicology and the Neuroscience Program, Michigan State University, East Lansing, MI 48824

Endothelin-1 (ET-1) is a vasoconstrictor peptide that acts on ET_A and ET_B receptors on smooth muscle cells (SMCs). Because vascular SMCs can express both receptors, it is difficult to study the localization and properties of each subtype. Therefore, we investigated the localization and function of ET_A and ET_B receptors transfected into HEK 293 cells. Immunocytochemistry was used to examine colocalization of ET receptors with the plasma membrane marker, pan cadherin. In cells transfected with ET_A receptors, 83 ± 2% of these receptors colocalized with pan cadherin. In ET_B receptor–transfected cells, 54 ± 2% of the receptor colocalized with pan cadherin. When ET_A and ET_B receptors were cotransfected, 97 ± 1% of ET_B receptors colocalized with ET_A receptors and 84 ± 2% of ET_B receptors colocalized with pan cadherin. ET-1 and sarafotoxin 6c (S6c, ET_B receptor agonist) increased $[Ca^{2+}]_i$ in cells transfected with ET_A or ET_B receptors; 100 n*M* of ET-1 and S6c caused maximal responses. When stimulated with ET-1, ET_B receptors desensitized faster ($t_{1/2}$ = 21 ± 1 sec) than ET_A receptors ($t_{1/2}$ = 48 ± 1 sec). S6c-induced increases in $[Ca^{2+}]_i$ desensitized in cells expressing ET_B receptors only ($t_{1/2}$ = 17 ± 1 s). Desensitization was eliminated in cells cotransfected with ET receptors. We conclude that ET_A receptors localize to the cell membrane, whereas ET_B receptors are in the membrane and intracellular compartments. Coexpressed ET receptors are in the membrane. ET_B receptors desensitize faster than ET_A receptors, but receptor coexpression eliminates desensitization. Finally, ET_A and ET_B receptors interact to change receptor trafficking which may modify ET receptor function in vascular SMCs coexpressing these receptors. Exp Biol Med 231:746–751, 2006

Key words: desensitization; endothelin receptor; trafficking

The study was funded by the National Institutes of Health grant P01HL70687 to J.J.G.

[1] To whom correspondence should be addressed at Department of Pharmacology and Toxicology, Life Science Building Room B308, Michigan State University, East Lansing, MI 48824. E-mail: daixiaol@msu.edu

Received September 26, 2005.
Accepted November 14, 2005.

1535-3702/06/2316-0746$15.00

Introduction

The endothelins (ETs) are a family of 21 amino acid peptides generated primarily by endothelial cells to produce contraction of vascular smooth muscle cells (SMCs). ET-1 plays a critical role in blood pressure maintenance and has been implicated in multiple cardiovascular functions and diseases, including hypertension and heart failure (1, 2). ET-1 levels are elevated in some hypertensive patients, especially in blacks, and the vasoconstrictive effects of ET-1 suggest that it may contribute to hypertension in these patients (3).

ET-1 binds to two receptor subtypes, ET_A and ET_B receptors, which are G-protein–coupled receptors (GPCR), to cause vasoconstriction (4). Messenger RNA and protein for ET receptors are found in SMCs (5). ET receptors have different recycling pathways after ligand stimulation. After internalization *via* caveolae or clathrin-coated pits, the ET_A receptor is recycled back to the cell surface (6, 7). In contrast, the ET_B receptor is internalized *via* a clathrin-dependent pathway and transported to late endosomes and lysosomes (6–9).

Although the dimerization of ET_A and ET_B receptor has never been proven, some experimental data fit well with the now widely accepted view that GPCRs can exist as heterodimers (10–13). Atypical ligand binding has been observed in cells coexpressing ET_A and ET_B receptors. In these studies, the ET_B receptor-selective ligand, sarafotoxin 6c (S6c), inhibits ^{125}I-ET-1 binding only in the presence of the ET_A receptor–selective antagonist, BQ123 in epithelial cells of the anterior pituitary (14). In astrocytes, ET_A and ET_B receptors cooperatively control ET-1 clearance, because only the combination of ET_A and ET_B receptor-selective antagonists, but not their individual application, increases ET-1 in the extracellular fluid (15).

Because vascular SMCs express both ET receptor subtypes, it is difficult to study the subcellular localization and functional properties of each subtype or interactions between receptors in SMCs. Therefore, we investigated the localization and function of ET_A or ET_B receptors transfected into HEK 293 cells. In these studies we attempted to

determine the function and localization of ET_A and ET_B receptors when they are expressed alone and the changes in localization and function when ET receptors are coexpressed.

Materials and Methods

Cell Culture. HEK 293 cells were obtained from American Type Culture Collection and maintained in advanced DMEM/F-12 medium supplemented with 10% fetal bovine serum and GluMax (Invitrogen, Carlsbad, CA) at 37°C in a 95% humidified air with 5% CO_2 incubator. Cells were passaged once every 3 days when they reached 90% confluence.

Transient and Stable Expression of ET Receptors. Plasmids (pcDNA3.1+, Invitrogen) containing the coding sequences of human ET receptors and human ET receptors with an N-terminal hemoagglutinin-epitope tag were purchased from University of Missouri, Rolla cDNA Resource Center (www.cdna.org). Cells growing on 35-mm Petri dishes at 90% confluency were transfected with 2.5 μg of plasmid DNA using 5 μl of the Lipofectamine transfection reagent (Invitrogen) following the manufacturer's guidelines.

Immunocytochemistry. Tissues were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS, 137 m*M* NaCl, 2.7 m*M* KCl, 1.5 m*M* KH_2PO_4, 8.0 m*M* Na_2HPO_4, pH 7.4) for 20 mins at room temperature. Cells were blocked and permeabilized with 5% goat serum and 0.1% Triton X-100 in PBS and incubated with primary antibodies. Antibodies for the ET_A receptor and ET_B receptor were purchased from Alomone (Jerusalem, Israel) and the antibody for pan cadherin was obtained from Abcam (Cambridge, MA). Primary antibodies in PBS with 5% serum were applied and incubated at 37°C for 2 hr. Cells were rinsed in PBS and incubated with secondary antibodies conjugated with sulfosuccinimidyl-7-amino-4-methylcoumarin-3-acetic acid (AMCA), fluorescein isothiocyanate (FITC), or Cy-3 (Jackson ImmunoResearch, West Grove, PA) in PBS for 1 hr. Cells then were rinsed in PBS, mounted on slides, and observed with a Nikon Eclipse fluorescence microscope. Images were captured using a Spot CCD camera (Sterling Heights, MI) at a resolution of 1024×1024 pixels as 8-bit color images. The colocalization of ET receptors with pan cadherin was analyzed using the image analysis software Metamorph software (Universal Imaging Corp., Downingtown, PA).

Measurement of Intracellular Calcium. HEK cells transfected with ET receptors on coverslips were loaded with 2 m*M* Fluo 4/AM and Pluronic acid (1%) (Molecular Probes, Eugene, OR) in Opti-MEM medium (Invitrogen) for 40 mins at 37°C and placed in a chamber mounted on the stage of a Leica confocal microscope. Cells were flushed continuously with HEPES-Ringer solution (140 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, 10 m*M* HEPES; pH 7.3) with or without drugs. The excitation and wavelength was 488 nm and a 500-nm long pass filter was used. Images were captured every 6 secs. Five baseline images were obtained and images were collected for 20 min during and after drug treatment. Changes in $[Ca^{2+}]_i$ in cells expressing ET_A or ET_B receptors were represented by changes in fluorescence intensity in individual cells after agonist stimulation. Fluorescence intensity was quantitated using Leica confocal software. Cells were treated with ET-1 or S6c with or without pretreatment with ET receptor antagonists for 5 mins.

To obtain a concentration response relationship, cells transfected with ET_A receptors were treated with ET-1 (1, 10, or 100 n*M*) for 2 mins. Other cells were treated with 10 n*M* ET-1 for 2 min after pretreatment with the ET_A receptor antagonist BQ610 (100 n*M*) for 5 mins. Another group of cells transfected with ET_B receptors was treated with 10 or 100 n*M* of S6c for 2 mins or with 10 n*M* ET-1 for 2 mins after pretreatment the ET_B receptor antagonist BQ788 (100 n*M*) for 5 mins. Desensitization was studied in cells transfected with ET_A or ET_B receptors and treated with ET-1 or S6c (100 n*M*) for up to 20 mins. Additionally, blank HEK-293 cells treated with ionomycin (0.1 μ*M*), a calcium ionophore, for up to 20 mins, were used as a control for fluorescence quenching or loss of Fluo-4 from treated cells.

Data Analysis and Statistics. For each type of experiment, data were obtained from six to eight dishes of cells. Data are presented as mean ± SEM. When comparing two groups, Student's *t* test was used. For multiple comparisons, an analysis of variance followed by the Bonferroni post hoc test was used. In all cases, a P value ≤ 0.05 was considered significant.

Results

ET_A and ET_B Receptors Have a Different Localization in Transfected HEK-293 Cells. ET_A and ET_B receptors were localized using immunocytochemical techniques. It was found that immunoreactivity (ir) for ET_A or ET_B receptors was present in about 25% of cells, indicating a low transfection efficiency. However, pan cadherin ir (a plasma membrane marker) was detected in all cells (Fig. 1). It was also found that ET_A and ET_B receptors were colocalized with pan-cadherin in some cells (Fig. 1). In ET_A receptor–transfected cells, 83 ± 2% of the receptors were colocalized with pan-cadherin. In cells transfected with ET_B receptors, 54 ± 2% of the receptor was co-localized with pan-cadherin. These data indicate that ET_A receptors were mainly localized on the cell membrane, whereas ET_B receptors were equally distributed between the cell membrane and intracellular compartments.

The Distribution of ET_B Receptors Changes When Coexpressed with ET_A Receptors. Because ET receptor antibodies are raised in the same host, cells cotransfected with ET_A receptor and ET_B-HA were used to examine colocalization using antibodies against the ET_A receptor and against HA. When ET_A and ET_B receptors

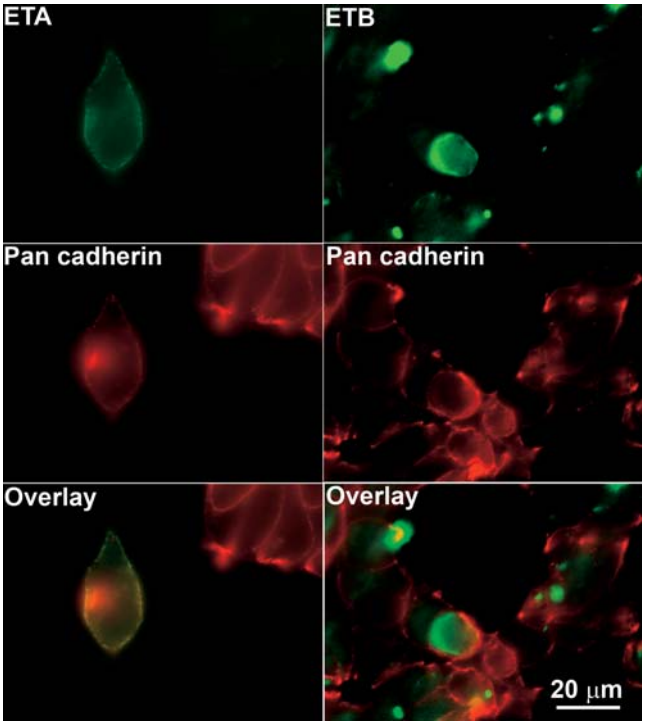

Figure 1. Immunocytochemistry for the ET_A or ET_B receptor costained with the cell membrane marker, pan cadherin. ET_A (left) and ET_B (right) receptors were transiently transfected into HEK 293 cells. ET_A receptors are localized on the cell membrane, whereas ET_B receptors are in the cell membrane and intracellular compartments. Most ET_A receptors (green) are colocalized with pan-cadherin (red); ET_B receptors (green) are colocalized with pan cadherin intracellularly.

were transiently cotransfected, 97 ± 1% of ET_B receptors colocalized with ET_A receptors and 84 ± 2% of ET_B receptors colocalized with pan-cadherin (Fig. 2).

ET_A and ET_B Receptors Transfected into HEK 293 Cells Are Functional. ET-1 was used to stimulate cells transfected with ET_A receptors; S6c was used to stimulate cells transfected with ET_B receptors. Both treatments caused an increase in $[Ca^{2+}]_i$ in a concentration-dependent manner. The fluorescence intensity for increases in $[Ca^{2+}]_i$ for ET_A receptor cells treated with 1, 10, or 100 n*M* ET-1 was 112 ± 6, 161 ± 5, and 158 ± 4 arbitrary fluorescence units, respectively. Fluorescence intensity for $[Ca^{2+}]_i$ for ET_B receptor cells treated with 10 or 100 n*M* ET-1 was 76 ± 3 and 104 ± 9 arbitrary fluorescence units, respectively. S6c did not increase $[Ca^{2+}]_i$ in cells transfected with ET_A receptors only. Cells transfected with ET receptors without or with a hemoagglutinin-epitope tag (ET_A-HA or ET_B-HA) were also used in this study. No difference in agonist-induced changes in $[Ca^{2+}]_i$ was observed between cells transfected with wild-type ET receptors or hemoagglutinin-tagged ET receptors (data not shown). Changes in $[Ca^{2+}]_i$ in cells transfected with ET_A or ET_B receptors were blocked by pretreatment with BQ610 or BQ788 (100 n*M*), antagonists of ET_A and ET_B receptors, respectively.

ET_A Receptors Desensitize More Slowly Than ET_B Receptors. A maximum concentration of ET-1 and S6c (100 n*M*) was used in desensitization studies, in which cells were treated with agonists for 20 mins (Fig. 3A). When stimulated with ET-1, ET_B receptors desensitized faster ($t_{1/2}$ = 21 ± 1 sec) than ET_A receptors ($t_{1/2}$ = 48 ± 1 sec). S6c-induced increases in $[Ca^{2+}]_i$ desensitized in cells expressing ET_B receptors only ($t_{1/2}$ = 17 ± 1 sec). There was no difference between cells transfected with wild-type or HA-tagged receptors (Table 1). To rule out variations coming from the decay of calcium indicator fluo-4/AM, control experiments were also done using the calcium ionophore, ionomycin, which caused a stable increase in the calcium signal for up to 20 mins (data not shown). The desensitization half-time ($t_{1/2}$) in cells cotransfected with ET_A and ET_B receptors in response to either ET-1 or S6c activation was greater than 400 sec (Fig. 3B).

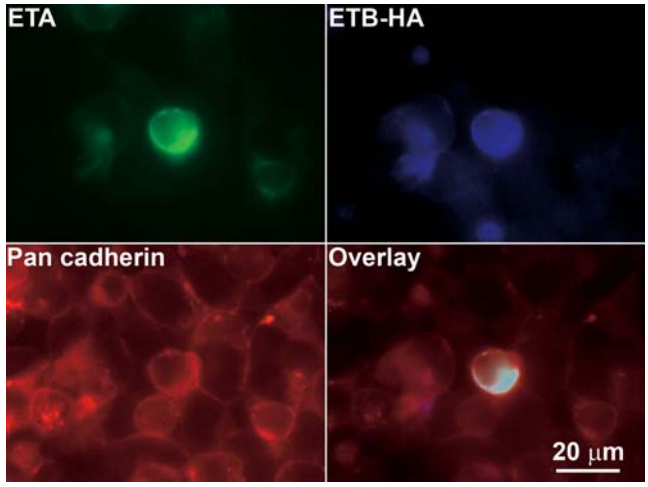

Figure 2. Immunocytochemical localization of ET_A, and ET_B receptors. The wild-type ET_A receptor was colocalized with pan-cadherin and HA-tagged ET_B receptors. Cells were costained with the cell membrane marker pan cadherin (red), ET_A receptors (green), and hemoagglutinin (blue). Cells coexpressing ET_A and ET_B receptors are colocalized in the same compartment and may form a dimer.

Discussion

We expressed human ET_A or ET_B receptors with or without a HA-epitope tag in HEK-293 cells and examined their cellular localization and function. We found that the ET_A receptor was mainly localized on the cell membrane, whereas ET_B receptors were not only localized on the cell membrane, but also in intracellular compartments. Our results also provide the first evidence that the distribution of ET_B receptors changes when they are coexpressed with ET_A receptors. We also show that ET_A receptors desensitized more slowly than ET_B receptors and that ET receptor desensitization is eliminated in cells coexpressing ET_A and ET_B receptors.

ET Receptor Localization. ET_B receptors have a different subcellular distribution than ET_A receptors. We localized ET receptors in HEK-293 cells by costaining the

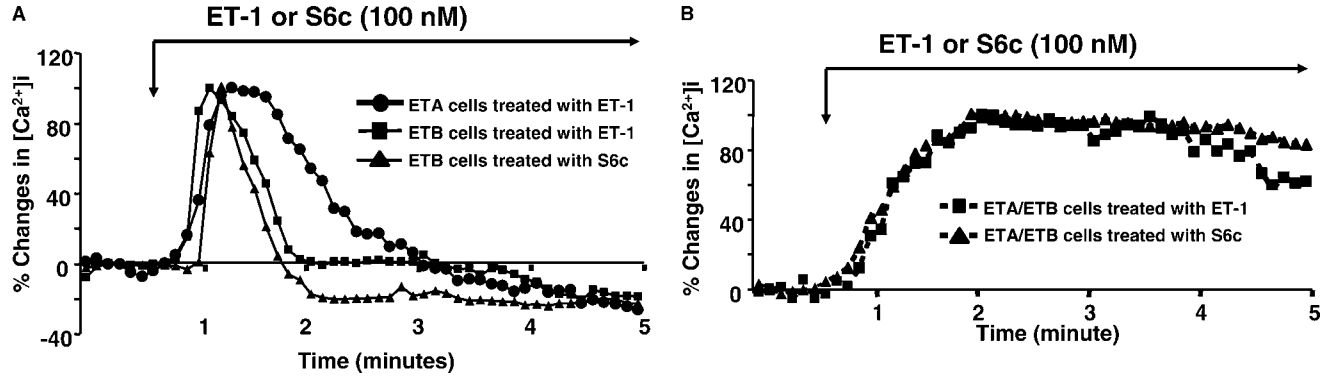

Figure 3. Agonist-induced increases in $[Ca^{2+}]_i$ in HEK-293 cells transiently transfected with ET_A or ET_B receptors. Maximal agonist concentrations (100 n*M* of ET-1 or S6c) were used in the desensitization study. A. When stimulated with ET-1, ET_B receptors desensitized faster than ET_A receptors. ET-1 and S6c-induced increases in $[Ca^{2+}]_i$ desensitized in cells expressing ET_B receptors only. B. In cells coexpressing ET_A and ET_B receptors the agonist induced desensitization $t_{1/2}$ was >400 sec.

receptors with pan cadherin, a cell membrane marker. We found that ET_A receptors were found predominately in the cell membrane, whereas ET_B receptors were distributed evenly between the cell membrane and intracellular compartments. Others have made similar observations in different cell lines transfected with green fluorescent protein-tagged ET receptors (6). These investigators found that, under steady-state conditions, ET_A receptors are localized to the plasma membrane, whereas ET_B receptors were localized to the cell membrane and to lysosomes where presumably they had been targeted for degradation. ET_A and ET_B receptors may have different subcellular localization because they are coupled to different signal transduction pathways in cell lines (16) and in isolated smooth muscle cells (17).

ET Receptor Function. ET_A and ET_B receptors contribute to vasoconstriction of vascular SMCs by coupling to an increase in $[Ca^{2+}]_i$ (18. 19). However, it is difficult to identify the contribution of each receptor in SMCs that constitutively coexpress ET_A and ET_B receptors. Studies using cell lines transfected with individual receptors provide an alternative approach for investigation of the function of individual receptors. We are the first to show that ET_A and ET_B receptors have different functional profiles when agonist-induced increases in $[Ca^{2+}]_i$ are measured in cells transfected with ET_A or ET_B receptors alone. ET_A receptors desensitized more slowly than ET_B receptors. The differences in desensitization of ET_A and ET_B receptors are not agonist-dependent because ET_B receptor cells desensitize similarly when activated with either ET-1 or S6c stimulation. We also show that there was little or no desensitization in cells coexpressing ET_A and ET_B receptors. ET receptors belong to GPCRs, in which the intracellular carboxy terminus of receptors contains sites for phosphorylation, and are used in the regulation of the receptor in internalization and desensitization (20). Coexpression of receptors may mask these phosphorylation sites and therefore impair receptor desensitization.

Receptor Dimerization. Several lines of evidence have suggested that different GPCRs can form heterodimers; receptors exhibiting this behavior include angiotensin AT1 receptor-bradykinin B2 receptor and β2-adrenergic-α2 adrenergic receptor (21, 22). These studies showed that the activation of one receptor modulates the ligand binding, receptor trafficking, and intracellular signaling of the other receptor. This interaction can come about through a physical interaction such as occurs with $GABA_{B1}$ and $GABA_{B2}$ receptors. Fluorescence resonance energy transfer (FRET) analysis has revealed the potential for similar heterodimerization of ET receptors (23). In the present study, we showed that ET_A and ET_B receptors are closely colocalized in the membrane of cells coexpressing these receptors. Although we did not demonstrate a direct physical coupling of the two receptors, our data are consistent with heterodimer formation. Heterodimer formation is also supported by our functional studies as desensitization of ET_A and ET_B receptors was eliminated in cells coexpressing ET_A and ET_B receptors.

Table 1. The $t_{1/2}$ (sec) in Cells Transfected With ET Receptors or ET Receptors Tagged with Hemoagglutinin (HA)[a]

Agonist	ET-1				S6c	
Receptor Mean ± SE	ET_A 48 ± 1.0	ET_A-HA 47 ± 3.4	ET_B 21 ± 1.2*	ET_B-HA 21 ± 2.2*	ET_B 17 ± 1.0*	ET_B-HA 21 ± 1.7*

[a] $P < 0.05$ is considered to be statistically significant; $n = 4–7$; *, versus ET_A treated with ET-1.

The molecular basis for ET receptor heterodimerization is unclear, but mounting evidence suggests that the dimerization of GPCRs is important for normal functioning of receptors. Heterodimerization of GPCRs is important during receptor synthesis and trafficking because dimerization masks specific retention signals that would otherwise keep the receptors in the endoplasmic reticulum, as shown for $GABA_BRs$ (24). In some cases, heterodimerization between closely related subtypes is essential for the formation of functional receptors, such as $GABA_BRs$ (24) and sweet (25) and L-amino acid (26) taste receptors. Another possibility is that the formation of an ET_A/ET_B dimer may occur in the presence of ET-1 as a bivalent ligand connecting two receptors (14, 27). However, our data show that in cells coexpressing ET_A and ET_B receptors the receptors are colocalized in the same subcellular compartments, suggesting that dimmer formation can occur in the absence of ET-1 binding (Fig. 3). ET_A receptors have the motif for an internal PDZ (postsynaptic density-95/disc-large/zona occludens) domain that contributes to receptor recycling (28). PDZ domains are ubiquitous protein-protein interaction domains comprising 70–90 residues and the most prominent role of PDZ-containing proteins appears to be the assembly of protein complexes at the plasma membrane, where they bind to the C-termini of membrane proteins (29).

Significance of ET Receptor Dimerization: Implication in Arterial and Venous Differences. ET_A and ET_B receptors influence contractility in mesenteric arteries and veins differently. ET_A receptors are the predominant receptor mediating arterial contraction, whereas ET_A and ET_B receptors contribute to venous contraction. ET-1 elicits arterial contraction while the ET_B receptor agonist, S6c, is inactive in arteries, whereas ET-1 and S6c cause venous constriction in veins (30–33). Veins are also more sensitive to the constrictor effects of ET-1 compared to arteries (34–36). In rat and murine mesenteric blood vessels, the ET_B receptor antagonist BQ788 blocks S6c-induced venous contraction. However, ET-1–induced venous contraction is reduced by the ET_A receptor antagonist BQ610, but not by BQ788, whereas the mixture of ET_A and ET_B receptor agonists further decreases venous contraction responses to ET-1 (36, 37). We speculate that the distinct arterial and venous responses to ET-1 may be due to the presence of the heterodimerization of ET receptors in veins, but not in arteries.

Our data supported that ET receptors transfected into HEK cells exist different trafficking and desensitization characteristics, which may or may not be different from the function of ET receptors in primary SMCs. Further studies of ET receptor trafficking and function need to be confirmed in vascular SMCs.

1. Haynes WG, Webb DJ. Endothelin as a regulator of cardiovascular function in health and disease. J Hypertens 16:1081–1098, 1998.
2. Touyz RM, Schiffrin EL. Role of endothelin in human hypertension. Can J Physiol Pharmacol 81:533–541, 2003.
3. Ergul A. Hypertension in black patients: an emerging role of the endothelin system in salt-sensitive hypertension. Hypertension 36:62–67, 2000.
4. Goddard J, Webb DJ. Plasma endothelin concentrations in hypertension. J Cardiovasc Pharmacol 35:S25–S31, 2000.
5. Mateo AO, de Artinano AA. Highlights on endothelins: a review. Pharmacol Res 36:339–351, 1997.
6. Abe Y, Nakayama K, Yamanaka A, Sakurai T, Goto K. Subtype-specific trafficking of endothelin receptors. J Biol Chem 275:8664–8671, 2000.
7. Bremnes T, Paasche JD, Mehlum A, Sandberg C, Bremnes B, Attramadal H. Regulation and intracellular trafficking pathways of the endothelin receptors. J Biol Chem 275:17596–17604, 2000.
8. Foster N, Loi TH, Owe-Young R, Stanley KK. Lysosomal traffic of liganded endothelin B receptor. Biochim Biophys Acta 1642:45–52, 2003.
9. Oksche A, Boese G, Horstmeyer A, Furkert J, Beyermann M, Bienert M, Rosenthal W. Late endosomal/lysosomal targeting and lack of recycling of the ligand-occupied endothelin B receptor. Mol Pharmacol 57:1104–1113, 2000.
10. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Rep 5:30–34, 2004.
11. Angers S, Salahpour A, Bouvier M. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annu Rev Pharmacol Toxicol 42:409–435, 2002.
12. Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2:274–286, 2001.
13. Filizola M, Weinstein H. The study of G-protein coupled receptor oligomerization with computational modeling and bioinformatics. FEBS Lett 272:2926–2938, 2005.
14. Harada N, Himeno A, Shigematsu K, Sumikawa K, Niwa M. Endothelin-1 binding to endothelin receptors in the rat anterior pituitary gland: possible formation of an ETA-ETB receptor heterodimer. Cell Mol Neurobiol 22:207–226, 2002.
15. Hasselblatt M, Kamrowski-Kruck H, Jensen N, Schilling L, Kratzin H, Siren AL, Ehrenreich H. ETA and ETB receptor antagonists synergistically increase extracellular endothelin-1 levels in primary rat astrocyte cultures. Brain Res 785:253–261, 1998.
16. Aramori I, Nakanishi S. Coupling of two endothelin receptor subtypes to differing signal transduction in transfected Chinese hamster ovary cells. J Biol Chem 267:12468–12474, 1992.
17. Hersch E, Huang J, Grider JR, Murthy KS. Gq/G13 signaling by ET-1 in smooth muscle: MYPT1 phosphorylation via ETA and CPI-17 dephosphorylation via ETB. Am J Physiol Cell Physiol 287:C1209–C1218, 2004.
18. Giulumian AD, Molero MM, Reddy VB, Pollock JS, Pollock DM, Fuchs LC. Role of ET-1 receptor binding and [Ca(2+)](i) in contraction of coronary arteries from DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 282:H1944–H1949, 2002.
19. Conant AR, Oo AY, Dashwood MR, Collard K, Chester MR, Dihmis WC, Simpson AW. Endothelin receptors in cultured and native human radial artery smooth muscle. J Cardiovasc Pharmacol 39:130–141, 2002.
20. Perez DM, Karnik SS. Multiple signaling states of G-protein-coupled receptors. Pharmacol Rev 57:147–161, 2005.
21. Rios CD, Jordan BA, Gomes I, Devi LA. G-protein-coupled receptor dimerization: modulation of receptor function. Pharmacol Ther 92:71–87, 2001.
22. Gomes I, Jordan BA, Gupta A, Rios C, Trapaidze N, Devi LA. G protein coupled receptor dimerization: implications in modulating receptor function. J Mol Med 79:226–242, 2001.
23. Gregan B, Jurgensen J, Papsdorf G, Furkert J, Schaefer M, Beyermann M, Rosenthal W, Oksche A. Ligand-dependent differences in the

internalization of endothelin A and endothelin B receptor heterodimers. J Biol Chem 279:27679–27687, 2004.

24. Grunewald S, Schupp BJ, Ikeda SR, Kuner R, Steigerwald F, Kornau HC, Kohr G. Importance of the gamma-aminobutyric acid(B) receptor C-termini for G-protein coupling. Mol Pharmacol 61:1070–1080, 2002.
25. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. Cell 106:381–390, 2001.
26. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. An amino-acid taste receptor. Nature 416:199–202, 2002.
27. Sakamoto A, Yanagisawa M, Sawamura T, Enoki T, Ohtani T, Sakurai T, Nakao K, Toyo-oka T, Masaki T. Distinct subdomains of human endothelin receptors determine their selectivity to endothelinA-selective antagonist and endothelinB-selective agonists. J Biol Chem 268:8547–8553, 1993.
28. Paasche JD, Attramadal T, Kristiansen K, Oksvold MP, Johansen HK, Huitfeldt HS, Dahl SG, Attramadal H. Subtype-specific sorting of the ETA endothelin receptor by a novel endocytic recycling signal for G protein-coupled receptors. Mol Pharmacol 67:1581–1590, 2005.
29. Beuming T, Skrabanek L, Niv MY, Mukherjee P, Weinstein H. PDZBase: a protein-protein interaction database for PDZ-domains. Bioinformatics 21:827–828, 2005.
30. White DG, Cannon TR, Garratt H, Mundin JW, Sumner MJ, Watts IS. Endothelin ETA and ETB receptors mediate vascular smooth-muscle contraction. J Cardiovasc Pharmacol 22(Suppl 8):S144–S148, 1993.
31. Wang HG, Shibamoto T, Miyahara T. Endothelin-1 selectively contracts portal vein through both ETA and ETB receptors in isolated rabbit liver. Am J Physiol 273:G1036–G1043, 1997.
32. Johnson RJ, Galligan JJ, Fink GD. Effect of an ET(B)-selective and a mixed ET(A/B) endothelin receptor antagonist on venomotor tone in deoxycorticosterone-salt hypertension. J Hypertens 19:431–440, 2001.
33. Watts SW, Fink GD, Northcott CA, Galligan JJ. Endothelin-1-induced venous contraction is maintained in DOCA-salt hypertension; studies with receptor agonists. Br J Pharmacol 137:69–79, 2002.
34. Cocks TM, Faulkner NL, Sudhir K, Angus J. Reactivity of endothelin-1 on human and canine large veins compared with large arteries in vitro. Eur J Pharmacol 171:17–24, 1989.
35. Yang Z, Bauer E, von Segesser L, Stulz P, Turina M, Luscher TF. Different mobilization of calcium in endothelin-1-induced contractions in human arteries and veins: effects of calcium antagonists. J Cardiovasc Pharmacol 16:654–660, 1990.
36. Johnson RJ, Fink GD, Watts SW and Galligan JJ. Endothelin receptor function in mesenteric veins from deoxycorticosterone acetate salt-hypertensive rats. J Hypertens 20:665–676, 2002.
37. Perez-Rivera AA, Fink GD, Galligan JJ. Vascular reactivity of mesenteric arteries and veins to endothelin-1 in a murine model of high blood pressure. Vasc Pharmacol 43:1–10, 2005.