Sustained agonist activation of MOPr rapidly initiates regulatory events including desensitization and internalization, that are thought to contribute to the development of tolerance. We studied the molecular mechanisms that mediate the trafficking of the MOPr following chronic morphine treatment (1μM; 72h). Using ELISA to quantify internalization in HEK 293 cells stably expressing HA-tagged MOPr, the extent of DAMGO-induced internalization was significantly reduced in cells that had been exposed chronically to morphine in comparison to control cells (14.6 ± 1.7% versus 25.2 ± 2.5%, respectively). This effect was due to chronic morphine as following morphine for only 24h the observed decrease in DAMGO-induced internalization was absent. Furthermore, in cells that have been exposed to morphine chronically, the MOPr did not recycle normally, only 0.8 ± 10.4% of the DAMGO-internalized receptor had recycled back after 30 min, whereas in control cells, 49.4 ± 8.4% of the receptor was found to recycle back to the plasma membrane. Chronic morphine treatment did not alter the expression of arrestins, dynamin or GRK2 in the cells; however, there was a substantial decrease (40.1±7.2%) in MOPr that could be prevented by inhibition of either the lysosomal or proteasomal pathways. Additionally, immunofluorescence microscopy indicated that chronic morphine induced colocalization of MOPr with lysosomes. Thus after chronic morphine, DAMGO induces significantly less internalization of MOPr, the receptor is unable to recycle and is targeted for degradation.