Characterization of a naturally occurring variant of the mu-Opioid Receptor (MOPr): L83I

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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 trafficking and signalling of the wild-type (WT) MOPr in comparison with that of the L83I variant.

All experiments were carried out on HEK 293 cells expressing either HA-MOPr or the HA-MOPr-L83I variant. Internalization of HA-tagged receptors was assessed by ELISA and immunofluorescence microscopy as previously described (Mundell *et al*., 2006). Phosphorylation of serine 375 was assessed by western blot following immunoprecipitation of HA-tagged receptors from whole cell lysates. cAMP measurements were performed using a cAMP enzyme immunoassay (EIA).

DAMGO stimulation of the receptors resulted in significant internalization of both the WT-MOPr and the L83I variant (33.9 ± 3.0% and 36.6 ± 4.3% respectively; *n=6*). In contrast, morphine induced significant internalization of the L83I variant but had little effect on the WT-MOPr (27.2 ± 4.1% and 1.6 ± 7.8% respectively; *n=6*). Marked internalization of the L83I variant in response to morphine was also seen by immunofluorescence confocal microscopy. Inhibition of dynamin by pre-treatment of cells with dynasore (40μM; 15 min) effectively inhibited the DAMGO-induced internalization of both the WT-MOPr and the L83I variant. Furthermore, dynasore also inhibited the morphine-induced internalization of the L83I variant (58.0 ± 6.6 % *v*. -8.6 ± 7.7%; *P*<0.001; *n=3*).

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% in the absence and presence of GRK2-DNM ; *P*<0.01*; n=4*) and morphine (41.4 ± 5.3% and 3.6 ± 9.6% in the absence and presence of GRK2-DNM; *P*<0.01) in addition to inhibiting the internalization of the WT-MOPr in response to DAMGO (52.3 ± 2.4% and 3.6 ± 4.7% in the absence and presence of GRK2-DNM; *P*<0.001*; n=4*). Of the numerous potential phosphorylation sites in the MOPr, serine 375 in the C-terminal tail has been the most extensively studied and identified as a probable GRK phosphorylation site. Following immunoprecipitation of the HA-tagged receptors, DAMGO induced substantial phosphorylation of this residue in both the WT-MOPr and the L83I variant, whereas, morphine induced far less phosphorylation of this residue but which was the same in the WT-MOPr and L83I (n.s.; *n=5*).

Investigations into the G-protein signalling of the L83I variant by measurement of cAMP inhibition revealed no significant change in the EC_{50} of either DAMGO or morphine when compared with values obtained for the WT-MOPr (n.s; *n=4*).

In conclusion these results show that unlike the WT-MOPr, the L83I variant rapidly internalizes in response to morphine in a GRK- and dynamin-dependent manner. The enhanced internalization of L83I in response to morphine is not due to increased phosphorylation of Serine 375, or an increased ability to signal via G-proteins.

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