Ethanol and opioid receptor signalling

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Summary. Ethanol may modulate endogenous opioid systems by disrupting opioid receptor signalling. Low concentrations of ethanol slightly potentiate \( \mu \)-opioid receptor binding by increasing receptor B_{max}, and, in some cases, chronic ethanol exposure decreases the density or affinity of the \( \mu \)-opioid receptors. By contrast, high concentrations of ethanol acutely decrease \( \delta \)-opioid receptor binding by decreasing receptor affinity, whereas chronic exposure of animals and neuronal cell lines to lower concentrations of ethanol leads to possibly adaptive increases in the density or affinity of the \( \delta \)-opioid receptors. In the neuronal cell line NG108-15, ethanol does not up-regulate the \( \delta \)-opioid receptor by blocking receptor degradation or endocytosis, but protein synthesis is required for this response. Up-regulation of the \( \delta \)-opioid receptor renders ethanol-treated NG108-15 cells 3.5-fold more sensitive to opioid inhibition of adenylyl cyclase. Long-term treatment with ethanol also increases maximal opioid inhibition in NG108-15 cells, possibly by decreasing levels of \( G_{\alpha} \) and its mRNA. Ethanol differentially modulates signal transduction proteins in three additional neuronal cell lines, N18TG2, N4TG1, and N1E-115. Ethanol-treated N18TG2 cells show the least up-regulation of the \( \delta \)-opioid receptor, little heterologous desensitization of adenylyl cyclase, and no changes in \( G_{\alpha} \) or \( G_{\beta} \). By contrast, ethanol-treated N1E-115 cells show the largest up-regulation of the \( \delta \)-opioid receptor, the most heterologous desensitization of adenylyl cyclase, and concentration-dependent decreases in \( G_{\alpha} \) and increases in \( G_{\beta} \). Further analysis of these related neuronal cell lines may help to identify the molecular elements that endow some, but not all, neuronal cells with the capacity to adapt to ethanol.

Key words. Endogenous opioid systems; ethanol; \( G_{\alpha} \)-proteins; receptor signalling; up-regulation; \( \mu \)-opioid receptor; \( \delta \)-opioid receptor.

Alcohol and endogenous opioid systems

There is considerable evidence that ethanol interacts with endogenous opioid systems to produce some of its central nervous system (CNS) effects \(^4,5,81\). ICI 174864, a selective \( \delta \)-opioid receptor antagonist, can block ethanol-induced hypothermia and sedation when microinjected into discrete brain regions \(^81\). Moreover, the opiate antagonist naloxone can attenuate the ethanol withdrawal syndrome when given during and after the administration of ethanol \(^5,6\). Heritable differences in susceptibility to alcoholism may also be related to an ethanol-opioid interaction. In inbred strains of mice, ethanol consumption correlates inversely with brain levels of [Met]enkephalin \(^5\). Ethanol could modulate the activity of endogenous opioid systems through effects on the synthesis, processing, or release of opioid peptides, or on opioid receptor signalling (fig. 1). Effects of ethanol on the biosynthesis and regulation of opioid peptides are described elsewhere in this volume \(^18\). Here, I will review the effects of ethanol on opioid receptor signalling in brain and in neuronal cells.
Opioid receptor signalling

Multiple opioid receptors
Pharmacological, anatomical, and physiological studies suggest that opioids interact with at least four different receptor subtypes, designated μ, δ, κ, and σ. Morphine and dihydromorphine (DHM) preferentially recognize the μ-opioid receptor; binding to this receptor is inhibited by nanomolar concentrations of the opiate antagonist naloxone. The synthetic opioid peptide H-Tyr-D-Ala(Me)Phe-NH₂-OH (DAGO) shows far greater μ receptor selectivity than morphine, whereas [D-Pen²,D-Pen⁶]enkephalin has excellent δ receptor selectivity. The peptidase-resistant derivative of [Leu⁶]enkephalin, [D-Ala²,D-Leu⁶]enkephalin (DADLE), is frequently used as a δ-selective ligand but has only 2–10-fold greater affinity for δ sites than μ sites. Naloxone binds δ sites 20-fold less potently than μ sites. The compound U50,488 binds κ sites more than 1000-fold more potently than μ or δ sites. The existence of a σ-opioid receptor was first suggested by the pharmacology of N-allylnormetacainine. This σ receptor may mediate the psychomimetic effects of benzomorphan opiates, but is often considered apart from the μ-, δ- and κ-opioid receptors because of its unique pharmacological profile and anatomical distribution.

The three major opioid receptor subtypes differentially recognize the opioid peptides encoded by the preproenkephalin, proopiomelanocortin (POMC), and prodynorphin genes. Preproenkephalin encodes the pentapeptides [Met⁶]enkephalin and [Leu⁶]enkephalin, which bind δ-opioid receptors with 10–25-fold greater affinity than μ-opioid receptors. β-endorphin is encoded by POMC and recognizes μ and δ sites with equally high affinity. Dynorphin, a product of the preprodynorphin gene, binds selectively to the κ-opioid receptor subtype.

Subtype-selective opioid ligands are endowed with unique pharmacological properties because of the differential regional localization of opioid receptor subtypes in the central and peripheral nervous systems and the preferential interaction of opioid receptor subtypes with specific effector systems. The co-existence of multiple opioid receptor subtypes within many brain regions, their occasional co-expression within the same neurons, and their interaction with multiple effector systems greatly complicates the study of how ethanol modifies opioid signalling. This task has been simplified somewhat by the identification of tissues that express only a single receptor subtype (such as the δ receptor in neuroblastoma cell lines) and the development of ligands with high selectivity for each receptor subtype, as described above.

Physiological effects of opioids
Many opioid receptors are located presynaptically and their activation decreases the release of various neurotransmitters and neuromodulators including acetylcholine, dopamine, norepinephrine, substance P, somatostatin, vasopressin and oxytocin. Several physiological actions of opioids may account for their modulation of transmitter release. Opioids increase an inwardly rectifying potassium conductance, decrease voltage-dependent calcium conductance, hyperpolarize neuronal membranes and decrease the spontaneous firing rate of neuronal cells. These actions have been observed in both central and peripheral nervous tissues, but vary among different brain regions and different neuronal cell types. Certain ionic conductances may be regulated by individual opioid receptor subtypes; for example, κ agonists inhibit voltage-dependent calcium conductance whereas both δ and μ agonists activate an inwardly rectifying potassium conductance.

Interaction of opioid receptors with G proteins
G proteins appear to mediate the effects of many neurotransmitters, neuromodulators, and growth factors through their interactions with adenylyl cyclase, phospholipases A and C, and ion channels. All G proteins are heterotrimeric membrane-associated proteins comprising α, β and γ subunits. The α subunits of different G proteins contain homologous domains for GDP binding and hydrolysis, and variable domains for receptor recognition and effector interaction. The βγ subunits show greater sequence homology and appear to interact with multiple α-subunits. Receptor activation promotes the exchange of GTP for GDP within the α subunit, leading to subunit dissociation and the development of ligands with high selectivity for each receptor subtype, as described above.

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