EDITORIAL VIEWS

sures that only one species of receptor is present and is a useful technique for defining the transduction pathways used by specific receptor subtypes. In two mammalian cell types they demonstrated that the μ- and δ-receptor subtypes activate extracellular signal-related kinase, one of the three defined mitogen-activated protein kinase species.

These findings have novel implications at the fundamental and clinical levels. At the fundamental level, it will be important to define which are the "downstream" effects of extracellular signal-related kinase activation and to determine if these can explain some of the excitatory effects of opioids. Such information may lead to therapeutic strategies that can interrupt the development of tolerance, dependence, and addiction. In addition, the fact that κ-agonists cannot stimulate extracellular signal-related kinase may provide insights into the different pharmacologic actions exhibited by drugs acting exclusively at the κ-receptor subtype.

I have one final thought: Even though nature is parsimonious in having but two endogenous opiate ligands and three opiate receptor subtypes, it can still introduce remarkable specificity by discriminating which molecular component in transduction pathways can "match" with one other component.

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Reference

Why Does Insensitivity to Opioid Narcotics Develop?

OPIATE receptors hold a place of prominence for practitioners of anesthesia. Not only are these targets for several analgesic and anesthetic drugs that are used commonly but the primary structure of each of the opiate receptor subtypes was first reported in 1993 by an anesthesiologist, Dr. Kazuhiko Fukuda working in Professor Kenjiro Mori's department in Kyoto, Japan. The isolation and cloning of this family of proteins proved particularly difficult because of its relatively low abundance. However, following the example of Fukuda's pivotal studies, molecular genetic reagents have been developed that have prompted a profusion of cell biology studies that has greatly increased our understanding of opioid action.

Of particular interest to investigators in Dr. Robert Peterfreund's laboratory at the Massachusetts General Hospital are factors that regulate sensitivity to opioid narcotics. Biochemical changes are induced in chronic pain states, which reduce the analgesic efficacy of opioids. In addition, patients develop tolerance to the analgesic properties of opioids after they are administered continuously. In both these settings, a similar cascade of neuroplastic changes are induced, resulting in activation of the NMDA receptor and activation and translocation of protein kinase C.

These investigators have directed their attention to the "downstream" effects of the activation of protein kinase C. In a model cell system derived from humans (SH-SY5Y cell line) that contains the μ-opioid receptor and the entire signaling system responsible for neuroplasticity, the authors directly activated protein kinase C with the phorbol ester. Then they determined

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whether transcription of the μ-opioid receptor was affected by this treatment by measuring the amount of messenger RNA (mRNA) using a technique called slot blot hybridization. This technique takes advantage of the fact that the mRNA is complementary to the template DNA with which it will "hybridize." Therefore, the amount of mRNA specific for the μ-opioid receptor can be determined by reacting the harvested mRNA with a 32P-labeled cDNA probe (i.e., the template for the μ-opioid receptor) and determining the amount of radioactivity that "sticks" by autoradiography followed by laser densitometry. Care was to taken to establish that the 32P-labeled cDNA probe directed against the μ-opioid receptor did not react nonspecifically with all mRNA. In addition, they confirmed that the same amount of mRNA was used by normalizing their data with the "housekeeping gene" β actin, whose content does not change under these conditions.

The authors report that the amount of μ-opioid receptor mRNA present is significantly reduced when protein kinase C activity is stimulated with phorbol esters. Further, they demonstrated that the decrement in transcription of the μ-opioid receptor did not depend on new protein synthesis, which effectively precludes a genetically mediated transcription factor in the pathogenesis of this event. Rather, the decrement in transcription is likely to be produced by posttranslational modification by phosphorylation of certain factors(s).

The importance of this finding is that the decrease in μ-opioid receptor mRNA is expected to cause a concomitant decrease in μ-opioid receptor protein expression or receptor downregulation. In turn, this may cause a decrease in sensitivity to opioids and thereby a loss in the analgesic properties of this class of compound.

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