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Opioid receptors

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Abstract

Mouse μ-opioid receptor carboxy-terminal splice variants have been shown to differ in their responses to agonists.

Significance and context

Opioid receptors belong to the G-protein-coupled receptor (GPCR) superfamily and are responsible for the transduction of diverse extracellular signals that regulate cell function. Their ligands are the endogenous opioid peptides and opiate drugs. Morphine, a highly addictive opiate, mediates its analgesic effect through the μ-opioid receptor. During repeated or continuous stimulation with agonists, μ-opioid receptors undergo rapid desensitization. Important general mechanisms for desensitization of other GPCRs are receptor phosphorylation and subsequent endocytosis, which removes the receptor-ligand complex from the cell surface. It has recently been suggested that such endocytosed receptors are mainly recycled and returned to the cell surface in activatable form, thus resensitizing the cell to the agonist. Unlike the endogenous opioid peptides, morphine can activate the μ-opioid receptor without causing its rapid internalization. Previous studies on the rat μ-opioid receptor indicated that the carboxy-terminus can influence agonist selectivity of receptor

internalization. Carboxy-terminal alternative splicing, which has been observed for a number of GPCRs, is thought to modulate several aspects of GPCR physiology, for example, cell- and tissue-specific expression, subcellular targeting and coupling to G proteins.

Three novel carboxy-terminal splice variants (MOR1C, MOR1D and MOR1E) of the mouse μ-opioid receptor (MOR1) have recently been identified. The differences between MOR1 and the new isoforms are restricted to the carboxy-terminal portion of the intracellular tail. The length of the tail as well as the number of potential phosphorylation sites varies: MOR1C, MOR1D and MOR1E contain seven, two and one new potential phosphorylation sites compare to MOR1, respectively. Koch *et al*. have investigated whether the differences in the carboxy-terminal tails of the μ-receptor splice variants influences endocytosis, phosphorylation rate, desensitization, resensitization and downregulation of the receptors in response to agonists.

Key results

The μ-opioid receptor splice variants were tagged with a hemagglutin (HA) epitope and stably expressed in HEK 293 cells. The ligands tested were the endogenous opioid peptide agonist [D-Ala^2, Me Phe^4, Glyol^5] (DAMGO) and morphine.

Treatment with DAMGO resulted in rapid internalization and similar desensitization and resensitization rates for all receptor isoforms. After exposure to morphine, however, only MOR1D and MOR1E were endocytosed, whereas MOR1 and MOR1C were highly resistant to endocytosis. Prolonged exposure to morphine led to complete desensitization of all receptor isoforms, but desensitization of MOR1 and MOR1C was markedly facilitated compared to that of MOR1D and MOR1E. After DAMGO-induced desensitization, all splice variants completely resensitized during agonist withdrawal. After morphine-mediated desensitization, MOR1D and MOR1E showed only partial resensitization during agonist withdrawal and MOR1 and MOR1C did not regain function.

To investigate whether receptor internalization affects agonist-induced downregulation, the monomeric forms of the receptors were determined after pretreatment with agonists. Exposure to morphine resulted in downregulation of MOR1D and MOR1E similar to that observed for DAMGO. No downregulation of MOR1 and MOR1C was observed after prolonged morphine treatment.

The μ-opioid receptor isoforms markedly differed in their morphine-induced phosphorylation rates. Morphine and DAMGO promoted similar levels of phosphorylation of MOR1D and MOR1E. Morphine-induced phosphorylation of MOR1 and MOR1C was, however, much lower compared to DAMGO-induced phosphorylation.

Conclusions

The results reinforce the hypothesis that receptor phosphorylation and internalization are required for opioid receptor reactivation, counteracting the effects of agonist-induced desensitization. Furthermore, the results show that differences in the carboxy-terminal tails of the μ-receptor splice variants influence their morphine-induced internalization. MOR1D and MOR1E showed morphine-induced internalization and were thus recycled and resensitized during morphine withdrawal; on the contrary, MOR1 and MOR1C were not internalized and thus not resensitized after morphine withdrawal.

Reporter's comments

This work is a good example of how important even small differences between splice variants can be. The authors did not discuss the fact that under normal conditions homodimers were found for MOR1 and MOR1C but not for MOR1D and MOR1E. What might be the explanation for this, and could this affect the agonist selectivity?

Table of links

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References

1. Koch T, Schulz S, Pfeiffer M, Klutzny M, Schröder H, Kahl E, Höllt V: C-terminal splice variants of mouse μ-opioid receptor differ in morphine-induced internalization and receptor resensitization. J Biol Chem. 2001, 276: 31408-31414 . 0021-9293