Endothelin-converting enzyme-1 (ECE-1) regulates the recycling and resensitisation of the neuromedin U 2 receptor (NMU2) following activation by neuromedin U-25 but not neuromedin S.

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Neuromedin U receptor 1 and NMU2 are family A, G protein-coupled receptors that mediate a wide range of physiological effects of the gut-brain neuropeptide, neuromedin U (NmU). More recently, neuromedin S (NmS) has been identified as a ligand of these receptors and its distribution within specific regions of the CNS suggests responsibility for the inhibitory effects of NMU2 activation on feeding behaviour. Both NMU1 and NMU2 couple to $G\alpha_{q/11}$ and $G\alpha_{i/o}$ proteins leading to increases in phosphoinositide/Ca^{2+} signalling and inhibition of adenylyl cyclase activity respectively. We have reported the essentially irreversible binding of NmU to its receptors and the subsequent internalisation of both ligand and receptor (Brighton et al, 2004). This suggests that the processing of internalised ligand could play a role in regulating the lifetime of the ligand-receptor complex and potentially regulate receptor recycling and resensitisation. Indeed, endosomal ECE-1 degradation of peptide ligands has been shown to be important in the recycling of several GPCRs (e.g. Roosterman et al, 2007). Here, we examined the role of ECE-1 in resensitisation of NMU2-mediated Ca^{2+} responses to either human NmU-25 or NmS-33 in HEK 293 cells recombinantly expressing human NMU2 using fluo-4-loaded cells and a NOVOstar microplate reader.

Acute exposure (5 min) to a maximum concentration of NmU-25 (30 nM) resulted in a marked desensitisation (reduced potency and E_{max}) of subsequent responses to NmU-25 that required 6 h to fully recover. This recovery was unaffected by inhibition of protein synthesis using cycloheximide (17.5 µM) but was significantly ($P<0.001$) attenuated by the internalisation inhibitor, dynasore (80 µM) (17$\pm$2% versus 69$\pm$1% recovery at 3 h; all data are mean$\pm$sem, n>3; statistical analysis by one-way or two-way ANOVA and Bonferroni's range test). Further, monensin (50 µM), an inhibitor of endosomal acidification, abolished recovery over the 6 h period. The ECE-1 inhibitor, SM-19712 (10 µM) significantly ($P<0.001$) delayed recovery (e.g. 17$\pm$3% versus 68$\pm$5% recovery at 3 h). Ca^{2+} responses to NmS-33 had similar potency and E_{max} values to NmU-25. However, resensitisation of responses to NmS-33 (5 min pretreatment with 30 nM) were significantly ($P<0.001$) delayed compared to those with NmU-25 (e.g.68$\pm$2% versus 97$\pm$2% recovery at 6 h). Although recovery of NmS-33 responses was inhibited by dynasore (29$\pm$3% versus 56$\pm$4% recovery at 6 h) and monensin (20$\pm$5% versus 58$\pm$7% recovery at 6 h), recovery was entirely unaffected by SM-19712.

These data suggest that following NMU2 activation and desensitisation by either NmU-25 or NmS-33, resensitisation requires dynamin-dependent internalisation and endosomal acidification. However, resensitisation following desensitisation by NmS-33 is delayed compared to that following NmU-25. Further, the data suggest that endosomal ECE-1 activity is required for resensitisation of NMU2 following desensitisation by NmU-25 but that this activity is not rate-limiting for recovery following desensitisation with NmS-33. These data highlight distinct ligand-dependent effects at the NMU2. Studies are currently underway to establish if such differences between ligands may also influence other aspects of receptor function such as G-protein-independent signalling by internalised receptors.

Brighton et al, (2004) *Mol Pharmacol* **66:** 1544-1556.

Roosterman et al, (2007) *Proc Nat Acad Sci USA* **104:** 11838-11843.