

5 Summary

The human endothelin B (ET_B) receptor comprises 442 amino acids, of which the first 26 function as a signal peptide. The signal peptide, which is essential for cell surface transport is cleaved off by a signal peptidase in the ER lumen during receptor biosynthesis. In addition, a second protheolytic cleavage within the extracellular N terminus (at R64↓S65) has been identified, which results in an N-terminally truncated receptor, lacking amino acids 26 to 64. The regulation and the physiological significance of this proteolysis were not known when this study was started. To gain more insight into the process of N-terminal proteolysis, ET_B receptor or ET_B-GFP fusion protein stably or transiently expressed in human embryonic kidney 293 (HEK 293) cells and in vascular smooth muscle cells (VSMCs) were analyzed. After incubation of cells with ¹²⁵I-ET-1 at 4°C, only the full-length ET_B receptor was detected at the cell surface. When cells were incubated at 37°C in the presence of endothelin-1, N-terminal cleavage was observed. The cleavage was not prevented by inhibitors of internalization (sucrose, phenylarsine oxide) or of serine and cysteinyl proteases. However, when cells were incubated with internalization and metalloprotease inhibitors (batimastat, inhibitor of TNF α -converting enzyme Ro32-7315) or metal chelators (EDTA, phenanthroline), the cleavage was blocked. The data show that metalloproteases mediate an agonist-dependent cleavage of the ET_B receptor at the cell surface.

Functional analysis of a mutant ET_B receptor lacking the first 64 amino acids (Δ 2-64 ET_B) revealed normal ligand binding properties and preserved G protein-signaling (increase of inositol phosphate formation and inhibition of forskolin-induced cAMP-formation) when compared to the wild-type receptor. However, the Δ 2-64 ET_B receptor showed a 15-fold reduced cell surface expression and an altered ability to activate ERK1/2. Although the wild-type and the Δ 2-64 ET_B receptor elevated an early phase of ERK1/2 phosphorylation (within 5 min), only the wild-type receptor induced a second phase of ERK1/2 activation (starting after 80 min). The second phase was mediated *via* $\beta\gamma$ subunit of G_i proteins and was abolished by inhibitors of matrix metalloproteases (batimastat and an inhibitor of TNF α -converting enzyme Ro32-7315).

The data presented in this study strongly suggest, that the N-terminal proteolysis of the human ET_B receptor is mediated by a metalloprotease in an agonist-dependent manner. Removal of the ET_B receptor's N terminus yields a receptor with a dramatically reduced cell surface expression and an altered ability to stimulate ERK1/2 activation. The data suggest, that the N-terminal cleavage of the ET_B receptor could be involved in the regulation of cell surface expression and of ERK1/2 activation. The functional role of the observed biphasic ERK1/2 activation *via* the full-length ET_B receptor and the monophasic ERK1/2 activation *via* the Δ 2-64 ET_B receptor requires further characterization.