Opioids and alcoholism

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Abstract

Although far from conclusive, evidence implicating the endogenous opioid system in the development and maintenance of alcoholism is growing. Currently available data suggest that ethanol increases opioid neurotransmission and that this activation is part of the mechanism responsible for its reinforcing effects. Findings from preclinical research indicate that ethanol consumption and ethanol-induced dopamine (DA) release are both reduced by opioid antagonists. Individual differences in endogenous opioid activity have been linked to inherited risks for alcoholism in studies comparing ethanol-preferring and nonpreferring rats, as well as in studies using targeted gene mutation (knockout) strategies. To a large extent, findings from human studies have paralleled those from the preclinical work. Persons who differ in family history of alcoholism have been shown to also differ in basal β-endorphin activity, β-endorphin response to alcohol, and subjective and HPA axis hormonal response to opioid antagonists. Findings from clinical trials indicate that opioid antagonists may reduce ethanol consumption in alcoholics, particularly in persons who have resumed drinking. Nevertheless, many questions remain unanswered about the use of opioid antagonists in alcoholism treatment and about the exact role of the opioid system in ethanol preference and reward. The progression of knowledge in this field suggests that many of these questions are imminently answerable, as our ability to characterize relationships between opioid activity and human behavior continues to develop. This paper summarizes both the progress that has been made and the gaps that remain in our understanding of the interactions between the endogenous opioid system and risk for alcoholism.

Keywords: Opioid peptides; Alcohol; Alcoholism; Opioid antagonists; β-endorphin; Family history of alcoholism; Naloxone; Naltrexone; ACTH; Cortisol; Reinforcement; Genetics

1. Introduction

It is estimated that approximately 14% of men and 5% of women in the United States will experience the symptoms of ethanol abuse or dependence sometime in their lives [1]. Processes involved in the development of alcoholism are thought to reside largely in the brain and are the result of complex interactions between genetic and environmental determinants. In recent years, there has been increasing interest in the neurobiological mechanisms that underlie ethanol reinforcement and the associated risks for the development of alcoholism. Considerable evidence has emerged suggesting that drugs of abuse derive their reinforcing properties by acting through a common pathway involving the brain neurotransmitter dopamine (DA) [2]. As with most other drugs of abuse, the rewarding effects of ethanol are thought to be associated with increased synaptic DA accumulation within the nucleus accumbens [3]. Although ethanol interacts with numerous neurotransmitter systems, its ability to increase mesolimbic DA release appears to depend on the integrity of the endogenous opioid system. In particular, findings showing that ethanol-induced DA release can be blocked by opioid antagonists have implicated opioidergic activity as an intermediary in this process [4]. Currently available data suggest that ethanol increases opioid neurotransmission and that this activation is part of the mechanism responsible for its’ reinforcing effects [5–9].

The evidence linking the endogenous opioid system to the development and/or maintenance of alcoholism has led to several theories regarding the possible nature of an opioid abnormality in this disorder [10]. The Opioid Deficit Hypothesis posits that low levels of endogenous opioid activity motivate compensatory ethanol consumption that serves to increase opioid activity in the brain [9,11]. In this scenario, the administration of an opioid antagonist blocks the ethanol-induced increases in opioidergic activity. Alternatively,
the Opioid Surfeit Hypothesis maintains that vulnerable individuals inherit or acquire an excess of endogenous opioid activity [12]. This opioid surfeit sets conditions for enhanced ethanol consumption, a process that also can be blocked by the administration of opioid antagonists. Theories about the directionality of an opioid abnormality in alcoholism have not yet been fully substantiated by empirical findings. However, in spite of their differences, the Opioid Deficit and the Opioid Surfeit hypotheses are both supported by data showing that ethanol-prefering subjects have a greater β-endorphin response to ethanol than do the nonpreferring subjects. Gianoulakis [5] maintained that this commonality in findings between the two theories leads to the possibility of a third hypothesis, which posits that vulnerability for increased ethanol consumption is determined more by individual differences in sensitivity of the opioid system to ethanol than by differences in the basal levels of endogenous opioid activity. Although our understanding about these complex processes is still in an evolutionary stage, considerable progress has been made in recent years to identify links between neurobiological function and human behavior. The purpose of this manuscript is to review findings of preclinical and clinical research that implicate the endogenous opioid system as a mediating force in the development and maintenance of alcoholism.

2. Reward pathways and drugs of abuse

To understand how the endogenous opioid system might mediate some of the reinforcing properties of ethanol, it is important to first appreciate the intrinsic function of the mesocorticolimbic DA reward pathway in drug reinforcement. Of the two major DA systems originating in the ventral midbrain, the mesocorticolimbic system, which serves as an interface between the midbrain and the forebrain, has been shown to play an important role in drug and ethanol reinforcement [13]. Findings from numerous preclinical studies (e.g., [14,15]), as well as from neuroimaging work with humans [16,17], have demonstrated that key structures mediating the rewarding properties of drugs include the ventral tegmental area (VTA), ventral striatum, nucleus accumbens, olfactory tubercle, amygdala, and frontal cortex. The nucleus accumbens, which is a structure located at the base of the striatum, is the key zone that mediates the rewarding effects of drugs such as amphetamine and cocaine, which act directly on dopaminergic terminals to increase the release of DA [18]. In contrast to the actions of these stimulants, morphine and heroin bind to opioid receptors on systems that regulate the activity of mesolimbic DA neurons [15]. In the VTA, opiates bind to μ- and, possibly, δ-receptors on GABA interneurons or GABA projection neurons that interact with DA neurons to cause the disinhibition of dopaminergic cell firing [15,19]. Opiate actions have not been as well characterized in the nucleus accumbens and may even be independent of the DA system [13,20]; however, all of the major types of opiate receptors are found in this region [10]. Importantly, the endogenous opioids β-endorphin and enkephalin also have rewarding properties and increase DA release within the nucleus accumbens [13,21]. Evidence implicating the DA system in ethanol reinforcement includes findings showing that self-administration is accompanied by increases in nucleus accumbens DA [22,23], that DA receptor antagonists dose dependently suppress ethanol self-administration in animals [24], and that D₁-receptor-deficient knockout mice show markedly less preference for ethanol than wild-type mice do [25]. In the absence of drug administration, the mesocorticolimbic system is thought to filter regional signals mediating basic biological drives and motivational behavior.

3. Opioid peptides and receptors

There are three major groups of endogenous opioid peptides, each derived from a specific precursor hormone: the endorphins from the β-endorphin/ACTH precursor proopiomelanocortin (POMC) [26]; the enkephalins from the precursor proenkephalin [27]; and the dynorphins and neoeendorphins from the precursor prodynorphin [28]. Each prohormone is the product of a distinct gene and a separate messenger RNA. The opioid peptides function as neurotransmitters or neuromodulators regulating a variety of brain functions including psychomotor stimulation, positive reinforcement, adaptive processes, drinking, eating, and sexual behaviors, pituitary function, thermoregulation, nociception, and mood [29–32].

Posttranslational processing of POMC produces several opioid peptides, including β-endorphin and some biologically active peptides, that are unrelated to opioid peptides, such as ACTH. The β-endorphin-producing neurons in the brain are located mainly in the ventromedial arcuate nucleus, which projects to widespread brain structures including many areas of the hypothalamus and limbic system. Other sites of POMC synthesis include the nucleus tractus solitarius, which is the likely origin of descending pathways that mediate analgesia, and the pituitary gland, which contains the highest concentration of POMC mRNA in the body. In contrast to the limited sites of POMC synthesis, the prohormone proenkephalin is produced in many brain regions and is processed into forms of Met–enkephalin and leucine–enkephalin. While some enkephalin-containing cells have long projections, many are small neurons that form local circuits. The highest levels of proenkephalin mRNA are found in the striatum, the ventral medial nucleus of the hypothalamus, and the dentate gyrus of the hippocampus. Prodynorphin and its peptide products are also found in many areas of the brain; in fact, its wide distribution often parallels that of proenkephalin [33–35]. Prodynorphin is the precursor for the dynorphins, alpha neoendorphins, and leucine–enkephalin [28].
The three classes of opioid peptides described above interact with at least three opioid receptor subtypes: mu (μ), delta (δ), and kappa (κ) [36]. β-Endorphin binds with about equal affinity to μ- and δ-opioid receptors, whereas the enkephalins bind with approximately 20-fold greater affinity to δ- compared with μ-opioid receptors. The prodynorphin peptide products tend to bind selectively to κ-opioid receptors. There are many types of opioid peptide and receptor interactions, and one should be cautious about generalizations. However, the activation of μ- or δ-receptors often seems to lead to similar patterns of neurotransmitter release, whereas the activation of κ-receptors often produces opposing patterns [21]. For example, in the mesolimbic system, β-endorphin and enkephalin peptides increase DA release within the nucleus accumbens through their interactions with the μ- and δ-opioid receptors and are, therefore, an intrinsic part of the process associated with reward and reinforcement [13]. Dynorphin, on the other hand, has been shown to decrease DA release secondary to activation of κ-opioid receptors, thereby producing aversive states. Although there seems to be homologous expression of opioid receptor subtypes in many brain regions of rodents and humans, some differences have been reported. These differences include a wider expression of κ-receptor mRNA in many brain regions, as well as the evidence of more κ- and μ-receptors in the cortex and hippocampus and more μ-opioid receptors in the hypothalamus of humans as compared with rats [37].

4. Ethanol and opioid interactions

The investigation of the relationship between endogenous opioids and alcoholism was a logical outgrowth of neurobiological studies showing that ethanol alters opioid peptide systems. To better understand the interactions between ethanol, endogenous opioids, and mesolimbic DA, it is important to know how ethanol alters opioid peptide systems and, in turn, how endogenous opioid activity modulates ethanol- and drug-seeking behaviors. This section summarizes what we currently know about ethanol’s effects on the endogenous opioid system.

The possibility that some effects of ethanol may be mediated through the endogenous opioid system was first proposed by Davis and Walsh [38], who discovered that morphine-like alkaloids (tetrahydroisouquinolones) are formed in vivo as a result of the interaction of the ethanol metabolite, acetaldehyde, with certain metabolites of DA. Further research demonstrated that these alkaloids could bind to opioid receptors and produce opioid-like effects [39–41]. However, the pharmacological relevance of these compounds in opioidergic processes remains unclear because their concentrations in brain tissues is extremely low, and they also seem to have direct effects on DA neurotransmission unrelated to opioid receptor binding [10].

In the past decade, a greater body of evidence has accumulated in support of speculations that ethanol interacts with opioid peptide systems by altering opioid peptide synthesis and secretion. Acute ethanol administration increases endorphin and enkephalin gene expression in discrete brain regions and increases the release of these peptides from the brain and pituitary of rodents [42–45]. In vitro studies have further shown that ethanol will stimulate β-endorphin release in a dose-dependent manner from the hypothalamus and the pituitary [43,46–48]. This effect is transient, lasting approximately 10–20 min. There is less data about the effects of ethanol on enkephalins and dynorphins than on its effects on β-endorphin [6]. Findings from some studies have indicated that short-term ethanol administration increases Met-enkephalin levels in the striatum and hypothalamus of rats, whereas findings from others have shown no significant changes in enkephalin levels in these brain regions [49–51].

Although acute ethanol exposure appears to stimulate brain and pituitary opioid peptide release, prolonged ethanol administration generally induces a decrease in endogenous opioid activity [52–55]. Chronic ethanol administration has been shown to decrease POMC gene expression and to alter the diurnal rhythm of POMC gene expression [56], β-endorphin release, hypothalamic levels of β-endorphin [57–60], μ-opioid receptor affinity, and, possibly, receptor binding [61]. In addition, chronic ethanol administration was shown to decrease dynorphin and α-neo endorphin expression in the hypothalamus and hippocampus, but not in the striatum, midbrain, or pituitary gland in one line of rats [51]. However, these effects may be specific to the tissue and to the species, strain, or line of animal being tested. Findings with another line showed increases in prodynorphin in the nucleus accumbens following chronic ethanol administration [62].

The evaluation of ethanol’s acute effects on the density or affinity of opioid receptors has been complicated by study differences in the types of opioid receptors and brain regions studied, the kinds of ethanol administration procedures used, the specificity of the study ligands, and the species, strains, and lines of animals tested. These differences have made it difficult to compare and contrast findings across studies. Furthermore, findings from the most recent autoradiographic techniques indicate that ethanol-induced changes on receptors appear to vary depending on the brain region and lineage of the animals used [6]. For example, ethanol consumption for 30 days increased binding to μ-opioid receptors in the caudate putamen of Sardinian rats [63], but induced a down-regulation of these receptors in nucleus accumbens and striatum of Wistar rats [64]. The results of studies examining the effects of chronic ethanol consumption on opioid receptor bindings also tend to be inconsistent and may reflect differences in the amount of ethanol consumed and in the experimental conditions during ethanol consumption [10].
5. Opioid neurotransmission and ethanol consumption

Although the evidence that acute ethanol administration increases the synthesis and secretion of opioid peptides raises the possibility that these effects may be causally related to the development and/or maintenance of alcoholism, it is far from conclusive. This possibility was made more plausible by findings from studies characterizing the endogenous opioid system in ethanol-prefering and non-prefering rodents, as well as from studies utilizing opioid receptor antagonists to alter ethanol consumption in rodents.

There are naturally occurring, as well as selective-bred, rodent lines that are ethanol preferring or nonpreferring. Ethanol-prefering rodents are animals that self-administer more ethanol than water in a free-living environment. Generally, the preference for ethanol is related to its intoxicating properties and not related to gustatory or olfactory preference. Several studies have shown that opioid peptide expression differs between ethanol-prefering and nonpreferring lines of rodents, supporting the hypothesis that genetic differences in opioid expression may be causally linked to differences in ethanol preference in ethanol-naïve animals [6]. For example, the selectively bred AA (ethanol preferring) rats have greater hypothalamic POMC mRNA expression compared with ANA (ethanol avoiding) rats [65,66]. Analogously, POMC mRNA expression is greater in the hypothalamus of ethanol-prefering C57BL/6 mice compared with ethanol-nonprefering DBA/2 mice [46,67,68]. Ethanol administration produces a greater increase in POMC mRNA in the pituitary of the selectively bred ethanol-prefering P rats compared with ethanol-avoiding NP rats [69]. Furthermore, ethanol exposure has been shown to induce greater release of β-endorphin from the hypothalamus of ethanol-prefering C57BL/6 mice [48] and AA rats [68] compared with nonprefering DBA/2 mice and ANA rats, respectively. Interestingly, the findings of one study also showed that differences in pituitary β-endorphin content correlated genetically with the severity of ethanol withdrawal symptoms in 16 lines of mice [70]. Collectively, these findings are suggestive of a relationship between endorphin expression and ethanol self-administration; however, they do not confirm the causative nature of the relationship.

The pattern of enkephalin levels in selectively bred lines of rodent does not necessarily the parallel endorphin levels in the same rodents [6]. For example, Met–enkephalin peptide levels were found to be lower in the nucleus accumbens, and Leu–enkephalin levels were found to be lower in the VTA of the AA compared with ANA rats [62]. The same is true for the hypothalamic enkephalin levels in C57BL/6 compared with DBA/2 mice [71]. Higher preenkephalin mRNA expression was found in the prefrontal cortex of the AA than ANA rats [65]. The C57BL/6 and DBA/2 mice have similar Met–enkephalin peptide levels in the hypothalamus, striatum hippocampus, medulla, and pons [72]. The selectively bred P and NP rats have similar levels of preproenkephalin-derived peptides. However, differences in preproenkephalin mRNA were noted in the nucleus accumbens of the P but not the NP line of rats 1 h after ethanol infusion [73].

Differences in μ-, δ-, and κ-opioid receptors expression have also been described in ethanol-prefering and ethanol-avoiding animals [74–76]. AA rats have a higher density of μ-opioid receptors in the shell region of the nucleus accumbens and prefrontal cortex, but a lower density of κ-opioid receptors in the ventromedial hypothalans than ANA rats have [65]. Inconsistent findings have been reported with respect to differences in δ-receptor binding between AA and ANA rats [74,77]. Ethanol-prefering C57BL/6 mice have higher δ-receptor density and lower κ-opioid receptor density in the nucleus accumbens compared with DBA/2 mice [75,76]. Ethanol-prefering P rats have higher density of μ-opioid receptors in some regions of the limbic system compared with the ethanol-avoiding NP rats [78]. No differences in μ-opioid receptor mRNA were reported between the HAD and LAD lines of rats [79]. Once again, these findings are suggestive, but do not clearly establish whether differences in opioid peptide/receptor expression produce the observed differences in patterns of ethanol self-administration between ethanol-prefering and nonprefering lines. In spite of the lack of consistency in opioid peptide/receptor levels between pairs of ethanol-naïve, ethanol-prefering, and nonprefering lines of rodents, acute ethanol exposure, in almost all cases, causes an increase in β-endorphin levels in serum and in multiple brain regions. This has not been shown for enkephalin peptides.

6. Antagonists and knock-out models

A more direct way to determine if there is causal linkage between the endogenous opioid system and ethanol consumption is to block or disrupt the opioid system. Indeed, there is a large body of literature demonstrating that opioid antagonist administration reduces ethanol consumption. Naloxone was the first opioid receptor antagonist shown to decrease ethanol consumption in rodents [12,80–84]. Similar findings were later reported with naltrexone and nalmefene [85–89]. Decreases in ethanol consumption with naloxone do not seem to be related to motor effects [90] or to effects on ethanol absorption [91,92]. Interestingly, Sinclair [8,93,94] observed that although opioid antagonists were effective in decreasing drinking in animal models, if they were given the drinks while ethanol was being consumed; opioid antagonists administered during abstinence did not reduce later drinking. The explanation was that blockade of ethanol-induced opioid release could only occur when ethanol was being consumed.

Naloxone, naltrexone, and nalmefene are nonselective opioid receptor antagonists with no intrinsic agonist activity. Antagonists that are selective for the μ- and δ-opioid receptor types also have been shown to decrease ethanol consumption...
self-administration in rodents and monkeys under a variety of experimental conditions [95–97]. In the P rat, the α-selective opioid receptor antagonists, naltrexone and naltriben, decrease ethanol consumption [96–98]. However, in the ethanol-prefering AA rats, the δ-opioid receptor antagonist CTOP, but not the α-opioid receptor antagonist ICI 174864, decreases consumption [99]. Despite these discrepancies, the use of the more selective opioid antagonists has helped to establish that both the β-endorphin and enkephalin systems are important for the maintenance of ethanol consumption [7].

Another piece of evidence supporting a role of the endogenous opioid systems in ethanol-seeking behaviors is the observation that ethanol-induced (applied focally) DA discharge in the striatum and the nucleus accumbens is decreased by opioid antagonists given either systemically [4,100] or focally [101]. This finding implicates opioidergic activity as an intermediary between ethanol exposure and the release of nucleus accumbens DA. However, it is important not to oversimplify the mechanisms governing reward. The effects of opioid agonists and antagonists on DA release have been shown to be, in part, dependent on which type of receptors are involved and whether the drugs are applied to the VTA or directly to the nucleus accumbens. Furthermore, the extended amygdala could be another site of opioid action, as a high proportion of cells in the medial nucleus of the amygdala and bed nucleus of the stria terminalis (BNST) express µ- and δ-opioid receptors [102]. The microinjection of an opioid receptor antagonist into the amygdala has been shown to decrease ethanol self-administration in nondependent rats [103]. This finding is part of a growing body of evidence suggesting that the extended amygdala may be integrally involved in both the positive and negative reinforcement associated with addiction [104–106].

Evidence that complements the findings from the opioid receptor blockade studies described above is derived from studies that have utilized strategies to disrupt opioid receptor and opioid peptide expression. For example, the microinjection of antisense oligonucleotides targeted to the µ-opioid receptor in the nucleus accumbens disrupted ethanol drinking by the ethanol-prefering ATP rats [107]. Targeted gene mutation (knockout) strategies have also produced mice that lack opioid peptides or receptors. µ-Knockout mice do not respond for ethanol [105]; whereas, δ-knockout mice self-administer ethanol [108]. Conceivably, the persistent ethanol administration in δ-knockout mice could be the result of a compensatory increase in µ-receptor activity in the absence of the δ-receptor [109]. In addition, C-57/B6 mice with a targeted disruption of the µ-opioid receptor showed a decrease preference for ethanol [110]. Mice with a targeted disruption of the POMC gene, therefore lacking β-endorphin peptide, also drink less ethanol than their wild-type littermates do [111,112].

In combination, the preclinical research findings make a compelling case for the role of the endogenous opioid system in ethanol preference and the development and maintenance of alcoholism. The next section will review how this work translates to the human condition.

7. Laboratory studies of β-endorphin levels in alcoholics and in persons at increased risk for alcoholism

The methods of examining central levels of endogenous opioids in humans have necessarily been more circuitous than in animals, and neurobiological findings have been somewhat more difficult to interpret. In humans, β-endorphin levels have been measured in cerebral spinal fluid [113] and blood plasma [114–117]. Gianoulakis [5] hypothesized that humans who differ in their genetic predisposition for alcoholism would also demonstrate inherited differences in β-endorphin sensitivity to ethanol. Findings from a series of studies by this investigator [118,119] showed that individuals with a family history of alcoholism (high risk) have lower basal plasma β-endorphin levels, but greater release of β-endorphin after exposure to a 0.5-g/kg dose of ethanol than do individuals without this history (low risk). Further support for the hypothesis that β-endorphin response to ethanol may represent a biomarker for increased genetic risk for alcoholism was provided by findings of a recent study of twins confirming that this response has significant heritability [120].

Although the degree to which changes in peripheral levels of β-endorphin mirror central opioid activity has not been firmly established, the clinical findings are consistent with findings of preclinical research suggesting that central opioid pathways are more sensitive to ethanol in high- than low-risk individuals [121]. Nevertheless, these findings are not ubiquitous, as a later study by Dai et al. [122] showed no significant β-endorphin response to a 0.5-g/kg dose of ethanol in either high- or low-risk groups. Interestingly, in the latter study, ethanol consumption attenuated stress-induced increases in plasma β-endorphin levels in both high- and low-risk subjects; the effect was somewhat greater in the low-risk group. There is considerable evidence that stress activates the endogenous opioid system [6,123] and that HPA axis hormones may interact with DA neurotransmission to mediate alcohol reinforcement [124–127]. Thus, the latter findings suggest that ethanol’s reported stress-reducing properties may be associated with its ability to prevent the increase in β-endorphin that generally results when an individual perceives a situation as stressful. Clearly, the exact nature of the relationships between endogenous opioid activity, stress, and risk for alcoholism are a long way from being fully understood.

8. Opioid modulation of the physiological stress response

To determine the validity of an opioid model for alcoholism in humans, it has been useful to generate additional
measures of endogenous opioid activity within the central nervous system. One technique that has been implemented is the induction of receptor blockade with an opioid receptor antagonist. To understand the utilization of this technique, it is necessary to understand the opioid modulation of the HPA axis stress response. Following the onset of stress, corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus receive convergent impulses from several major neurotransmitter systems that contribute to the dynamic regulation of the HPA axis. This regulatory input includes stimulatory signals from serotonergic and noradrenergic neurons, as well as inhibitory signals from centrally located GABA- and β-endorphin-releasing neurons [128,129]. Once CRH is released, it stimulates β-endorphin and ACTH secretion from the anterior pituitary, which is followed by ACTH stimulation of cortisol release from the adrenal cortex. Cortisol forms a negative feedback loop with the pituitary, hypothalamus, and other brain regions, playing a critical role in reestablishing homeostasis following stress exposure.

Stress-induced opioid neurotransmission may play a role in counteracting potentially detrimental effects of sustained stress by facilitating the termination of the HPA axis stress response [122,130,131]. Both acute and chronic administration of opiates suppress HPA axis hormones in humans; whereas, levels of ACTH, cortisol, and β-endorphin are all increased during acute opiate withdrawal [132]. Naloxone, a nonselective opioid receptor antagonist, induces a rise in ACTH and cortisol levels by blocking the opioid component of the inhibitory activity directed at the CRH-producing neurons. Whereas these effects are thought to result primarily from the direct blockade of opioid pathways from the arcuate nucleus (β-endorphin and enkephalins) to the hypothalamic CRH neurons [133–135], a blockade of opioid inhibition of NE neurons in the LC may also be involved [131,136,137].

Studying the release of HPA axis hormones provides a window on CNS function and can uncover differences in neurotransmitter systems as a function of both alcoholism and family history of alcoholism. Because of the powerful inhibitory effect of opioid neurons on CRH activity, an acquired or inborn abnormality in opioid activity would alter inhibitory tone on CRH neurons. Thus, an individual’s response to opioid receptor blockade by naloxone provides a noninvasive, functional assessment of his/her hypothalamic opioid activity (Fig. 1).

Fig. 1. β-Endorphin neurons in the arcuate nucleus both inhibit CRF release from the hypothalamus and stimulate DA release in the nucleus accumbens. Naloxone administration induces the release of cortisol by blocking opioidergic input on the CRF neuron. GABA indicates γ-aminobutyric acid; NE indicates norepinephrine. Reprinted by permission of the American Medical Association from Ref. [149].
9. Relationship between the endogenous opioid system, the HPA axis, and reward

Interestingly, β-endorphin neurons in the arcuate nucleus of the hypothalamus both inhibit CRH release in the PVN of the hypothalamus [128,129] and simultaneously stimulate DA release in the nucleus accumbens [2,10,121]. Because the opioids are involved in regulating both of these systems, it is plausible that a lesion in opioid neurotransmission could cause a simultaneous derangement of both physiological processes. In recent years, a body of research has accumulated that provides evidence of deranged HPA axis function in drinking alcoholics [138], in acute withdrawal and more prolonged abstinence [139–142], and in nondependent individuals with a family history of alcoholism [143–145]. Furthermore, glucocorticoids, which are released during stress and following ethanol-induced activation of the HPA axis, modulate the activities of the opioidergic, CRH, and mesolimbic DA systems [146,147] and have been shown to interact with the rewarding properties of a number of drugs of abuse [123–126]. Several investigators have speculated that diminished opioid activity, which is either the result of alcoholism or genetically linked to the risk of alcoholism, could induce hypercortisolemia, alter mesolimbic DA production, and lead to abnormal ethanol reinforce-

10. Laboratory studies of naloxone administration in alcoholics

To examine the significance of the opioid deficiency hypothesis in humans, response to opioid receptor blockade with naloxone has been examined in abstinent alcoholics, ethanol abusers, and nonalcoholic offspring of families with a high density of alcoholism. The first use of the naloxone challenge to examine HPA axis function in alcoholics was reported by Kemper et al. [153] who administered a single 20 mg dose of naloxone intravenicularly to 20 male alcoholics receiving inpatient abstinence treatment, 10 ethanol abusers, and 10 healthy controls. Although naloxone increased cortisol levels in all three groups, findings suggested that the time course of the response was different in the control group than in the two groups of drinkers. Furthermore, baseline cortisol levels and cortisol response to naloxone were significantly lower in the alcoholics after four weeks of abstinence than during acute withdrawal. No group differences were found in the levels of β-endorphin-like immunoreactivity in any of the analyses. Inder et al. [142] administered the same dose of naloxone to eight male and one female alcoholics who were between 10 days and 6 weeks of abstinence. Although these investigators found no difference in cortisol response to the challenge between the group of alcoholics and a group of nine healthy controls, the alcoholics showed a blunted incremental ACTH response.

Similar differences were found between groups in response to an administration of ovine CRH. The interpretation of the results of these studies is restricted by the fact that only a single dose of naloxone was administered. Furthermore, the ability to definitively conclude that the findings were the result of events occurring at, above, or below the level of the hypothalamus is hampered by methodological limitations in humans. Nevertheless, it is reasonable to assume that the impact of opioid blockade may be diminished in alcoholics, secondary to decreased levels of central opioid peptides and decreased tonic inhibition on the HPA axis.

11. Laboratory studies of naloxone administration in persons at increased risk for alcoholism

One question not addressed in the designs of the previous studies was whether the reported abnormalities represented biological markers of an underlying genetic predisposition to alcoholism or whether they were a consequence of chronic ethanol exposure. To examine the etiological significance of the endogenous opioid system in alcoholism, Wand et al. [149–151,154] conducted a series of studies comparing the HPA axis responses to opioid blockade in individuals from families with a high density of ethanol-dependent persons (FHP) with those from families with no history of alcoholism (FHN). The participants were healthy individuals between the ages of 18 and 25 who were free from DSM-IV Axis I diagnoses, as well as free from diagnoses of ethanol and drug abuse or dependence. In an initial study with 26 FHP and 22 FHN participants, three doses of naloxone (0, 125, and 375 μg/ kg) were administered in double-blind, randomized order on three separate days [149]. The participants who were fasting from 09:00 a.m. reported for sessions at the General Clinical Research Unit (GCRC) and had an intravenous catheter inserted into a forearm vein at 01:00 p.m. Naloxone was administered for 1 min as a bolus dose 1 h later. Blood samples were obtained 15 min before drug administration, immediately before drug administration, and at 15, 30, 45, 60, 90, and 120 min. Findings showed that FHN participants had graded cortisol responses to each dose of naloxone; that is, cortisol response to high-dose naloxone was greater than the cortisol response to low-dose naloxone, which, in turn, was greater than cortisol response to placebo. In contrast, the cortisol responses of the FHP participants were maximally stimulated to the same level as the FHN participants following the low dose of naloxone, with no further increase following the high dose. The findings were not associated with age, race, body mass index, educational level, ethanol drinking history, gender, or plasma naloxone concentration. Baseline cortisol levels did not differ between groups. Furthermore, no group differences were found in response to the ACTH analog cosyntropin, suggesting that the findings were not related to the differences in adrenal responsiveness to ACTH.
Fig. 2. Hormone responses to five doses of naloxone by dose and family history. (Top) ACTH responses. All doses were significantly different from placebo ($P < .001$). ACTH responses differed between FHP and FHN subjects at doses of 375 ($P < .001$) and 500 µg ($P = .02$) after adjusting for placebo responses. (Bottom) Cortisol responses. All doses were significantly different from the placebo ($P < .001$). Cortisol responses differed between FHP and FHN subjects at doses of 125 ($P = .004$), 375 ($P < .001$), and 500 µg ($P = .001$) after adjusting for placebo responses. Reprinted by permission of Lippincott Williams and Wilkins from Ref. [154].
Subsequent findings from our group and others have largely replicated these observations, providing further confirmation that nonalcoholic offspring from families with a history of alcoholism have altered cortisol responses to naloxone provocation [151,154,155]. Findings for ACTH have been somewhat equivocal, whereas we found a greater ACTH response to naloxone in FHP participants [150], Hernandez-Avila et al. [155] observed no differences in ACTH response between FHP and FHN individuals. Baseline cortisol levels were also found to differ as a function of family history of alcoholism in the latter study. We have not observed differences in ACTH or cortisol levels at baseline (before drug or placebo administration), during placebo administration, or with adrenal stimulation by the ACTH analog cosyntropin on the basis of family history of alcoholism. In addition, unprovoked cortisol secretion, monitored over a 24-h interval, did not distinguish FHP from FHN participants [151]. Interestingly, we observed that obsessive-compulsive symptomatology was positively associated with the endogenous opioid tone in FHP participants [152]. This finding is consistent with those of studies showing that naloxone decreases obsessive-compulsive behaviors [156–158], as well as with findings showing that opioid agents affect stereotypic behavior [159–161]. It is also consistent with hypotheses linking alterations in the endogenous opioid system, with enhanced risk for the development of alcoholism.

Despite the striking qualitative similarities that we have observed in findings across studies, some unexplained quantitative differences in hormonal responses have been observed. For example, findings from a study [154] involving a five-dose range of naloxone (0, 50, 125, 375, and 500 µg/kg) differed from those of the initial study [149] in showing that family history differences in cortisol and ACTH responses occurred primarily at the higher doses of naloxone (375 and 500 µg/kg) rather than at the 125-µg/kg dose. The findings from the study using five doses of naloxone are shown in Fig. 2. Although FHP participants generated greater ACTH and cortisol levels than FHN participants did, the dose of naloxone required to produce half-maximal hormonal responses did not differ by family history. Another difference that has been noted across our studies has been the presence or absence of an interaction between family history and gender. In one study, enhanced ACTH responses to naloxone were found in FHP men, but not in FHP women [150]. No such interactions were found in the later study [154].

The findings discussed in this section may have several different interpretations. First, participants who are at high risk for excessive ethanol consumption by virtue of their family history may have an inherited or acquired deficiency in activity of the endogenous opioid system. These deficits could be the result of less synaptic opioid content, reduced opioid receptor density, and/or differences in the type or binding affinities of opioid receptors in specific brain regions. If high-risk participants have less inhibitory tone on CRH neurons, they would be maximally blocked by a lower dose of naloxone, resulting in a lower plateau in serum cortisol levels compared with FHN individuals. Reduced opioid tone could lead to diminished basal levels of DA within the nucleus accumbens and/or to the diminished accumulation of DA following ethanol ingestion. Thus, FHP individuals might require higher blood ethanol levels to stimulate the opioid-mesolimbic DA cascade. A second possible explanation for the findings is that blocking opioid inhibitory tone unmasked family history differences in serotonin, GABA, and/or noradrenergic tone on the CRH neuron. All of these neurotransmitter systems have been implicated as “candidates” in the genetic vulnerability for alcoholism and/or HPA axis dysfunction by virtue of their involvement in modulating both mesolimbic DA pathways and CRH secretion. Third, differences in cortisol response to naloxone could be the result of the differential expression or sensitivity of CRH receptors or the differing levels of releasable CRH and/or arginine vasopressin (AVP) in FHP as compared with FHN individuals. In other words, the observed differences in the HPA axis function could be the result of processes occurring downstream of neurotransmitter modulation of hypothalamic CRH neurons. The similarities that Inder et al. [142] observed between ACTH responses to naloxone and ovine CRH in alcoholics suggest that alcoholics might have reduced pituitary sensitivity to CRH and/or AVP. In concordance with those findings, Waltman et al. [145] reported that FHP men had lower basal ACTH levels and lower ACTH responses to ovine CRH than FHN men did.

12. Clinical trials using opioid antagonists for the treatment of alcoholism

Based primarily on findings from preclinical work implicating the endogenous opioid system in alcoholism, several clinical trials were conducted in the early 1990s to examine the effectiveness of opioid antagonists for the treatment of recently abstinent ethanol-dependent participants. Two 12-week, double-blind clinical trials reported the effectiveness of naltrexone in combination with psychosocial treatment in decreasing both relapse and amount of ethanol consumption [162,163]. Naltrexone is an opioid antagonist that acts primarily not only at µ-opioid receptors, but also at δ and κ. In contrast to naloxone, naltrexone is administered orally, has a longer half life (4–9 h vs. 1–2 h), and has an active metabolite, 6-γ-naltrexol, with a half-life of approximately 12 to 18 h [164–167]. The primary effect of naltrexone in these initial clinical trials was a reduction in the frequency and amount of ethanol consumption as compared with placebo and lower rates of full-blown relapse (consumption of five or more drinks per occasion for men and four or more for women) among patients who resumed drinking. Based, in part, on the findings of these studies, naltrexone was approved by the
Food and Drug administration (FDA) in 1995 for the treatment of ethanol dependence.

Findings from several subsequent single-center trials [168–170] and two multicenter trials [171,172] have tended to support the major findings of the two initial studies in demonstrating naltrexone’s efficacy for short-term use (12 weeks) as an adjunct to standardized psychosocial treatments. However, one large-scale, multicenter study reported contradictory findings showing a lack of benefit of either short- (13 weeks) or long-term (52 weeks) naltrexone treatment in patients with chronic, severe ethanol dependence [173]. Outcome measures used in the latter study were consistent with those used in earlier studies; possible reasons why the results differed include differences in participant characteristics or in the types of psychosocial interventions administered. There is some evidence of an interaction between naltrexone and the type of psychosocial therapy administered, with most findings showing that relapse to heavy drinking is least likely to occur when naltrexone is given in conjunction with coping skills training [162,174]. A reputed limitation of naltrexone treatment is that its benefits are evident primarily in persons who are motivated enough to maintain relatively high levels of medication compliance [171,175,176]. Levels of 6-β-naltrexol have been found to be negatively correlated with both frequency of drinking [177] and study dropout [171]. Additionally, the clinical utility of this medication may be limited in some patients by gastrointestinal side effects [178] and, rarely, dose-related hepatocellular toxicity [179]. In patients who can tolerate the medication, the use of a sustained-release formulation of naltrexone has been shown to reduce the frequency of heavy drinking [180] and shows promise as a tool for circumventing problems with noncompliance.

Currently, there are no clearly established guidelines as to the optimal length of treatment; most trials have examined the efficacy of a 50 mg/day dose for 3- to 6-month periods of time. Findings from at least two studies indicated that treatment benefits began to fade once the medication is discontinued, suggesting that, at least, some ethanol-dependent individuals may benefit from longer term treatment [170,181]. In support of this contention, benefits of naltrexone have been shown to persist when it is taken either p.r.n. for craving [174] or on a daily basis [182] for periods that extend up to one year. However, another area that requires further research is whether chronic naltrexone administration might lead to an increase in opioid receptor density in humans, as has been shown to occur with opioid antagonist administration in some preclinical studies [183,184], and what the clinical implications of such effects would be [130].

Nalmefene is a 6-methylene analog of naltrexone that has also been examined as a potential pharmacological treatment for ethanol dependence. Nalmefene is a nonspecific opioid antagonist that has a longer half-life (8 to 11 h) than naltrexone does, is more potent at the μ-receptor, has a somewhat greater affinity for κ- and δ-receptors, and has less risk of hepatotoxicity [130,185,186]. Interestingly, it also produces greater HPA axis activation than naltrexone does [185]. Mason et al. [187] conducted a 12-week double-blind, placebo-controlled safety and efficacy trial with two doses of this medication (20 or 80 mg/day) in 105 ethanol-dependent participants, who also received cognitive behavioral therapy. Similar with findings with naltrexone, results indicated that patients treated with either dose of nalmefene were less likely to relapse to heavy drinking and had fewer relapses than those treated with placebo.

The mechanisms underlying the potential usefulness of opioid antagonists in alcoholism treatment is unknown [169]. Volpicelli et al. [188] found that among alcoholics who reinstated drinking following detoxification, a larger proportion reported that the "high" from ethanol was significantly less than usual if they were taking naltrexone than if they were taking placebo. This suggests that opioid antagonist administration acts by interfering with ethanol reinforcement and that this reinforcement is mediated by the endogenous opioid system. Sinclair [94] maintained that findings from double-blind, placebo-controlled studies have also supported the contention that opioid antagonists act by blocking ethanol reinforcement in that the primary effects of these drugs are seen in patients who have resumed drinking. According to Sinclair [94], ethanol drinking behavior and craving are gradually extinguished as patients experience reduced reinforcement with ethanol over time. Nevertheless, findings related to craving have been somewhat ambiguous, and the role of opioid antagonists as anticraving drugs is still unresolved. Anton et al. [168] attributed at least some of the inconsistencies related to craving to the inadequacy of the commonly used one-item analog scales. Using a measure with improved psychometric qualities, these investigators demonstrated that naltrexone-treated individuals had greater ability to resist thoughts, urges, and behavior associated with continued drinking than individuals taking placebo.

It is also possible that opioid antagonists decrease ethanol consumption by mechanisms that do not specifically involve the modification of the positive or negative reinforcement properties of ethanol. For example, there has been some suggestion that the suppressant effects of opioid antagonists on ethanol drinking may be related, at least partially, to the nausea or fatigue that may accompany their use [164,189,190]. It is still unclear whether the gastrointestinal disturbances associated with naltrexone are a direct result of side effects of the medication or of its interactions with ethanol [191]. Endogenous opioids are involved in regulating autonomic functions, such as respiration, vomiting [192], and cardiovascular activity [193], as well as having inhibitory effects on pain pathways [194] and mediating stress-induced analgesia [195]. There is evidence from preclinical studies showing that opioid peptides can induce feeding and drinking in rats [196,197], whereas opioid antagonists decrease consummatory behavior [90,96,198–200]. These findings suggest that opiate antag-
onists may mediate ethanol consumption through nonspecific mechanisms. The information that is available about the nonspecific effects of opioid antagonists on ethanol consumption in humans has been derived primarily from human behavioral pharmacology studies as discussed below.

13. Human laboratory studies examining effects of opioid antagonists on subjective responses to alcohol and on alcohol consumption

Because the laboratory setting allows greater control of confounding factors than is usually possible in the outpatient setting, human behavioral pharmacology studies are thought to provide the most sensitive tests of pharmacological effects of opioid antagonists in humans. In the majority of these studies, ethanol has been administered under laboratory conditions to evaluate the effects of naltrexone pretreatment on subjective responses to ethanol. Findings have been somewhat equivocal. Some studies have reported that acute doses of naltrexone (25 and/or 50 mg) had no effects on subjective responses to a range of ethanol doses in social drinkers [190,201,202]. However, one group of investigators observed reduced stimulant and increased sedative effects of ethanol following naltrexone (50 mg) administration in this population [203].

If the increased activity of the endogenous opioid system mediates ethanol reinforcement, then, high-risk individuals would be expected to show a greater response to opioid antagonists than low-risk individuals do. Consistent with this hypothesis, the findings of one study showed that acute naltrexone (50 mg) attenuated the stimulant effects of ethanol in participants with a family history of alcoholism, but not in participants without this history [204]. Naltrexone-induced decreases in vigor ratings have also been found to be greater in FHP than in FHN participants [205]. Furthermore, decreased positive mood states, increased sedative effects, and/or decreased liking have been reported in response to ethanol following 7 days of naltrexone administration in heavy drinkers [189,191]. In one of the latter studies [191], a within-subject design was used to examine the direct and interactive effects of three chronic (7 days) doses of naltrexone (0, 50, and 100 mg) on responses to three doses of ethanol (0, 0.5, and 1.0 g/kg doses). Findings showed that pretreatment with a 50-mg dose of naltrexone reduced desire to drink in the absence of ethanol, and pretreatment with a 100-mg dose decreased desire to drink following ethanol ingestion. The high dose of naltrexone (100 mg) also interacted with the high dose of ethanol (1 mg/kg) to produce the greatest decreases in liking and best effects. O’Malley et al. [206] extended this line of research by examining naltrexone’s effects on craving in ethanol-dependent participants. Non-treatment-seeking volunteers received naltrexone (50 mg) or placebo for six days prior to an ethanol self-administration session. Findings indicated that naltrexone-treated participants had lower levels of craving at baseline and during ethanol self-administration than did the participants who received placebo. In general, the qualitative similarities in the findings across these studies provide some support for contentions that naltrexone decreases the reinforcement value of ethanol, particularly in persons who are at greatest risk for alcoholism.

Only a limited number of laboratory studies have examined naltrexone’s effects on actual ethanol consumption, and procedures have yielded mixed results. In one study, social drinkers were given a single 50-mg dose of naltrexone or placebo one hour before choice sessions in which they were permitted to regulated both the type (ethanol or placebo) and the amount of beverage that they consumed [190]. Findings showed that naltrexone did not alter ethanol preference. However, it produced mild sedative effects and nausea in some participants and reduced both ethanol and placebo consumption. These findings led to general conclusions that naltrexone may reduce ethanol consumption by a nonspecific, possibly aversive, mechanism. However, it should be noted that half of the participants who reported any unpleasant naltrexone effects in this study reported them only during active ethanol sessions. King et al. [204] had previously found that young adults who experienced vomiting following a 50-mg dose of naltrexone did so only after consuming ethanol.

In a paradigm in which heavy drinkers were allowed to drink ad libitum in a bar/restaurant setting, seven days of pretreatment with naltrexone (50 mg) was associated with greater nausea and less ethanol consumption, as well as with decreased urges to drink, greater time to consume each drink, and earlier termination of drinking [189]. Because no placebo doses of ethanol were included in this paradigm, it is unclear whether these findings were related to specific or to nonspecific effects of the medication. McCaul et al. [191] found that chronic pretreatment with naltrexone was associated with greater drink refusal and a slower pace of drinking during a fixed-dose ethanol administration in heavy drinkers; these effects were not observed during the placebo drinking session. Although naltrexone appeared to increase sedation irrespective of whether participants received active or placebo ethanol, naltrexone-precipitated nausea was not present at baseline, nor was it noted following the administration of placebo ethanol. In contrast, the ratings of nausea and unpleasant/sick effects were increased by naloxone during active ethanol administration. Furthermore, O’Malley et al. [206] found that naltrexone decreased ethanol self-administration in ethanol-dependent participants; individuals receiving naltrexone drank fewer drinks and consumed their drinks more slowly than did the participants receiving placebo. Ratings of nausea were low in the sample as a whole, and no differences in nausea were found between naltrexone- and placebo-treated groups.

A review of these findings suggests that there are still no definitive answers to questions about whether naltrexone-induced nausea occurs to a clinically meaningful
14. Genetic differences in the endogenous opioid system and risk for alcoholism

Taken together, the data presented in this article suggest that nonalcoholic persons with a family history of alcoholism may have lower basal levels of \( \beta \)-endorphin, greater \( \beta \)-endorphin response to ethanol, and altered subjective and neuroendocrine responses to opioid blockade and to opioid antagonist administration than did the persons without this history. In conjunction with an abundance of epidemiological evidence showing inherited vulnerability for alcoholism [210–212], these findings embody rather strong support for notions that genetically determined differences in opioid activity confer vulnerability for alcoholism.

Considerable progress has been made in recent years by molecular biologists in identifying polymorphisms in genes that may be responsible for the functional differences in the endogenous opioid system and putative risks for alcoholism [213]. Because of the physiological relevance of the \( \mu \)-opioid receptor, much of this work has been targeted towards this gene. One polymorphism of interest involves a common A118G nucleotide exchange in exon 1 of the \( \mu \)-opioid receptor gene that causes an Asn40Asp substitution in the extracellular N-terminal domain of the receptor [214,215]. In vitro studies have shown that the Asp40 variant (118G allele) binds \( \beta \)-endorphin three times more avidly than does the common variant (118A allele) and also induces a threefold increase in the agonist-induced activation of G protein-coupled potassium channels [216]. Thus, it is plausible that this polymorphism alters processes under opioidergic regulation [213]. This polymorphism has a prevalence rate of approximately 10% in the general population, although racial differences have been noted.

Findings from genetic association studies have yielded somewhat equivocal findings with respect to the relationship between the A118G polymorphism and alcoholism [214,217–220]. Some investigators have reported greater frequencies of the 118A allele and the +118A/A genotype in alcoholics than in nonalcoholic controls; whereas, others have found no differences in the allelic frequencies on the basis of this diagnosis. In a recent review of the literature, LaForge et al. [213] concluded that existing evidence suggests that the 118G allele has either no or a protective effect with respect to the development of alcoholism. Other investigators have maintained that findings from genetic association studies converge, with other lines of evidence in linking hyposensitivity of the endogenous opioid system to alcoholism [221]. Because the 118A allele binds \( \beta \)-endorphin less avidly than the 118G allele does, individuals who are homozygous for the 118A allele could hypothetically have an intrinsic opioid response deficit that influences the patterns of ethanol consumption. One problem with genetic association studies is that disorders with a behavioral component, such as alcoholism, are phenotypically variable and are likely to be influenced by multiple genes, each gene carrying only a small amount of variance for the phenotype [213]. Indeed, it has been argued that under these circumstances, inconsistencies in findings across studies is the expected outcome [222]. In contrast to some of the other reports, Rommelspacher et al. [218] observed a trend for increased frequency of the Asp40 (118G allele) variant in a group of 327 alcohol-dependent participants compared with 340 healthy controls. No differences in the allelic frequen-
cies of this polymorphism were noted between the control participants and subsets of the alcohol-dependent participants who had a family history of alcoholism, early-onset alcoholism, or severe withdrawal symptoms.

Additional information about the functional relevance of this polymorphism has come pharmacological challenges and treatment studies using opioid antagonists. Findings from our laboratory (Fig. 3) showed that healthy male participants expressing the 118G allele had greater ACTH and cortisol responses to opioid receptor blockade with naloxone than did the participants without the 118G allele [223]. If the 118G variant receptor produces greater tonic inhibition when more tightly bound to endogenous \( \beta \)-endorphin, then, the removal of this inhibition by naloxone might induce greater activation of the HPA axis, as seen in this study. Consistent with these findings, Oslin et al. [224] recently reported a differential response to 12 weeks of naltrexone treatment in alcohol-dependent participants as a function of the A118G polymorphism. Participants of European descent who were homozygous or heterozygous for the 118G allele had significantly lower rates of relapse and a longer time to return to heavy drinking than those who were homozygous for the 118A allele. Thus, both healthy, normal and alcohol-dependent participants have demonstrated differential response to opioid antagonists on the basis of this polymorphism.

Using a different methodology, Rommelspacher et al. [218] reported that seven days after withdrawal, alcohol-dependent participants with the 118G allele had a greater growth hormone (GH) response to the DA receptor agonist apomorphine than did the alcohol-dependent participants without this variant. Because findings from animal studies suggest that there is an up-regulation of D2 receptors after ethanol withdrawal, the authors hypothesized that the greater GH response could be the result of reduced dopaminergic activity and greater compensatory up-regulation of DA receptors. The variant group in this study also showed a trend toward lower novelty-seeking scores on the Tridimensional Personality Questionnaire, which is consistent with low DA activity in the theme of Cloninger et al. [225]. In another line of investigation, Lotsch et al. [226] found that the A118G polymorphism decreased the pupillary constricted effect of morphine-6-glucuronide, an active metabolite of morphine. The relevance of this finding awaits further determination since the polymorphism had no effect on the potency of morphine itself, and the sample size was small (six participants carrying the G allele and six controls).

15. Conclusions

The aggregate findings of the research presented in this paper provide a rather broad base of support for theories of opioid involvement in ethanol reinforcement and associated risks of alcoholism. The preclinical findings, particularly those from studies using opioid antagonists and gene knockout models, present a compelling case for the involvement of the \( \mu \)-opioid receptor in the development of this disorder. Many of the findings from the animal literature also seem to translate remarkably well to the human condition. Nevertheless, the fact that the findings can often be used to simultaneously support the Opioid Surfeit and the Opioid Deficit Hypotheses speaks to a continued lack of clarification about the specific biological mechanisms underlying the relevant relationships. Some of the strongest evidence for a causal relationship between endogenous opioid activity and alcoholism come from studies showing that opioid antagonists decrease alcohol consumption in both animals and humans. Yet, opioid antagonists are not uniformly efficacious, and it has not been clearly established

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**Fig. 3. Cortisol responses to naloxone by the \( \mu \)-opioid receptor genotype.** The participants were administered five incremental doses of naloxone (0, 50, 100, 200, and 400 \( \mu \)g/kg) within a single session. “G” denotes subjects who were either homozygous or heterozygous for the 118G allele (\( n = 10 \)). “A” denotes subjects who were homozygous for the 118A allele (\( n = 29 \)). Reprinted by permission of Elsevier Science from Ref. [223], American College of Neuropsychopharmacology.
even why they work when they do. Nevertheless, the abundance of evidence suggests that the problems in defining the relationships between endogenous opioids, DA, and alcohol consumption lie more in the technical limitations of our current assessment methods than in the nonexistence of such relationships. With greater ability to quantitatively assess alterations in the endogenous opioid system will come greater understanding of its role in alcoholism and greater refinement of pharmacological treatment approaches.

It should also be kept in mind that individual differences in the endogenous opioid system probably account for only a portion of the total variance in ethanol-seeking behavior. Alcoholism is a multifaceted disorder that seems to be the result of complex interactions among a number of neurotransmitter, neuromodulatory, and second messenger systems, which are further impacted by the environmental variables. For example, there is evidence that the DA system itself may be altered in alcoholics. Using PET scan technology, Volkow et al. reported reduced D 2 receptor density in both alcoholics [227] and cocaine abusers [228] as compared with healthy controls. Low D 2 receptor density was also found to be associated with increased subjective liking of methylphenidate in a small group of nondrug-abusing participants, suggesting that low receptor levels might predispose a person to use drugs to compensate for decreased activation of reward circuits [229]. Children of alcoholics have been shown to have altered prolactin response to an alcohol challenge as compared with children of nonalcoholics, also suggesting alterations in DA neurotransmission [230]. Additionally, reduced D 2 receptor Bmax has been linked to a polymorphism in the D 2 receptor gene; this polymorphism has been positively associated with both alcohol and drug abuse severity and family density of alcoholism [231].

Other neurotransmitters, such as γ-aminobutyric acid (GABA), glutamate, and serotonin (5-HT) have also been shown to play a role in ethanol’s reinforcing effects. To date, the only two drugs that have been approved for the treatment of alcoholism are naltrexone and acamprosate. Although acamprosate’s mechanism of action is not precisely understood, the excitatory neurotransmitter glutamate is probably involved [232]. Interactions between opioid peptides and other neurotransmitters in the brain add yet another level of complexity to our understanding of the neurobiological processes involved in alcoholism. Opioid peptides coexist and are often coreleased with other neurotransmitter molecules from presynaptic neurons. They also act as neuromodulators of neurotransmitter release from postsynaptic target neurons, reportedly inhibiting the release of acetylcholine, DA, and norepinephrine, as well as having both inhibitory and excitatory effects on serotonin and GABA [233]. Thus, much work remains to be done to disentangle the role of the endogenous opioid system in alcoholism and to determine the conditions under which manipulations of this system might be most efficacious for the treatment of this disorder.

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