Role of carboxyl terminus of mu- and delta-opioid receptor in agonist-induced down-regulation.

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Chronic exposure of mu- and delta-opioid receptors to their agonists leads to different rates in receptor down-regulation. In order to analyze the role of the carboxyl terminus of mu- and delta-opioid receptors in the difference in the rate of down-regulation, two chimeras of these receptors were generated by swapping the carboxyl termini; MORTAGDT and DORTAGMT. These chimeras were tagged at the N-terminus with hemagglutinin (HA) epitope (YPYDVPDYA), which can be recognized by the monoclonal antibody 12CA5, and then stably expressed in Neuro 2A (N2A) cells. The swapping of the carboxyl termini did not alter the ligand selectivity of these receptor chimeras. However, they did exhibit a reduction in agonist potency to inhibit forskolin-stimulated adenylyl cyclase activity for all agonists tested except etorphine which had a potency comparable to that of wild type receptors. Treatment of the N2A cells expressing MORTAGDT with 50 nM etorphine produced a faster rate of receptor down-regulation when compared to the wild type mu-opioid receptor. Immunofluorescence microscopy of the MORTAGDT chimera using a monoclonal antibody against HA confirmed internalization of the receptors after treatment with etorphine for 1 and 6h. There was a reduction in the HA-immunoreactivity at the cell surface of the MORTAGDT chimera concurrent with more noticeable HA-immunoreactivity inside the cell compared to the wild type receptor. On the other hand, the rate of down-regulation of DORTAGMT receptors was seen to be the same as the wild type delta-opioid receptor after etorphine treatment. Immunofluorescence studies showed more reduction in cell surface staining of the DORTAGMT chimera compared to the wild type receptor. These data suggest the involvement of the carboxyl terminus in agonist-induced down-regulation and internalization of the nu-opioid receptor. However, different mechanisms that are unrelated to the carboxyl terminus may operate in the down-regulation of delta-opioid receptor.

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