Effects of Human Peptide Endothelin-1 and Two of its Sterically Unrestrained C-Terminal Fragments on CoronaryVascular Smooth Muscle

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Abstract. Clearance of human peptide endothelin-1 (ET-1) has been proposed to follow a receptor pathway involving a cascade of ET-1 receptor endocytosis and lysosomal degradation by a family of proteinases expressed constitutively by most cells. Genetically distinct endopeptidases produce ET-1 and degrade mature peptide. The ET-1 degradation products were considered to be inactive, however, recent evidence suggests that ET-1 fragments sustain most of the homeostatic response produced by parent peptides. The purpose of this study was to establish whether the overall structure of human ET-1 or the structure of its C-terminus is responsible for the subtype-selectivity, down-regulation and clearance of endothelin, and whether D-aminoacid substitution in the moiety of synthetic peptide is involved in effective ET-1 antagonism in coronary vascular smooth muscle. To characterize specific mechanism(s) leading to subtype-selective ET-receptor down-regulation and/or to ET-1 antagonism, ligand binding studies were accomplished with radioactive human (1–21)ET-1 and with C-terminal ET-1 fragments, both peptide agonists and antagonists, in adult male porcine coronary artery vascular smooth muscle (CVSM). The subcellular membranes of CVSM were isolated by isopycnic gradient centrifugation. Exposure of porcine coronary artery to exogenous ET-1 induced endothelin-ET_B selective down-regulation. ET_A-mediated subtype-ET_B down-regulation was observed with distribution of ligand-ET_B receptor complexes in light, endosomal, membranes. The ET_A selective PD151242 significantly attenuated [^{3}H]-thymidine incorporation, and the ET_B selective an-

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tagonist BQ788 blocked down-regulation observed in porcine vascular fibroblasts (PF). Preincubation of coronary arteries with ET_B selective BQ3020 was accompanied with a more intense down-regulation.

Conclusion: our data are indicative of short-term ET_B selective down-regulation of endothelin receptors in coronary vascular smooth muscle after exposure to ET-1. The presence in the carboxy-terminus of ($Ala^{11,15}$) substitution in peptide fragments IRL1620 and BQ3020 determined the differential specificity of ET_B-receptor coupling and was important for subtype-ET_B-receptor down-regulation. The activation of the dominating ET_A-receptor by ET-1 facilitated mitogenic responses to ET-1 in porcine vascular fibroblasts.

Key words: Endothelin-1 — ET_A- and ET_B-receptors — Down-regulation — Porcine coronary artery — Vascular smooth muscle — Vascular fibroblasts

Introduction

With its vasoconstrictor activity and mitogenicity, human peptide endothelin-1 (ET-1) could play an important role as a local mediator in cardiovascular diseases (see the review by Gray and Webb 1996). In patients with angina pectoris, the constitutive plasmatic ET-1 concentration correlated well with the severity of coronary artery disease (Salomone et al. 1996). Elevated ET-1 levels were reported in myocardial infarction and congestive heart failure (Stewart et al. 1991). The effects of ET-1 are presumably mediated by two types of G protein-coupled receptors (ET_A and ET_B), (Alexander and Peters 1999), expressed abundantly on vascular smooth muscle cells (Hasdai et al. 1997; Bunneman et al. 1999). The endothelin-ET_A-receptor is the dominant subtype in coronary arteries of most species, including man (Opgaard et al. 1996; Pierre and Davenport 1998). However, in adult porcine heart, coronary artery vascular smooth muscle membranes (VSM) showed significant non-ET_A contractile response (Harrison et al. 2000) and nearly proportional distribution of endothelin ET_A/ET_B-receptor subtypes (Dřímal et al. 2000). In vascular smooth muscle, the human ET-1, a potent agonist at ET-receptors, binds preferentially and with high affinity to the ET_A-receptor and with much lower affinity to the ET_B-receptor. The peptide is produced and also cleared from the circulation by two genetically distinct metallopeptidases (Grantham et al. 1999). Widely different, linear peptide fragments, (8–21)ET-1 (IRL1620) and its (6–21)ET-1 derivative (BQ3020), both resistant to degradation by neutral endopeptidases and carboxypeptidases, bind exclusively to ET_B-receptors (Takai et al. 1992). The present study was therefore designed to investigate: a) the subtype-specific down-regulation of endothelin in porcine vascular smooth muscle with three different agonists: the native human/porcine [^{125}I]ET-1 and the endothelin-ET_B selective [^{125}I]IRL1620 and BQ3020, and b) the selectivity of endothelin antagonism in porcine vascular smooth muscle with D-amino-acid substituted peptide fragments. To achieve this aim, we performed ligand binding experiments with subcellular membranes of

porcine vascular smooth muscle isolated from coronary arteries preincubated with the ET-1 and/or ET-1 fragments, being endothelin agonists and antagonists.

Materials and Methods

(3-[^{125}I]Tyr^{13})ET-1, specific activity 2200 Ci/mmol; [^{125}I]Tyr^{13}-Suc-[Glu^{9}, Ala^{11,15}] ET-1(8–21); ([^{125}I]IRL1620), specific activity 2200 Ci/mmol (both from NEN Life Sci. Products); [^{3}H]-thymidine, specific activity 29 Ci/mmol (ICN). N-Acetyl-[Ala^{11,15}] ET-1(6–21) (BQ3020); BQ788; PD151242; pertussis-toxin (all RBI, Natur, MA, USA); human/porcine ET-1(1–21); (Sigma, Aldrich). Serum for cell cultures and other cell culture reagents were from Gibco (Grand Island, NY, USA).

Coronary artery

Adult male porcine hearts of approximately 450 g were obtained from the local abattoir, and transported to the laboratory in ice-cold Buffer I (in mmol/l: 250.0 sucrose solution, containing 0.4 KCl and 10.0 imidazole, pH = 7.5). A thin catheter was inserted into the right and left coronary artery, and the epicardial portion of the artery was isolated and quickly excised. The medial part of the coronary artery was separated (free of adventitia and intima) and the preparation was minced with scissors and homogenized in nine volumes of Buffer I for 3 × 20 s at 2/3 of setting in an Ultraturrax homogenizer. The crude homogenate was centrifuged at 1000 × *g* for 10 min at 5 ℃ in Buffer II (in mmol/l: Tris-HCl 50.0 and $MgCl_2$ 5.0, pH = 7.51). The resulting postnuclear supernatant was filtered through a stainless steel mesh and through three layers of gauze and centrifuged at 10,000 × *g* for 10 min in Buffer II. The pellet was discarded and the supernatant was centrifuged at 105,000 × *g* for 60 min in Buffer II and defined as soluble microsomal fraction. In separate experiments specimens of coronary arteries were fixed in isometric conditions (under small preload 5–10 mN) in a temperature controlled tissue bath (37 ℃) containing HEPES-buffered physiological salt solution (PSS, in mmol/l): NaCl 135, $CaCl_2$ 1.25, $MgCl_2$ 1.0, KH_2PO_4 0.44, NaH_2PO_4 0.34, $NaHCO_3$ 2.6, HEPES 20.0, glucose 5.5, with protease inhibitors PMSF 0.1, phosphoramidon 0.01, (in μg/ml): pepstatin 5.0, saturated with a 95 % mixture of O_2/5 % CO_2, giving pH 7.51) and were incubated (60 min) with endothelin agonists or antagonists. The coronary artery was then denuded and treated as described above. The microsomal subfractions were prepared from porcine coronary artery by the method of Kwan et al. (1984), modified by using medial layers only (Dřímal et al. 1991).The microsomal vesicular preparation was layered on a discontinuous sucrose density gradient comprising 15 %, 30 %, 60 % sucrose. The loaded sucrose gradient tubes were centrifuged at 130,000 × *g* for 2 h with a swing-out rotor. Two major microsomal subfractions were collected at 15/30 and 30/60 sucrose interface. In coronary artery smooth muscle membranes 120 min equilibrium binding assays were carried out at 25 ℃ in 250 μl volumes containing 50 mmol/l Tris-HCl and 5 mmol/l $MgCl_2$ (pH = 7.51) and 100 μl of coronary artery microsomal membranes (40–90 μg of protein). Radioligands [^{125}I]ET-1 or [^{125}I]IRL1620 were added in the concentration range

from 0.05 to 3.2 nmol/l for 120 min. The concentrations of the various pharmacologically active drugs or ions used are indicated where appropriate. After the end of the incubation, the bound drug was separated from the free fraction by rapid filtration through 25 mm Whatman GF/B glass microfiber filters, followed by three 1.5 ml washes with Buffer II at 5 ℃. The filters were dried, placed in scintillation vials with 10 ml liquid scintillant (Bray). To determine the dissociation constant by competition, binding was accomplished with 50 μg of membrane protein and nonspecific binding was obtained adding 10 μmol/l of unlabeled human ET-1. After the end of 60 min incubation, the bound radioligand was separated from the free one by rapid filtration through glass microfiber filters, washed with cold assay Buffer II, and the radioactivity bound to glass filters was collected in Bray scintillation cocktail and counted in a Packard Tri-Carb-300CD scintillation counter (Packard Instruments, Downers Grove, IL, USA). Binding data were analyzed by the curve fitting programs "Ligand" and "Inplot", and Hill analysis predicted the probability of a single binding site versus two sites of the population of endothelin receptors.

Porcine coronary fibroblasts (PF)

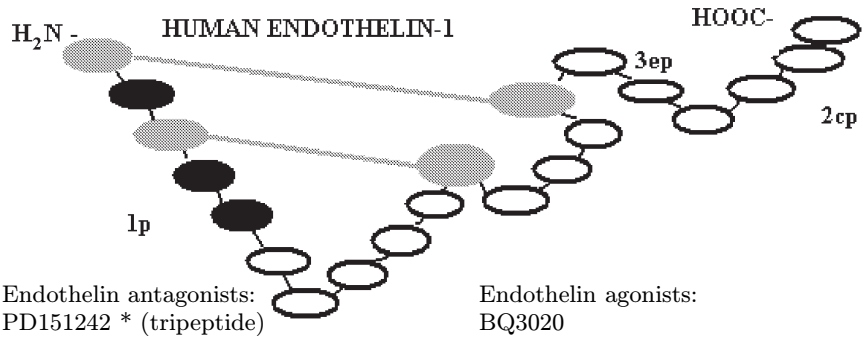
Vascular fibroblasts were isolated from medial layer of coronary arteries. PF were grown in Dulbeco's modified Eagle's medium (DMEM) supplemented with 10 % calf serum, penicillin and streptomycin in a humidified incubator at 37 ℃ with 5 % CO_2. The cultures were routinely passaged twice a week at a seeding density of approximately 1–2 $\times$ 10^5 cells/ml. At sub-confluence (approximately 95 % of grown cells), PF were isolated by short (1min) enzymatic digestion in DMEM containing 0.1 % trypsin and 100 U/ml Collagen solution 2 (Worlington Biochem.). The isolated cells were pooled and centrifuged for 3 min at 2000 $\times$ g. The resulting pellet was resuspended in DMEM without serum. Cell viability was assayed by the Trypan exclusion test, and altered stainability with acridine orange. The routine use of fibroblasts, typification, morphology, viability and the total number of receptors on the cells were described elsewhere (Dřímal et al. 1991; Dřímal 1992).

Other measurements

Protein concentrations were measured by the method of Bradford (1976), using bovine serum albumin as standard. Serum endothelin concentrations were measured using commercially available ELISA kits. The significance of differences between groups of data was assessed by Student's t-test.

Results

The structures of the human helical peptide ET-1 and of four ET-1 fragments, the latter being a potent endothelin antagonists, and one nonpeptoid, butenolide-type compound used in the present study, are shown in Fig. 1. The numbers along



Endothelin antagonists:
PD151242 * (tripeptide)
BQ123 * (cyclic peptapeptide)
BQ788 * (ET_B selective tripep.)
PD142893 * (nonselective peptide type)
PD145065 *
PD155080 (is butenolide)
* mostly D-aminoacid substitution

Endothelin agonists:
BQ3020
IRL1620
ET-1(6–21) §
ET-1(8–21) §
Cys
Ser
§ ($Ala^{11,15}$)

Figure 1. Structure of human peptide endothelin-1 (1–21) and endothelin fragments. Human endothelin-1 is presumably degraded to peptide fragment by peptidases (1p), carboxy-peptidases (2cp), endopeptidases (3ep, i) in three or four sequentional steps.

the ET-1 moiety indicate the assumed sequence of steps in the cleavage of mature human ET-1. The nicked peptides with conserved carboxy-terminus exhibited pharmacological activity comparable to that of the intact peptide (see Dřímal et al. 1999, 2000). The shorter peptide fragments, mostly potent peptide antagonists, used in the present study, were substituted with one or two D-amino-acid (s): a) with -Trp- in penta-peptide, or b) with two amino-acids (-Trp- and -Tyr-) in tripeptide PD151242, and with two amino-acids (-Trp- and -Nle-) in tripeptide BQ788.

In equilibrium binding experiments with native membranes of porcine coronary arteries, the nonselective human [^{125}I]ET-1 identified two different population sites. The high affinity sites with $K_D = 0.3 \pm 0.1$ nmol/l represented $35 \pm 4\%$, whereas the low affinity binding sites with $K_D = 2.6 \pm 1.0$ nmol/l represented $54 \pm 8\%$ fmol/mg protein. Subtype ET_B receptors and associated signalling in CVSM were further analyzed in saturation experiments with linear-truncated type of peptide, (8–21)ET-1 analogue, with the endothelin-ET_B selective [^{125}I]IRL1620. Scatchard analysis of the binding isotherms indicated that truncated ET_B selective peptidic ligand was bound to a single site with a mean dissociation constant $K_D = 0.58 \pm 0.11$ nmol/l and a capacity of 40 ± 17 fmol/mg protein. In competition experiments, the combined ET_A selective antagonists PD151242 ($ET_{(Ax)}$ 0.1 μmol/l) and PD155080 ($ET_{(ax)}$ 0.1 μmol/l) displaced the high affinity [^{125}I]ET-1 binding to porcine coronary artery vascular smooth muscle membranes (in a monophasic manner), whereas the low affinity binding was $B_{max} = 115 \pm 11\%$ of its control values. When the membranes of porcine coronary artery were preincubated with the ET_B selective antagonist BQ788 ($ET_{(Bx)}$ 0.1 μmol/l), the low affinity specific binding was significantly reduced to $B_{max} = 22.1 \pm 9\%$ of its control values ($p < 0.05$).

The membrane characteristics and distribution of subtypes of endothelin receptors

To characterize in detail the proportion of endothelin receptor subtypes and their agonist-induced down-regulation in preincubated porcine coronary artery smooth muscle, the membrane fractions were subjected to centrifugation in discontinuous sucrose density gradient, specially constructed to harvest two major microsomal subfractions in vascular smooth muscle: light vesicular, presumably endosomal, and heavy membrane fraction, presumably plasmalemmal. Table 1 summarizes the characteristics of the two main subcellular membrane fractions of porcine coronary artery membranes isolated from the discontinuous sucrose density gradient and used in our studies: (a) distribution of protein, (b) marker enzyme activity (5'-nucleotidase and Mg^{2+}-ATPase), (c) specific dihydropyridine-binding and (d)/(e) the distribution of endothelin subtype-ET_A and ET_B receptor subtypes ([^{125}I]ET-1, [^{125}I]ET-1(8–21) receptor binding).

The competitive binding experiments with porcine coronary vascular smooth muscle membranes and [^{125}I]ET-1 showed that the nonselective endothelin agonist was displaced by ET-1 in a biphasic manner, whereas BQ788, an endothelin-ET_B selective antagonist, significantly increased the total number of sites identified in plasmalemmal membranes of the coronary artery ($B_{\max BQ788} = 144 \pm 9\,\%$, $p < 0.05$). As the next step the ligand-binding experiments were conducted with purified subcellular plasmalemmal and light microsomal membranes isolated from native porcine coronary arteries and from coronary arteries preincubated with nmolar concentration of the (6–21)ET-1 fragment BQ3020, an endothelin-ET_B selective agonist. The representative saturation curves obtained with two different endothe-

Table 1. Characteristics of membrane fractions isolated from medial layers of porcine coronary vascular smooth muscle

Preparation (Procedure)	Plasmalemmal Membranes	Light Endosomal Fraction
(a) Protein Content (mg/g tissue w/w)	0.60 ± 0.07	0.40 ± 0.05
(b) 5'-nucleotidase (μmol/mg/h)	92 ± 4	10 ± 3 *
Mg^{2+} ATP-ase (μmol/mg/h)	69 ± 10	119 ± 12 *
(c) Photoaffinity Label		
(fmol/mg of p. of [^{3}H]-azidopine)	233 ± 18 *	142 ± 13
(d) [^{125}I]ET-1	11.4 ± 3.5	8.7 ± 1.8
(e) [^{125}I]IRL1620		
Receptor Binding (fmol/mg p.)	21.1 ± 0.3*	23.6 ± 2.6*

Explanation and symbols: Values are mean ± S.E.M. Specific enzyme activities are expressed as micromoles of phosphate released/mg protein/h. Density of specific high affinity (-)-[^{3}H]-azidopine binding sites (in fmol/mg protein, photolabeled presumably on inactivated Ca^{2+} channels, for details of photolabeling and effects of ET-1 see (Dřímal 1991)). Total number of [^{125}I]ET-1/[^{125}I]IRL1620 binding sites on plasma membranes and on endosomal vesicles are expressed as fmoles/mg protein. * Significant change ($p < 0.05$).

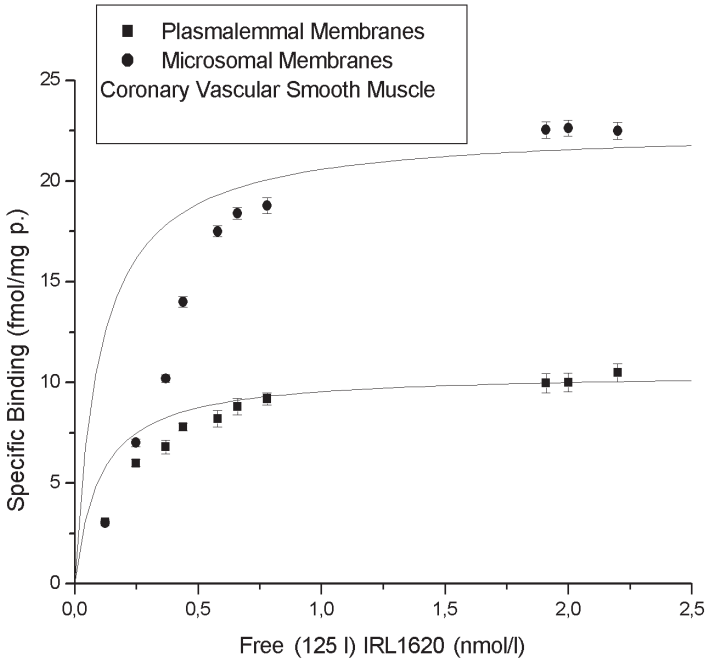


Figure 2. The proportion of specific endothelin ET_B endothelin-binding sites (fmol/mg protein) identified in two subfractions, plasmalemmal and microsomal, isolated from porcine coronary vascular smooth muscle preincubated (60 min) with (6–21)ET-1 agonist BQ3020 (10.0 nmol/l). The ET_B-selective radioiodinated (8–21)ET-1 fragment [^{125}I]IRL1620 (peptide fragment with at least 10 aminoacids preserved in the carboxy-terminus) was used for identification of subtype-specific endothelin binding sites. Fractional increase in specific ET_B-receptor binding in microsomal membranes isolated from coronary arteries preincubated with endothelin fragment BQ3020. Values are mean $\pm$ S.E.M. ($n = 12$).

lin agonists, [^{125}I]ET-1 and its (6–21) fragment, ([^{125}I]IRL1620) in plasmalemmal membranes of porcine coronary artery vascular smooth muscle are shown in Figure 2.

The subcellular membranes, prepared from coronary arteries preincubated with 0.1 nmol/l of ET-1 or its (6–21)ET-1 fragment (for 60 min) showed the total number of specific high affinity [^{125}I]ET-1 binding sites identified in plasmalemmal membranes to be significantly reduced ($78 \pm 19\,\%$ ($p < 0.05$) at reduced affinity. The preincubation of coronary arteries with ET-1 of BQ3020 significantly increased the total number of specific [^{125}I]ET-1 binding sites identified in microsomal membranes (Table 2). BQ788 and pertussis-toxin (PTX) attenuated increased binding in microsomal fraction induced by BQ3020 preincubation ($27 \pm 11\,\%$, $p < 0.05$). Preincubation of coronary arteries with endothelin antagonists resulted in

Table 2. [^{125}I]ET-1 binding sites identified in porcine coronary artery microsomal membranes after preincubation of coronary arteries with ET-1 and (6–21)ET-1 (fragment BQ3020). Porcine coronary arteries were preincubated also with phorbol myristate acetate (PMA), pertussis toxin (PTX), and BQ788 (ET_B – selective endothelin antagonist)

Preincubation	Specific ET-1 Binding Sites in Microsomal Membranes				
(100 nmol/l)			PMA+	PTX+	BQ788+
(0.1nmol/l)	ET-1	BQ3020	BQ3020	BQ3020	BQ3020
B_{max} (%^{c}) [^{125}I]ET-1	128 ± 4 *	144 ± 8 *	135 ± 4 *	52 ± 6 *	23 ± 7 *

Explanation and symbols: ^{c} values are mean ± S.E.M expressed as percentage of control. (Control density in coronary microsomal membranes was B_{max} = 19.2 ± 0.8 fmol/mg protein, $n = 20$, * significant change in B_{max}, $p < 0.05$).

Table 3. Effects of preincubation of quiescent porcine vascular fibroblasts (QVF) with human peptide endothelin-1 (ET-1) and with (6–21)ET-1 fragment BQ3020 on proliferation (measured as incorporation of [^{3}H]-thymidine[1]

Preincubation of QVF with (nmol/l)	ET-1 10.0	ET-1(6–21) 10.0
Incorporation of [^{3}H]-thymidine (% of Control)	128 ± 5 *	112 ± 6 *

Explanation and symbols: values are mean ± S.E.M. (n = 18), * significant difference from control. Porcine cardiac fibroblasts were seeded in 1% PDS in DV/F 12 medium 2 days *prior* to the addition of endothelin-1 or its (6–21)ET-1 fragment. [^{3}H]-thymidine incorporation was measured during 2 hrs sequential pulses of [^{3}H]-thymidine.

reduction of total numbers of binding sites in declining order: BQ788 > PD142893 > PD151242 >> PD155080. Affinity dropped in the following order: PD142893 > PD155080 > PD151242 > BQ788. The combined preincubation of the porcine coronary arteries with three ET-1 antagonists, i.e. endothelin-ET_A selective PD141242 and butenolide PD151080, endothelin-ET_B selective BQ788, and with nonselective peptide type antagonist PD142893 (all in 0.1 nmol/l), significantly reduced the total specific [^{125}I]ET-1 binding in plasmalemmal membranes to 15 ± 6% ($p < 0.05$) of control values.

Discussion

The following major findings were obtained from this study: 1. both, endothelin (6–21)ET1 and (8–1)ET-1 fragments with preserved carboxy-terminus, induced competent down-regulation and redistribution of membrane receptors, 2. ET-1 fragments produced redistribution of endothelin-ET_B-receptors to microsomal membranes and reduced their affinity, 3. linear ET-1 fragments acted as agonists on endothelin receptors (Dřímal et al. 1999), and 4. they increased the proliferation

rate in quiescent vascular fibroblasts. There are also three lines of evidence for multiple binding of ET-1 and its fragments to specific binding sites in CVSM and porcine vascular fibroblasts: firstly, the 21 aminoacids containing molecule of ET-1 is possibly cleared by neutral peptidases and carboxypeptidases in several steps (according to suggestions published by Sokolovsky et al. 1990, the degradation steps starts at the amino-terminal part of the peptide moiety). Our present results, showing significant pharmacological activity (down-regulation and redistribution of ET_B selective binding with two ET-1 fragments, both with nicked amino-terminal part and preserved carboxy-terminus supported the view of sequentional degradation of the peptide and confirmed at least two binding domains in the peptide moiety. Secondly, the observation obtained in the present study, indicating that very short two- or tripeptide fragments with one or two D-aminoacid substitutions in the molecule may bind to endothelin receptors and competitively antagonize binding of the 21 aminoacids containing molecule of human peptide ET-1 and/or effectively antagonize the binding of (8–21)ET-1 fragment, is convincingly indicative of multiple binding sites of human peptide ET-l and its fragments. Thirdly, concerning the down-regulation and subcellular distribution of endothelin receptors in porcine vascular smooth muscle,the total density of ET-1 receptors identified in the present study in porcine coronary vascular smooth muscle membranes was lower, while the proportion of subtypes was similar to that reported in a previous study (Peter and Davenport 1995). Both, the use of only medial part of the porcine coronary artery and the higher purification of membranes may account for the lower density found in the present study. Autoradiographic studies with human coronary artery showed that ET_A-receptors predominated and ET_B-receptors were difficult to detect in "normal" human coronary arteries (Dashwood et al. 1995; Davenport et al. 1995; Russell et al. 1997). The mRNA for both subtypes of endothelin receptor detected from the media of human coronary arteries and endothelin production were found to be markedly increased in human atherosclerotic coronary arteries (Timm et al. 1995). Some of the functional studies with ET-1 done on porcine coronary arteries described ET-1 induced contracture and suggested that both Ca^{2+} entry and Ca^{2+} sensitization contributed to the integrated ET-1 response (Elmosehi and Grover 1997). The fact that in our study [^{125}I]IRL1620 binding in porcine coronary artery membrane preparation displayed significantly lower K_D indicated a higher affinity and a more homogenous binding of the ET_B selective ET-1 fragment. The displacement of the high affinity [^{125}I]ET-1 binding to the low affinity high density receptor sites in microsomal membranes isolated from porcine coronary arteries preincubated with the ET_A selective antagonist PD141242 may reflect the predominance of the subtype-ET_B selective down-regulation in the porcine coronary artery. The preferential high affinity [^{125}I]ET-1 binding in porcine coronary arteries preincubated with the ET_B selective antagonist BQ788 in our recent study (Dřímal et al. 2002, in press) may indicate an exclusive property of ET_B selective down-regulation of ET-1 receptors in porcine coronary arteries. What is particularly interesting about the down-regulation pattern of ET-receptors in vascular smooth muscle in our study is that the synthetic, linear-type peptides BQ3020 and

IRL1620, i.e. truncated endothelin-ET_B selective peptides, induced a more intensive ET_B-receptor down-regulation. This may suggest that in comparison with the N-terminal domain, antiparallel β-sheet with its short β-turn, and the whole helical suppport in the molecule of ET-1, the linear part of the ET-1 and its C-terminus are probably more important for the induction of ET_B-receptor down-regulation. Independently, three different pathways/mechanisms were suggested in vascular smooth muscle stimulated with ET-1: ET_A mediated stimulatory transcription and subsequent translation of the contractile ET_B subtype of receptor (Moller et al. 1997), ET_A mediated inhibition of NO synthase by IL-1β stimulated pathway, and finally constitutive ET_B mediated NO production (Ikeda 1997). The last two seemed to be blocked by the ET_B selective antagonist.

In conclusion, in the present study we outlined a common mechanism of natural, helical ET-1 and its ET-1 fragment: the induction of ET_B selective down-regulation of endothelin receptors occuring at the short-term agonist exposure. This mechanism is subtype-ET_A and -ET_B specific, dependent on the presence of both N- and C-termini in the molecule of the ET-agonist. It was demonstrated that both, human and porcine vascular smooth muscle receptors undergo ET_B selective down-regulation after exposure to natural ET-1. The process is mediated by ET-1 receptor activation and is reversible. Both ET-1 fragments, truncated peptide agonists BQ3020 and IRL1620 induced similar reductions in plasmalemmal endothelin receptors, the ET_A selective antagonists PD151242 and PD155080 blocked [^{3}H]-thymidine incorporation in human fibroblats, and BQ788 attenuated ET-1 induced down-regulation of ET_B-receptors in coronary vascular smooth muscle.

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