Trafficking of L83I, a Naturally Occurring Variant of the Mu-Opioid Receptor (MOPr).

Alexandra E. Cooke, Sue Oldfield, Eamonn Kelly, Stuart J. Mundell, Graeme Henderson

School of Physiology and Pharmacology University of Bristol, BS8 1TD Bristol, United Kingdom

In HEK 293 cells, DAMGO-induced desensitisation of the Mu-opioid receptor (MOPr) is mediated primarily by G-protein-coupled receptor kinase 2 (GRK2) and results in rapid MOPr internalization, whereas morphine-induced desensitization is largely PKC-dependent and results in little MOPr internalization (Bailey et al., 2003; Johnson et al., 2006). A recently identified naturally occurring variant (L83I) has been observed to undergo significant internalization in response to morphine (Ravindranathan et al., 2009). The current study examined the molecular regulation of internalization of the wild-type (WT) MOPr in comparison with that of the L83I variant.

HEK 293 cells were transiently transfected with either HA-tagged MOPr or the MOPr-L83I variant, and internalization of HA-tagged receptors was assessed by ELISA as described previously (Johnson et al., 2006). For investigations of constitutive internalization, cells were prelabelled with the primary antibody at 4°C for one hour prior to incubation with serum free DMEM.

Agonist stimulation of the WT receptor for 5 min resulted in substantial internalization of the WT-MOPr in response to DAMGO (10µM; 39.8 ± 7.5% internalization) but not to morphine (30µM; 3.2 ± 4.6% internalization). In contrast, there was substantial internalization of the L83I variant in response to both DAMGO (37.7 ± 5.3% internalization) and morphine (28.9 ± 4.7% internalization; P<0.01). Marked internalization of the L83I variant in response to morphine was also seen by immunofluorescence confocal microscopy. No significant difference in the constitutive internalization between the WT-MOPr and the L83I variant measured over a 30 minute time course was observed (15.0 ± 6.0% and 21.3 ± 3.7% internalization at 30 minutes, respectively). Pre-treatment of cells with dynasore (40µM; 15 minutes), a dynamin inhibitor, effectively inhibited the internalization of both the WT-MOPr and the L83I variant in response to DAMGO. Dynasore pre-treatment also inhibited morphine-induced internalization of the L83I variant.

Differential phosphorylation of MOPr by opioid agonists has been well documented (El Kouhen et al., 2001; Schulz et al., 2004). Antagonism of GRK2 with a dominant negative mutant (DNM) GRK2 (K220R) attenuated the internalization of the L83I variant in response to both DAMGO (50.2 ± 5.7% and 7.9 ± 4.6% internalization in the absence and presence of GRK2-DNM; P<0.05) and morphine (41.4 ± 5.3% and 3.6 ± 9.6% internalization in the absence and presence of GRK2-DNM; n.s.) in addition to inhibiting the internalization of the WT MOPr in response to DAMGO only (52.3 ± 2.4% and 3.6 ± 4.7% internalization in the absence and presence of GRK2-DNM; P<0.05).

In conclusion these results show unlike the WT MOPr the L83I variant internalizes rapidly in response to morphine. The agonist-induced internalization of the L83I variant is GRK- and dynamin-dependent. Work is currently underway to investigate the extent of morphine-induced phosphorylation of the L83I variant and to investigate whether the observed results can be explained by differential efficacy of morphine for coupling at the L83I variant as compared to the WT MOPr.


