

Role of the Endogenous Opioid System on the Neuropsychopharmacological Effects of Ethanol: New Insights About an Old Question

Carles Sanchis-Segura, Judy E. Grisel, M. Foster Olive, Sandra Ghozland, George F. Koob, Amanda J. Roberts, and Michael S. Cowen

This article presents the proceedings of the symposium "Endogenous Opioids and Voluntary Ethanol Consumption: What Have We Learnt From Knock-out Mice?" presented at the meeting of the International Society for Biomedical Research on Alcoholism held in Heidelberg/Mannheim, Germany, in September/October 2004. The organizers and chairpersons were Michael S. Cowen and Carles Sanchis-Segura. The presentations were as follows: (1) Regulation of the Opioid System by Alcohol: Comparison of Alcohol-Preferring and -Nonpreferring Strains by Michael S. Cowen; (2) Endogenous Opioids and Alcohol: Lessons From Microdialysis and Knock-out Mice by M. Foster Olive; (3) From Neurochemistry to Neuroanatomy: The Hypothalamic Arcuate Nucleus as a Main Site for Ethanol-Opioids Interaction by Carles Sanchis-Segura; (4) Sensitivity to Ethanol Is Modulated by β -Endorphin in Transgenic Mice by Judy E. Grisel, Amanda J. Roberts, and George F. Koob; and (5) The μ -Opioid Receptor Modulates Acute Ethanol Sensitivity and Ethanol Withdrawal Severity by Sandra Ghozland.

Key Words: Ethanol, Opioids, Endorphins, Enkephalins, Nociceptin, Knock-Out Mice.

THE ENDOGENOUS OPIOID system (EOPS) has been one of the neurotransmitter systems more often proposed as a major determinant of the activating/reinforcing effects of ethanol administration and consumption. Several lines of evidence support that proposal (for a review of these topics, see Herz, 1997; Koob et al., 1998; Cowen and Lawrence, 1999). First, ethanol promotes the release of several endogenous opioid peptides (such as β -endorphins), modifies their synthesis rate, and alters the binding properties of the opioid receptors. Furthermore, a large number of studies have shown that low doses of opioid agonists, especially μ -receptor ligands, can enhance the neuropsychopharmacological effects, whereas an even higher number of studies have demonstrated that μ - and δ -opioid antagonists reduce them. Indeed, this latter finding has been of major clinical significance with the adoption of naltrexone as a major therapeutic alternative in treatment of the alco-

holic patient. On the other hand, there is also genetic evidence that suggests a main role of the EOPS in ethanol alcohol consumption and alcoholism. Thus, human associative studies reveal that the EOPS of subjects at a higher risk of excessive ethanol consumption/alcoholism has distinctive characteristics (i.e., prevalence of specific alleles) compared with the EOPS of subjects at a lower risk. Similarly, rodent strains selected by higher ethanol consumption present inborn differences and display differential adaptive changes after chronic ethanol consumption than their corresponding nonpreferring strains. In summary, there is an extensive corpus of evidence that supports a major concourse of the EOPS on the mediation of several of the neuropsychopharmacological effects of ethanol, including those that initiate and maintain its consumption.

The development of new animal models that were engineered to specifically target components of opioid peptides or receptors was regarded as a major opportunity to definitively characterize the role of EOPS in alcohol consumption beyond the obvious limitations of pharmacological and association-based genetic approaches. However, until now, these models have been restricted to the generation of peptide and receptor gene knock-out mice. In addition, the use of these models has produced conflicting findings. For example, whereas mice deficient for the μ -opioid receptor had decreased ethanol consumption (Roberts et al., 2000), mice deficient for β -endorphin had, under certain circumstances,

From the Howard Florey Institute (MSC), University of Melbourne, Australia, Department of Psychology (JG), Furman University, Greenville, SC; Neuropharmacology Department (SG), The Scripps Research Institute, La Jolla, CA; Ernest Gallo Clinic and Research Center (MFO), Department of Neurology, UCSF, Emeryville, CA; Area de Psicobiologia (CS-S), Universitat Jaume I, Castello, Spain.

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Carles Sanchis-Segura, PhD, Department of Psychopharmacology, Zentral Institut für Seelische Gesundheit (Central Institute for Mental Health), Mannheim, J5, 68159, Germany; E-mail: sergura@zi-mannheim.de

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greater consumption of ethanol (Grahame et al., 2000; Grisel et al., 1999). The purpose of this symposium was therefore to provide a forum to discuss these conflicting data as well as integrate them with other recent data obtained in pharmacological and lesion studies with the final aim of providing new alternatives in the investigation of this field.

REGULATION OF THE OPIOID SYSTEM BY ALCOHOL: COMPARISON OF ALCOHOL-PREFERRING AND - NONPREFERRING STRAINS

Michael S. Cowen

Opioidergic signaling is believed to be involved in the reinforcing properties of alcohol and may be involved in the predisposition to consume alcohol (Cowen et al., 2004). Therefore, a comparison was made of preproenkephalin (Penk) and pro-opiomelanocortin (POMC) mRNA expression in the forebrain of alcohol-preferring Fawn-Hooded (FH) and alcohol-nonpreferring Wistar Kyoto rats. Compared with Wistar Kyoto rats, FH rats had elevated POMC mRNA in the arcuate nucleus and elevated Penk mRNA in the central nucleus of the amygdala. We hypothesized that the elevated levels of opioid peptide gene expression in these brain regions of the alcohol-preferring FH rats may predispose toward enhanced ethanol consumption. In contrast, FH rats had lower levels of Penk in the caudate putamen and nucleus accumbens (Cowen et al., 1998). Interestingly, 56 days of free-choice alcohol consumption (2×28 days with a two-week hiatus) by FH rats led to a further increase in Penk mRNA expression in the central and intercalated nuclei of the amygdala but a decrease in Penk mRNA expression in the nucleus accumbens and olfactory tubercle (Cowen and Lawrence, 2001); however, POMC mRNA was unaffected. As suggested below (presentations by Olive and Sanchis-Segura), β -endorphin may play a role in the properties of ethanol other than reinforcement.

The convergence of β -endorphin (Finley et al., 1981) and enkephalin terminal fibers in the nucleus accumbens and central nucleus of the amygdala is suggestive of redundancy in alcohol-mediated opioidergic signaling. However, because a recent study indicated that C57BL/6 mice lacking both β -endorphin and enkephalin learned to self-administer ethanol (Hayward et al., 2004), other neurotransmitters such as endomorphin or dynorphin may also be recruited to mediate the reinforcing effects of ethanol. Alcohol consumption by FH rats also led to increases in μ -opioid receptor density in number of brain regions such as the nucleus accumbens (Cowen et al., 1999; Djouma and Lawrence, 2002); interestingly, these changes were in some case enhanced with cessation of alcohol consumption (Djouma and Lawrence, 2002), suggesting the possibility of sustained responsiveness or sensitization of the opioidergic system to ethanol.

ENDOGENOUS OPIOIDS AND ALCOHOL: LESSONS FROM MICRODIALYSIS AND KNOCK-OUT MICE

M. Foster Olive

One of the most widely distributed endogenous opioid peptides in the brain is β -endorphin, an endogenous agonist of μ - and δ -opioid receptors. β -Endorphin is derived from the POMC precursor peptide, which is produced by neurons that are largely restricted to the arcuate nucleus of the hypothalamus and nucleus of the solitary tract (Bloom et al., 1978; Finley et al., 1981). However, arcuate POMC-containing neurons send widespread endorphinergic projections throughout the brain, including limbic regions known to be involved in ethanol reward and reinforcement such as the nucleus accumbens and central nucleus of the amygdala. Along these lines, it has been shown that micro-injections of nonselective opioid receptor antagonists into the central nucleus of the amygdala and nucleus accumbens reduce ethanol consumption in rats (Foster et al., 2004; Heyser et al., 1999), suggesting that neurotransmission mediated by endorphins (or other opioid peptides) in these regions maintains ethanol consumption behavior.

Although many studies have shown that acute exposure to ethanol increases the secretion of β -endorphin from the hypothalamus-pituitary axis, we utilized in vivo microdialysis coupled to highly sensitive solid-phase radioimmunoassay procedures to demonstrate that acute exposure to ethanol (2 g/kg intraperitoneally) in nondependent rats increases extracellular endorphin levels in the ventral forebrain region of the nucleus accumbens (Olive et al., 2001), a region thought to play a critical role in the positive reinforcing effects of ethanol. Other investigators have replicated these findings and shown this effect to only occur at higher doses of ethanol (Marinelli et al., 2003). Given that μ - and δ -receptor agonists produce conditioned place preference when locally administered in the nucleus accumbens and positively modulate dopamine release in this region, it is tempting to speculate that drug-induced increases in endorphin release in the nucleus accumbens contribute to the rewarding and positive reinforcing effects of ethanol.

However, a potential limitation of this hypothesis is the fact that several recent studies have shown that aversive stimuli such as tail pinch (Marinelli et al., 2004), footshock (Zangen and Shalev, 2003), and extinction of intravenous heroin self-administration (Zangen and Shalev, 2003) also increase extracellular levels of β -endorphin in the nucleus accumbens as measured by microdialysis. Thus, as with nucleus accumbens dopamine, increased β -endorphin release in the nucleus accumbens may not necessarily reflect the rewarding and positive reinforcing effects of ethanol per se but may perhaps encode other cognitive processes such as stimulus salience. To further explore this possibility, we are currently conducting studies examining fluctuations in β -endorphin release in the nucleus accumbens during voluntary ethanol consumption as well as during reinstatement of self-administration after extinction. In addition,

studies are also under way examining the effects of selective immunoneutralization of β -endorphin in the nucleus accumbens and other limbic brain regions on ethanol self-administration. These studies will hopefully shed light on the importance of central β -endorphin neurotransmission in the maintenance of ethanol consumption and its possible dysregulation in rodent models of alcoholism.

Other types of abundant endogenous opioid peptides that bind preferentially to the μ - and δ -opioid receptors are the enkephalin pentapeptides, primarily met- and leu-enkephalin, which are derived from the Penk precursor. To examine a potential role for these peptides in ethanol consumption and reward, we obtained mice lacking the Penk gene as originally described by König et al (1996). Wild-type and Penk knock-out mice were exposed to varying concentrations of ethanol in a homecage two-bottle test paradigm for four days at each concentration. Surprisingly, we observed no genotypic differences in ethanol consumption or preference at any of the ethanol concentrations tested (Koenig and Olive, 2002), and similar negative results were recently reported in an operant self-administration paradigm in Penk knock-out mice (Hayward et al., 2004). In our study, we also examined the ability of systemically administered ethanol (2 g/kg intraperitoneally) to produce a conditioned place preference to ethanol in wild-type and Penk knock-out mice (Koenig and Olive, 2002). Again, to our surprise, no genotypic differences in the degree of ethanol-induced conditioned place preference were observed. From these findings, one might be tempted to come to the conclusion that Penk-derived peptides, including met- and leu-enkephalin, do not play a role in voluntary ethanol consumption or ethanol reward. However, these negative data must be viewed in light of several reports that Penk knock-out mice have significant up-regulations in μ - and δ -opioid receptor expression in various brain regions (Brady et al., 1999; Clarke et al., 2003), which is likely a result of physiological compensation for decreased overall levels of these endogenous μ - and δ -opioid agonists during development. Thus, components of other endogenous opioid systems also appear to be altered in Penk knock-out mice.

In summary, ethanol can stimulate the release of β -endorphin in the nucleus accumbens, although the physiological as well as behavioral significance of this phenomenon needs to be determined, because it is currently unclear whether β -endorphin release in the nucleus accumbens contributes to the positive reinforcing effects of ethanol and the maintenance of ethanol consumption behavior. Similarly, the role of enkephalin-related peptides in the control of ethanol consumption and reward also requires further studies utilizing more temporally defined manipulations of this peptide system. Developmental compensation issues in mice with embryonic deletion of the Penk gene limit any definitive conclusions regarding the role of enkephalin peptide neurotransmission in regulating

ethanol consumption behaviors and the rewarding effects of ethanol.

FROM NEUROCHEMISTRY TO NEUROANATOMY: THE HYPOTHALAMIC ARCUATE NUCLEUS AS A MAIN SITE FOR ETHANOL-OPIOIDS INTERACTION

Carles Sanchis-Segura

When studying the interaction between ethanol and the EOPS, genetic (i.e., transgenic animals) and pharmacological studies have been mainly focused on the functional consequences of the selective inactivation of an opioid peptide or receptor. Unfortunately, most of these studies have taken a “whole brain” approach, and consequently, very little is known about the molecular mechanisms and anatomic sites underlying this interaction.

In this regard, the hypothalamic arcuate nucleus is the only site of β -endorphin synthesis in the forebrain (Herz, 1997), and ethanol stimulates β -endorphin release from hypothalamic cultures. The ability of ethanol to stimulate this release is mediated by catalase (the main enzymatic system mediating ethanol oxidation in the brain), and acetaldehyde (the breakdown product of this ethanol metabolism) promotes even more potently this β -endorphin release (Reddy and Sarkar, 1993; Reddy et al., 1995). Interestingly, there is a clear parallelism between the effects of brain catalase inhibition and EOPS antagonism in several ethanol effects, including neuroendocrine changes (Pastor et al., 2005; Sanchis-Segura and Aragon, 2002b), voluntary alcohol consumption (Aragon and Amit, 1992; Höltter and Spanagel, 1999), and ethanol-induced locomotor changes in rodents (Sanchis-Segura et al., 1999a,b; Sanchis-Segura et al., 2004). On the basis of these observations, we have proposed (Miquel et al., 2003; Sanchis-Segura and Aragon, 2002b; Sanchis-Segura, 2005) that the hypothalamic arcuate nucleus could be the anatomic site for a functional system involving ethanol metabolism via catalase and, in a second step, the acetaldehyde formed in this nucleus could stimulate β -endorphin release that could act over opioid receptors located in brain areas receiving hypothalamic arcuate nucleus efferent projections (i.e., nucleus accumbens, amygdala, or VTA).

For the initial evaluation of this model, ethanol-induced locomotion was preferred as the dependent variable. This behavior was chosen because of its objectivity, its dependence on both the EOPS and catalase activity (Sanchis-Segura et al., 1999a,b), and its less dependence on free will than others (i.e., self-administration), consequently providing a more straightforward interpretation of the results. Thus, in the first series of experiments, it was demonstrated that extensive lesions of the hypothalamic arcuate nucleus secondary to monosodium glutamate or aureoethioglucose administration resulted in a blockade of the stimulant effect of low and moderate doses on mice locomotion (Sanchis-Segura and Aragon, 2002a), without affecting open field behavior after saline or caffeine injections. In-

terestingly, an almost complete blockade of ethanol-induced locomotor activity was also found after restricting the hypothalamic arcuate nucleus lesion to those neurons synthesizing β -endorphins (Sanchis-Segura et al., 2000). This effect was again observed in the absence of any alteration of spontaneous propanolol (an alcohol that does not release β -endorphins from the hypothalamic arcuate nucleus) or caffeine-induced locomotion. On the other hand, the impact of an acute ethanol injection on rat locomotion was attenuated after the selective injection of the catalase inhibitor sodium azide into the hypothalamic arcuate nucleus, then replicating the effects previously observed after its intraperitoneal or ICV administration. Therefore, there is an incipient body of evidence pointing to involvement of the β -endorphin release (produced after ethanol oxidation to acetaldehyde) from the hypothalamic arcuate nucleus in ethanol-induced locomotion and, more likely, other ethanol effects.

At present, the following steps of this neuropharmacological circuit are unclear, but the results of a second series of experiments (Pastor et al., 2005; Sanchis-Segura et al., 2004) suggest that the μ -opioid receptor is critically involved in the promotion of at least ethanol-induced motor behavior. Thus, it was demonstrated that naltrexone, at doses that primarily block the μ -opioid receptor, reduced ethanol-induced locomotion. Conversely, after repeated naltrexone injections, a transient boost of ethanol-induced locomotor activity was observed. This pattern of results is coincident with the previously described changes in MOR activity after acute and chronic naltrexone administration. Indeed, the same treatment conditions produced similar changes in the locomotion of mice after a challenge with morphine but not after tert-butanol (another alcohol that does not release β -endorphins; Reddy et al., 1995) administration. Indeed, selective μ -opioid, but not δ -opioid, receptor blockade also results in a dose-dependent antagonism of ethanol-induced locomotion (Pastor et al., 2005). Future experiments will be aimed at studying the anatomic location of the μ -opioid receptors involved in ethanol-induced locomotor changes and/or other effects of this drug.

SENSITIVITY TO ETHANOL IS MODULATED BY β -ENDORPHIN IN TRANSGENIC MICE

Judy E. Grisel

There is ample evidence supporting a mutually influential relationship between endogenous opioid systems and alcohol. We have been investigating the effect of β -endorphin on ethanol sensitivity using transgenic mice that were created by insertion of a premature stop codon onto the POMC gene and therefore do not produce this peptide (Rubinstein et al., 1996). The mutation has been fully backcrossed onto the C57BL/6J strain, and we have used these mice to investigate sensitivity to reinforcing [self-administration (Grisel et al., 1999), conditioned place

preference], sedative (loss of righting reflex), ataxic (stationary dowel, Roto-rod), anxiolytic (light/dark box, plus maze, novelty emergence), hypothermic, and analgesic (tail withdrawal) properties of alcohol. Most of these effects appear to be influenced by β -endorphin, often in a sex-dependent manner. In the present study, we evaluated ethanol-induced changes in locomotor activity in C57BL/6J, β -endorphin-deficient and heterozygote mice. On day one, subjects were habituated to an activity chamber for 10 min. On day two, they were injected with saline or 1.0, 1.5, 2.0, or 2.5 g/kg ethanol and immediately placed in the chamber for 10 min. Although there were no apparent differences between male subjects in movement time, female C57BL/6J, β -endorphin-deficient and heterozygote mice were less active after ethanol injections than their wild-type counterparts. Taken with previous data, these findings further the hypothesis that the effects of ethanol on behavior depend upon both β -endorphin and sex.

THE μ -OPIOID RECEPTOR MODULATES ACUTE ETHANOL SENSITIVITY AND ETHANOL WITHDRAWAL SEVERITY

Sandra Ghozland, Amanda J. Roberts, and George F. Koob

The μ -opioid receptor has been implicated as a mediator of the rewarding effects of ethanol. The fairly recent availability of mice lacking this receptor has made it possible to more fully explore this role (Matthes et al., 1996). Previously, it was shown that mice lacking the μ -opioid receptor do not consume ethanol under several different conditions (Roberts et al., 2000). We have demonstrated that experimentally naive μ -opioid receptor knock-out mice have decreased anxiety-like behavior in several tests (Filliol et al., 2000). Therefore, we were interested in investigating the relationship between ethanol drinking behavior and anxiety-like behavior under conditions of acute ethanol exposure as well as ethanol withdrawal and protracted abstinence. We performed a dose-response study of acute affective responses in the light/dark transfer paradigm and examined affective signs of ethanol withdrawal in these mice. A major finding was that μ -opioid receptor knock-out mice do not have any acute anxiolytic-like response to ethanol at any of the doses that elicit strong anxiolytic-like responses in their wild-type littermates. This suggests that μ -opioid receptor knock-out mice may not consume ethanol because they are less sensitive to the intoxicating effects of ethanol. Results examining affective behavior during ethanol withdrawal suggest that μ -opioid receptor knock-out mice have increased anxiety-like responses in earlier withdrawal tests than controls, suggesting that the knock-out mice may be more sensitive to the development of ethanol dependence. Therefore, the μ -opioid receptor may confer risk in the initial stages of ethanol exposure but may have a more protective role in the development of dependence, at least in terms of affective responses.

SUMMARY

The different presentations of this symposium lead to the main conclusion that the role of the EOPS in the determination of ethanol effects, consumption, and alcoholism is far from being completely understood. To the contrary, the data obtained using transgenic animals and their comparison with those obtained by other research strategies have revealed that the involvement of the EOPS on ethanol-induced behaviors is more complex than the classically proposed mediation of ethanol's reinforcing properties. This neurotransmission system can highly differ depending on which effect of ethanol is considered and even can play opposing roles according to several conditionals. In this regard, converse to expectations, the use of knock-out mice targeted for opioid peptides or receptors has not supposed a confirmatory strategy but has been extremely useful to highlight the necessity of considering EOPS genetic variations in a broader frame. Thus, as a result of our experience with these new genetic models and their phenotypic variation according to their genetic backgrounds, it seems essential to pay special attention to the possible epistatic effects of EOPS-related genes and/ or adaptive changes. On the other hand, it also seems essential to incorporate an anatomic view to the study of the interaction between ethanol and the EOPS as well as to consider the reciprocal gain changes between the EOPS with other neurochemical systems. Finally, the role of different components of the EOPS that have been more recently identified (i.e., nociceptin, endomorphins) must be clarified and that knowledge needs to be incorporated into the present proposals that are mainly based on the concurrence of endorphins, enkephalins, and dynorphins and their respective receptors. In summary, as stated by the neuropsychologist Antonio Damasio, "when it comes to explaining behavior and mind, it is not enough to mention neurochemistry. We must know whereabouts the neurochemistry is, in the system presumed to cause a given behavior. Without knowing the cortical regions or nuclei where the chemical acts within the system, we have no chance of ever understanding how it modifies the system's performance. . . . Moreover, the neural explanation only begins to be useful when it addresses the results of the operation of a given system on yet another system." Fortunately, these issues can be addressed by incorporating new refinements of our ability to engineer transgenic animals (i.e., conditional knock-out models) and other techniques (i.e., microinjection, local immunoneutralization).

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