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The opioid receptor system and alcoholism: a genetic perspective

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Abstract

Over the past decade, mounting evidence has implicated the endogenous opioid receptor system as a central player in the etiology of alcohol drinking behavior in animals and alcoholism in humans. Much of this work is a product of a pharmacological approach, where differences in opioid receptor pharmacology have been found to predict drinking behavior in animal models of alcoholism, including rats and mice selectively bred for alcohol preference and avoidance. This review considers the opioid receptor system and alcoholism from a genetic standpoint, and discusses investigation into opioid receptor pharmacology in animal models of alcoholism as work that paved the way for the more recent molecular genetic studies implicating the δ-, and particularly, the μ opioid receptors as genetically linked to alcoholism-associated phenotypes in animal models of the disease. These genetic studies are set within the broader context of the candidate gene approach for alcoholism, where opioid receptor genes are taken to be partial, rather than complete, risk factors for alcoholism. Building upon these findings, the recent genetic association between alcoholism and the μ opioid receptor gene in humans is discussed. Finally, the translation of such genetic association studies between opioid receptor genes and alcoholism to a pharmacogenetic approach, allowing for the evaluation of putative relationships between genotype and pharmacological response profiles, is suggested to address the etiological question of what the molecular mechanism is underlying opioid receptor genetic risk for alcoholism phenotypes.

Keywords: Alcohol; Opioid receptor; Genetics; Alcoholism; Quantitative trait locus

1. Introduction

Different aspects of alcoholism and other drug abuse disorder traits (phenotypes) such as building tolerance to drug, chronicity of disease, and severity of withdrawal are known to be partially genetically determined. However, as alcoholism and drug abuse phenotypes are distributed continuously on such measures rather than dichotomously, it is generally accepted that the contribution of genetic factors to these phenotypes is multi-factorial (polygenic with environmental influence), with each gene contributing partial risk for phenotype as opposed to a simple Mendelian model, where a single gene accounts for essentially all of the genetic risk for a phenotype. Based on this assumption, strategies aimed at evaluating multiple genes as candidate risk factors for disease have generally been employed. One such genetic strategy that has shown promise is the quantitative trait locus (QTL) approach, which determines which genomic regions contribute to a phenotype. The underlying precept of the QTL approach is that, while each QTL confers only a small amount of risk for a particular disease-associated phenotype, together all of the QTLs for a particular disease phenotype explain essentially all of the genetic risk for that phenotype aside from non-genetic risk factors (which also account for a large proportion of risk variance for disease phenotype) (Paterson et al., 1988).

Much progress has been made in identifying endogenous opioid receptors as candidates, at the protein and gene levels, for alcoholism-associated phenotypes in animal models of the disease and for alcoholism in humans. The general trend in this research has been from an opioid receptor pharmacological approach in rat and mouse models of alcoholism to a genetic approach, where opioid receptor genes of the μ and δ classes have been implicated as genetic candidates for alcoholism-associated phenotypes in animals and for alcoholism risk in man. As a logical extension of this trend, future research will include phar-

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macogenetic approaches, where opioid receptor genotypes are related to pharmacological response profile phenotypes in alcoholics. In this way, the molecular basis of the relationship between genotype and alcoholism phenotype will be dissected, yielding valuable clues to alcoholism etiology.

2. Opioid receptor system involvement in alcoholism: clues from animal models

2.1. Animal models

Over the past decade, a chain of research in animal models of alcoholism has substantiated the involvement of the endogenous opioid receptor system in alcohol drinking behavior. Much of this research was prompted by the findings that, at low concentrations, ethanol can modulate endogenous opioid system receptor binding density (B_{max}) (for a review see Charness, 1989), that certain metabolites of alcohol, known as tetrahydroisoquinolines, can bind to opioid receptors (for a review, see Trachtenberg and Blum, 1987), that alcohol can induce conformational changes in peptide ligands for opioid receptors (Rapaka et al., 1986a,b; Bhargava et al., 1988), and that opioids can modify alcohol consumption (for a review see Herz, 1997). Rats bred for alcohol preference [such as the Alko, Alcohol (AA), Fawn–Hooded, and alcohol-preferring (P) rats] and alcohol avoidance [such as the Alko, Non-Alcohol rats (ANA) and alcohol non-preferring (NP) rats] are the preferred animal models, although alcohol-seeking and -avoiding mice have also been employed. Two main types of pharmacological studies have been performed with these animal models: investigation of the effects of opioid receptor antagonism on alcohol drinking behavior, and comparison of the pharmacological profiles of opioid receptor subtypes between different animals bred for alcohol preference and avoidance. Using such animal models, each of the three classes of endogenous opioid receptors (μ, δ, and κ) has been evaluated as potential molecular candidates underlying alcohol drinking behavior, although the major focus has been on opioid receptors of the μ and δ classes, which are generally thought to be involved in alcohol-induced euphoria (for a review, see Herz, 1997).

2.2. Opioid receptor antagonism and alcohol drinking in animal models of alcoholism

In order to investigate the potential involvement of μ and δ opioid receptor types in alcohol drinking behavior in AA rats, Hyytia (1993) treated these animals with selective μ opioid [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Phe-Thr amide (CTOP)] or δ opioid [*N*, *N*-diallyl-Tyr-Aib-Aib-Phe-Leu (ICI 174864)] receptor antagonists and found that only antagonism of μ opioid receptors resulted in suppression of alcohol drinking behavior. These results were confirmed in a following report by Honkanen et al. (1996), who showed that the selective μ opioid receptor antagonist naloxonazine, but not the selective δ opioid receptor antagonist naltrindole, resulted in decreased alcohol consumption in AA rats. Furthermore, these authors showed that, 4 days after cessation of drug treatment, only naloxonazine-treated animals nearly doubled their alcohol consumption, suggesting a rebound effect where these animals seek alcohol in order to activate antagonist-induced hypopotentiated μ-, but not δ-, opioid receptors. In another line of alcohol preferring rats (HAD line), a similar effect of selective μ opioid antagonism with β-funaltrexamine was observed on decreased alcohol drinking (Krishnan-Sarin et al., 1998). Yet, in three other reports which employed P rats, selective antagonism of the δ opioid receptor (using ICI 174864, naltrindole, and naltriben) resulted in attenuation of alcohol drinking (Krishnan-Sarin et al., 1995a,b; Froehlich et al., 1998). Recently, in a study employing μ opioid receptor knockout mice, Roberts et al. (2000) showed that these animals do not self-administer alcohol compared to wild-type controls but do demonstrate an aversion to ethanol under several experimental conditions. When taken together, these data implicate the μ opioid receptor as critical in the reinforcing effects of alcohol. One suggestion is that after μ opioid antagonism, receptor density increases, correlating with an increase in alcohol consumption once the antagonist is withdrawn. Yet, the apparent discrepancy concerning the involvement of δ opioid receptors in alcohol drinking in rats may reflect different genetic backgrounds of the lines employed, which may predict altered pharmacological properties of endogenous opioid receptors.

2.3. Opioid receptor pharmacology in animals bred for alcohol preference and avoidance

Regarding the investigation into brain opioid receptor pharmacological profiles of alcohol preferring and avoiding animals, recent studies have set out to evaluate possible differences in these profiles that may co-vary with alcohol drinking behavior. As an example, three reports (Soini et al., 1998, 1999; Marinelli et al., 2000) compared brain-binding profiles of various μ and δ opioid receptor antagonists between AA and ANA rats using quantitative autoradiography. These reports showed a decrease in μ and δ opioid receptor binding densities in some limbic areas in AA vs. ANA rat brains, while other limbic areas (the substantia nigra, striatal clusters, and nucleus accumbens) showed increased μ-, but not δ-, opioid receptor densities. With the exception of the limbic system, labeling patterns and distributions of these receptors were not measurably different between AA and ANA animals, suggesting a region-specific correlation between μ opioid receptor

density and alcohol drinking. In a similar study, quantitative autoradiography revealed that μ opioid receptor densities were increased (20–25%) in certain limbic areas (the nucleus accumbens, amygdala, and olfactory tubercle) when comparing P to NP rat brains (McBride et al., 1998), suggesting that differences in μ opioid B_{max} values between P and NP rats may be factors contributing to their different alcohol drinking behaviors. It is generally regarded that μ opioid receptors in the limbic ventral tegmental area positively regulate dopamine release in the nucleus accumbens, thereby promoting reinforcement for alcohol drinking behavior (Koob and Le Moal, 1997). Noteworthy is the finding that AA rats demonstrate higher μ opioid receptor densities in the ventral tegmental area and related limbic regions compared to ANA rats (De Waele et al., 1995), suggesting a molecular mechanism for such observed differences in alcohol consumption between these strains.

In a different experimental paradigm designed to measure ethanol-dependent opioid receptor gene expression, C57BL/6 (B6, alcohol-preferring) and DBA/2 (D2, alcohol-avoiding) mice were allowed a free-choice between a 10% ethanol solution or water for 4 weeks, and some of these mice went an additional 3 weeks of only water drinking prior to sacrifice. Winkler et al. (1998) then observed that alcohol-freely drinking D2 mice manifested markedly lower levels of δ opioid receptor mRNA in the striatum compared to alcohol withdrawn D2 mice, and found a positive association in both mouse strains between alcohol drinking behavior and μ opioid receptor mRNA in the hypothalamus. Cowen et al. (1999) also found a positive association between μ opioid receptor density (most pronounced in the nucleus accumbens) and ethanol consumption in alcohol-preferring Fawn–Hooded rats using a similar experimental paradigm, further substantiating the involvement of the endogenous opioid receptor system, in particular the μ and δ opioid receptor classes, in the rewarding effects of alcohol.

3. Genetic opioid receptor variants and alcoholism

3.1. The candidate gene approach

Based on such data obtained from animal models of alcoholism implicating the endogenous opioid receptor system in the etiology of the disease, investigators have sought genetic links between this receptor system and alcoholism. Alcoholics typically show diverse responses to and different consumption rates of alcohol, indicating that the alcoholic phenotype is qualitatively rather than dichotomously distributed (Crabbe et al., 1999). Following from this idea, it has become widely recognized that alcoholism is a heterogenous disorder of polygenic inheritance, as its pattern of genetic transmission is not readily explained by single gene models (Merikangas, 1990; Kendler et al., 1992). Therefore, the general strategy in identifying putative genes associated with the disease, such as the opioid receptor genes, has been a *candidate* gene approach, whereby each genetic candidate is assumed to only predict a minor degree of risk for disease. One commonly used rational strategy for identification of genetic candidates is the QTL approach, where several chromosomal regions are simultaneously evaluated based on linkage, or not, to disease phenotype. In mice, this strategy is often realized by crossing alcohol-preferring and -avoiding animals to give recombinant inbred mouse strains [such as when alcohol-preferring B6 mice are crossed with -avoiding D2 animals (BXD)], which display varying degrees of alcoholic-associated phenotypes. At this point, genetic linkage between these phenotypes and specific chromosomal regions can be evaluated. Once a region is identified as being genetically linked to a disease phenotype, small genetic variations (polymorphisms), located proximal to or within specific genes in that chromosomal region, can be examined for their association, or not, with alcohol-associated phenotypes in mice and/or alcoholism in humans.

3.2. Genetic linkage to the μ opioid receptor in mice

While, to date, direct evidence of strong genetic linkage between the chromosomal regions containing opioid receptor genes and alcohol drinking has not been reported, Belknap and Crabbe (1992) and Belknap et al. (1995) have shown that response to morphine-induced analgesia in mice is controlled by a locus on murine chromosome 10 wherein the μ opioid receptor resides (Kozak et al., 1994; Giros et al., 1995). Furthermore, in BXD recombinant mouse inbred strains showing the highest sensitivity to analgesia, there was a significant correlation between μ opioid receptor density and analgesic response. Taken together, these findings suggest that genetic variation in the μ opioid receptor gene may exercise phenotypic variation by controlling μ opioid receptor density. Alternatively, the changes in receptor density may be secondary to genetically determined sensitivity of the μ opioid receptor to endogenous opioids. In relation to alcohol preference, it is interesting to note that the D2 mouse strains are the most sensitive to analgesia and least alcohol preferring (and the converse with the B6 strains) (Belknap et al., 1995). This, again, would be consistent with the hypothesis that an innate sensitivity of the μ opioid receptor to endogenous opioids and/or alcohol results in reduced alcohol preference. Additional QTL studies affirmed that the μ opioid receptor gene is a candidate for morphine preference, again using intercrosses between D2 and B6 mouse strains, finding a strong maximum likelihood of the odds score of 15.0 to a marker proximal to the μ opioid receptor gene (Berrettini et al., 1994a, Alexander et al.,

1996). Additionally, as D2 mice show a strongly decreased preference for both morphine and alcohol consumption compared to B6 mice (Berrettini et al., 1994b), the possibility arises that the μ opioid receptor may underlie both behaviors.

3.3. Genetic association between the human μ opioid receptor gene and alcoholism

As a next step in the candidate gene approach for alcoholism, research has focused on evaluating putative genetic association between polymorphisms within the human μ opioid receptor gene and alcohol abuse. These studies have generally followed the case-control paradigm, where unrelated alcoholics and controls are sampled and subsequently genotyped for μ opioid receptor polymorphisms. Two groups have now sequenced the human μ opioid gene (Berrettini et al., 1997; Bergen et al., 1997) and have identified four DNA sequence polymorphisms, two of which were extremely rare and therefore uninformative while two others (a functional coding +118A/G substitution and an intronic +691G/C polymorphism) showed population prevalence rates of greater than 10%. Bergen et al. found that both polymorphisms were not significantly associated with alcoholism in Finnish Caucasians ($n = 324$) and Southwest American Indians ($n = 367$). However, while the +691A/G polymorphism was clearly not associated with alcoholism in their U.S. Caucasian sample ($n = 100$), these authors found that alcoholics from that sample demonstrated a marked 20% elevation in the +118A/A genotype compared to non-alcoholic controls.

While this trend towards +118A/A-associated risk for alcoholism did not reach statistical significance in the Bergen et al. sample (most likely due to poor statistical power associated with small sample size), these data led our laboratory to further investigate the possible association between +118A/G status and alcoholism in a larger U.S. Caucasian sample ($n = 227$, Town et al., 1999). We found a 13.2% increase in +118A/A genotype in alcoholics compared to controls which was statistically significant ($P = 0.020$), predicting a greater than two-fold risk for alcoholism incurred by the +118A/A genotype. The risk incurred by the +118A/A genotype appears to be specific to alcoholism and is not associated with smoking, either when comparing smoking to non-smoking alcoholics ($n = 144$, $P = 0.810$) or smoking vs. non-smoking controls ($n = 98$, $P = 0.430$) (unpublished observations). Furthermore, combined analysis ($n = 327$) of our sample and the Bergen et al. data set again revealed a greater proportion of alcoholics with the +118A/A genotype compared to controls (13.4%), which was highly statistically significant ($P = 0.005$) and comparable to the results obtained with our data set, predicting a 2.2-fold increased risk for alcoholism associated with possession of the +118A/A genotype. The genetic association between +118A carriers and alcoholism seems to be specific to U.S. Caucasians, as two other groups have investigated the +118A/G polymorphism in alcoholic and control samples of European and German descent (Gelernter et al., 1999; Sander et al., 1998) and were unable to detect the association.

An additional polymorphism at the μ opioid gene, a non-coding (CA)n repeat with nine common repeat alleles, was examined by Kranzler et al. (1998) in both African–American ($n = 108$) and U.S. Caucasian ($n = 320$) subjects, and these authors found evidence of allelic repeats that were significantly ($P = 0.030$) different between U.S. Caucasian poly-substance (including alcohol and addictive drugs) abusers and controls. No significant differences were observed in their African–American sample, again suggesting that risk for alcoholism (and/or other drug dependence) incurred by genetic variation at the μ opioid receptor locus is restricted to U.S. Caucasians. Yet, additional evaluation of this (CA)n repeat polymorphism in a larger samples of U.S. Caucasian confirmed alcoholics and controls is needed to confirm the specificity of this association to alcoholism vs. other substance abuse.

The functional significance of the +118A/G polymorphism at the receptor protein level has been demonstrated by Bond et al. (1998), who showed that the +118G-predicted receptor isoform (Asp^{40}) binds endogenous β-endorphin approximately three times more tightly than the +118A-predicted receptor isoform (Asn^{40}). Further, Inder et al. (1998) showed that alcoholics demonstrate significantly less β-endorphin blood plasma immunoreactivity than unaffected controls, and individuals from families with a high incidence of alcoholism exhibit diminished endogenous hypothalamic-opioid activity (Wand et al., 1998). When taken together, these lines of evidence converge on the suggestion that the molecular mechanism for the +118A association with alcoholism involves hyposensitivity of the endogenous μ opioid receptor system, where such a phenotype leads to increased consumption of alcohol in order to compensate for this intrinsic opioid response deficit. Clearly, however, studies designed to correlate response to alcohol with +118A/G status are needed to confirm or deny this hypothesis.

3.4. Other opioid receptor genes and alcoholism

QTL strategies similar to those employed in studies implicating the μ opioid receptor as a locus for alcohol-associated phenotypes in mice have also provided some evidence of murine linkage between alcohol drinking phenotypes and the δ opioid receptor. For example, Risinger and Cunningham (1998) utilized BXD recombinant inbred mice previously described and found linkage between alcohol conditioned taste aversion and a region on murine chromosome 4 near the gene encoding the δ opioid recep-

tor. An additional study also reported significant linkage in BXD recombinant mice between chromosome 4 and acute alcohol withdraw convulsions (Buck et al., 1997). Following from this work, Franke et al. (1999) assessed a putative association between a silent 921T/C polymorphism in the coding region of the human δ opioid receptor gene and alcoholism ($n = 435$). These authors were unable to detect such an association in their homogenous German sample. Yet, just as racial and ethnic differences on +118A/G status have been reported (Bond et al., 1998), it remains possible that 921T/C status might be associated with alcoholism in other populations, and further study in the area is warranted to either confirm or deny the lack of association between the 921T/C polymorphism and alcoholism. Additionally, investigation into putative genetic associations between other human δ opioid receptor polymorphisms and alcoholism would be useful in evaluating whether or not this opioid receptor is a risk factor contributor to the disease.

4. Future direction: from genetic association to pharmacogenetics

Genetic association studies between the μ opioid receptor gene and alcoholism have provided evidence that variants of this receptor predict risk for the disease. Yet, what is the molecular basis underlying this risk? To begin to address this question, a paradigm shift from gene association to pharmacogenetics is warranted. Such a strategy would allow for direct correlation between genotype and disease-associated phenotype, thereby giving clues as to which molecules mediate the effects echoed by genetic variation at the protein level. Smolka et al. (1999) have recently taken this approach, showing that the +118A/G polymorphism predicts altered dopaminergic sensitivity after alcohol withdraw. Specifically, they demonstrated that alcoholics with the +118A/G genotype had a twofold increased dopaminergic sensitivity (assessed by apomorphine-induced growth hormone secretion) 1 week following alcohol withdrawal compared to alcoholics with the +118A/A genotype. It is generally recognized that the endogenous opioid receptor system mediates the reinforcing properties of alcohol via interconnected activation of the mesolimbic dopamine system. Therefore, these data not only provide evidence that the +118A/G polymorphism within the μ opioid receptor gene influences the neurobiology of alcoholism, but also suggest a mechanism for the +118A association with alcoholism. Specifically, +118A-associated hypo-potentiation of the dopaminergic system may result in alcohol drinking as a compensatory behavior to stimulate the endogenous dopamine pathway. Additionally, such an intrinsic opioidergic deficit might promote initial exposure to alcohol. Clearly, additional studies relating μ opioid receptor genotype to pharmacological response profiles in alcoholics will aid in elucidating the molecular mechanisms behind genetic associations with alcoholism, such as that with the μ opioid receptor gene.

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