
Index

A

Antagonists, *see* Endothelin receptors,
Ligands

Antibody,

concentration, 11

generation, 4

ET-1 and big ET-1, 151–153

ECE-1 isoforms, 111–118

secondary, 17

specificity, 10, 40

Antisera, *see* Antibody

Arachidonic acid release, 193

Autoradiography,

quantitative, 64–66

B

Big endothelin-1, 3–7, 22, 37, 107–109,
125, 147–149, 165–167,

antibody generation, 151–153

measurement by,

ICC, 3–19

RIA, 24–36

sandwich immunoassay, 147–164

Big endothelin-2,3–7, 22, 166,

measurement by,

ELISA, 22

ICC, 3–19

Big endothelin-3, 3–7, 22–23, 166,

measurement by ICC, 3–19

C

Chronic heart failure, 217,

endothelin antagonists, 217

Competition binding assays, 56–61, 80,
189–190

data analysis, 58, 60–61, 189

Coronary artery ligation, 218, 221–222,
echocardiography, 222

endothelin-1, 218

endothelin receptors, 218–219

infarct size, 218, 223–225

left ventricular pressure

measurements, 222–223

plasma sampling, 222–223

C-Terminal fragment (CTF), 23–25,
109, 121,

measurement by RIA,
24–36

D

DNA synthesis, 190–191

E

ECE, *see* Endothelin-converting
enzyme

ECE activity assays,

HPLC, 113, 120

fluorescence, 113, 120–121

sandwich immunoassay, 147–164

ECE-1, 3–7, 107–111, 125–127,
147–148, 165,

antisense oligonucleotide, 148

detection of isoforms by ICC, 3–19,
110–118

isoforms, 3–7, 108, 110–111,
125–128, 165–166,

generation of antibodies, 111–118

oligonucleotide primers, 130

knockout, 148

- ECE-2, 3, 5–7, 107, 125,
detection by ICC, 3–19
- ECE-3, 107
- ELISA, *see* Enzyme-linked
immunosorbent assay
- Endothelin-1, 3–7, 22, 37, 71, 107–109,
125, 147–149, 165, 167, 181,
217, 229,
antibody generation, 151–153
measurement by,
ELISA, 25–29
ICC, 3–19
RIA, 24–36
sandwich immunoassay, 147–164
- Endothelin-2, 4, 22, 37–38, 71, 166, 181,
measurement by HPLC and RIA,
24–36, 38
- Endothelin-3, 4, 22–23, 37, 71, 127,
166, 182,
measurement by,
ELISA, 23, 40
ICC, 3–19
HPLC and RIA, 24–36, 38
- Endothelin converting enzyme, 3–7,
107–111, 125–127, 147–149, 165,
see also ECE-1, ECE-2, ECE
activity assays,
antibody generation, 4, 111–117,
inhibitors, 148, 166, 217,
evaluation by,
sandwich immunoassay,
147–164
in vitro pharmacology,
165–177
smooth muscle ECE, 165–177
- Endothelin peptides, *see also*
Endothelin-1, Endothelin-2,
Endothelin-3,
purification by HPLC, 38
tenacious binding, 73–75
- Endothelin receptors, *see also* ET_A
receptor, ET_B receptor, Receptor
binding assays,
antagonists, 72–73, 76, 181–182,
208–209, 217,
binding properties, 75–77
handling, 73, 79–80
IC₅₀ values, 76
interaction with serum albumin, 77
characterization, 45, 46, 48–49,
71–91, 181
distribution, 47
mRNA, 94
primer design, 95
primer sequences, 95
splice variants, 47
subtypes, 45–46, 71, 77–79, 93, 181
- Enzyme-linked immunosorbent assay,
21–36, 115–116
- ET_A receptor, *see also* Endothelin
receptors, 22, 45–49, 71, 75, 127,
181–187, 208, 218,
antagonists, 72, 181–182, 208–213
autoradiography, 64–67
binding assays, 50–64, 67, 79–88
cDNA mutant, 96–98
ligands, 49, 74, 76
radioligands, 49, 183
- ET_B receptor, *see also* Endothelin
receptors, 22–23, 45–49, 71, 75,
127, 181–187, 208, 218,
antagonists, 72, 208–213
autoradiography, 64–67
binding assays, 50–64, 67, 79–88
cDNA mutant, 98
ligands, 49, 76
radioligands, 49, 183
- ET_C receptor, 46, 48, 71
- Experimental heart failure, 217–227
- F**
- Forearm vasoconstriction, 229–237,
endothelin, 229–230
endothelin-1 infusion, 231–234
endothelin antagonists, 230

G

G-protein coupled receptors, 45, 71, 93, 181

H

HPLC,
ECE activity assay, 113, 120
separation of ET isoforms, 37–42

I

IgG,
affinity purification, 148–149,
153–155
biotinylation, 149, 155–156
Immunocytochemistry (ICC), 3–19,
112, 117–118,
controls,
positive, 10
negative, 10
tissue,
preparation, 12
fixation, 15–16
immunofluorescent visualization,
13–14
Intimal hyperplasia, 199, 205–206,
207–211
In vitro pharmacology, 165–177,
data analysis, 171–172
evaluation of ECE inhibitors, 170
normalization procedure,
169–170
In vitro transcription, 99

K

Kinetic binding assays, 61–64, 74,
association assay, 62–63
dissociation assay, 63–64

L

Ligand characterization, 181–199,
functional assays, 184–187

O

Organ culture, 199, *see also* Saphenous
vein organ culture

P

Phosphatidylinositol hydrolysis,
191–193
Phosphoramidon, 125, 147, 165, 167
Polymerase chain reaction (PCR),
see also RT-PCR, 135–140,
amplification efficiency, 98–100
Protein determination, 32

R

Radioimmunoassay, 21–36, 170, 172
Radioligands, 48–49
Receptor binding assays, 45–70, 71–91,
see also Competition binding
assays, Kinetic binding assays,
Saturation binding assays
RIA, *see* Radioimmunoassay
RNA,
isolation, 131–132
quantification, 132
RT-PCR, 93, 127,
competitive, 93–104
quantitative, 127–128, 135–141
semi-quantitative, 127, 132–134, 141
specificity, 141

S

Sandwich immunoassay, 147–149,
156–158,
evaluation of ECE inhibitors,
158–159
Saphenous vein organ culture, 199–214,
effect of ET-1 on, 205–207
endothelin antagonists, 207–211, 213
measurement of neointima, 204–205
vein harvesting, 200, 212
Saturation binding assays, 50–56, 66,
80–87, 190–192,

- data analysis, 54–56, 82–86
- graphical analysis, 52–54, 83
- Site-directed mutagenesis, 96–98
- Species differences,
 - ET peptides, 23
 - ET receptors, 46

T

- Tissue preparation,
 - cultured cells, 12, 15

- cryostat sections, 12, 14–15
- membranes, 81
- solid phase extraction, 25–28
- vascular, 166–169,
 - spontaneous activity, 173

V

- Venous occlusion plethysmography,
 - 229–237,
 - data analysis, 231, 235