

Table of contents

	page
Summary English	1
Summary German	3
1. Introduction	5
1.1 Signal transduction	5
1.2 G protein-coupled receptors	5
1.3 Endothelins and endothelin receptors	12
1.4 The aim of this study	14
2. Materials and Experimental procedures	15
2.1 Materials	15
2.1.1 Chemicals, antibodies, cDNA's and receptor ligands	15
2.1.2 Apparatus and software	18
2.1.3 Cells	19
2.1.4 Commonly used buffers	19
2.1.5 Plasmids/constructs	21
2.2 Experimental procedures	23
2.2.1 Cell culture	23
2.2.2 Peptide synthesis and fluorescence labeling	23
2.2.3 Transient and stable transfection of HEK293 cells	24
2.2.4 Generation and affinity-purification of polyclonal antibodies	25
2.2.5 Immunoblots for the detection of ET _B .YFP and ET _A myc.CFP	28
2.2.6 Immunoprecipitation experiments	28
2.2.7 Fluorescence resonance energy transfer (FRET)	29
2.2.8 Receptor sequestration assay	30
2.2.9 Fluorescence microscopy and image analysis	31
2.2.10 ¹²⁵ I-ET-1 displacement binding experiments	31
3. Results	32
3.1 Generation of HEK293 cell clones stably expressing ET _A and ET _B receptors	32

3.2	Functional analyses of fluorescent endothelin receptor fusion proteins	33
3.2.1	Saturation binding experiments	33
3.2.2	LSM analysis of HEK293 cell lines expressing fluorescent endothelin receptor fusion proteins	35
3.2.3	Characterisation of polyclonal ET _A and ET _B receptor antibodies	37
3.3	Immunoprecipitation	49
3.3.1	Antibodies for immunoprecipitation studies	49
3.3.2	Selection of HEK293 cell clones for immunoprecipitation studies	49
3.3.3	Immunoprecipitation analysis reveals ET _A /ET _B heterodimers	50
3.4	Fluorescence resonance energy transfer analysis of protein-protein interactions in living cells	54
3.4.1	The principle of FRET	54
3.4.2	FRET analyses demonstrate homo- and heterodimerisation of endothelin receptor subtypes in living HEK293 cells	55
3.4.3	Specificity of ET _A /ET _B receptor heterodimerisation	59
3.5	Endothelin receptor subtypes display similar ligand-binding affinities when expressed individually or in combination	63
3.6	Heterodimerization results in a decreased rate of ET-1 mediated ET _B receptor sequestration	66
3.7	Intracellular trafficking of the ET _B receptor in HEK293 ET _B flag.YFP/ET _A myc.CFP cell clones	70
3.8	Influence of receptor ligands on ET _A /ET _B heterodimers	72
3.9	Dissociation of ET _A /ET _B heterodimers occurs along the endocytic pathway	74
4.	Discussion	76
4.1	Homo- and heterodimerisation of ET receptor subtypes	76
4.2	Regulation of ET _A /ET _B dimerisation	77
4.3	Dimerisation of GPCRs. A general phenomena among all GPCR families?	78
4.4	Functional role of GPCR dimerisation	82

4.5	Outlook, enhancing and disrupting GPCR oligomerisation	85
5.	References	87
6.	Appendix	101
6.1	Publications	101
6.2	Abbreviations	101