Summary

Endothelin-1 (ET-1) is a potent vasoactive peptide that acts on endothelin A (ET_A) and endothelin B (ET_B) receptors. Although both receptor subtypes are co-expressed in numerous cells, little is known about their ability to form heterodimers. In this study it was shown that both receptors were co-immunoprecipitated with an ET_B-specific antibody using extracts from HEK293 cells stably co-expressing a fusion protein consisting of a myc-tagged ET_A receptor and CFP (ET_Amyc.CFP) and a fusion protein consisting of an ET_B receptor and YFP (ET_B.YFP). Co-immunoprecipitation was also observed with extracts from HEK293 cells transiently co-expressing FLAG-tagged ET_B and myc-tagged ET_A receptors, thereby excluding that heterodimerisation is mediated by the CFP/YFP moieties. Heterodimerisation was further confirmed in fluorescence resonance energy transfer (FRET) analysis of HEK293 cells transiently co-expressing ET_Amyc.CFP and ET_B.YFP receptors. Additionally homodimerisation of ET_A and ET_B receptors could be demonstrated in FRET analysis of HEK293 cells transiently co-expressing ET_Amyc.CFP and ET_Amyc.YFP or ET_B.CFP and ET_B.YFP receptors. FRET efficiencies were between 12 and 18% in untreated and antagonist- or ET-1-treated cells, indicating constitutive homo- and heterodimerisation. Prolonged stimulation (30 min) with the ET_B receptor-selective agonist BQ3020 decreased FRET efficiency by 50%. This decrease was not observed when internalisation was inhibited by co-expression of dominant-negative K44A.dynamin I or incubation with 450 mM sucrose. Enzyme-linked immunosorbent assay and laser scanning microscopy of cell clones stably co-expressing ET_Amyc.CFP/ET_Bflag.YFP receptors revealed a slower sequestration of the ET_B.flag.YFP receptors upon stimulation with ET-1 than with BQ3020. No difference in ET-1 or BQ3020-mediated sequestration was observed with cell clones expressing ET_Bflag.YFP alone. The data suggest that ET_A and ET_B receptors form constitutive heterodimers, which show a slower sequestration upon stimulation with ET-1

than with BQ3020. Heterodimer dissociation along the endocytic pathway only occurs upon ET_B receptor-selective stimulation.