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Perspective

Neuronal nicotinic acetylcholine receptors are important targets for alcohol reward and dependence

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Neuronal nicotinic acetylcholine receptors are important targets for alcohol reward and dependence. Alcoholism is a serious public health problem and has been identified as the third major cause of preventable mortality in the world. Worldwide, about 2 billion people consume alcohol, with 76.3 million having diagnosable alcohol use disorders. Alcohol is currently responsible for the death of 4% of adults worldwide (about 2.5 million deaths each year), and this number will be significantly increased by 2020 unless effective action is taken. Alcohol is the most commonly abused substance by humans. Ethanol (EtOH) is the intoxicating agent in alcoholic drinks that can lead to abuse and dependence. Although it has been extensively studied, the mechanisms of alcohol reward and dependence are still poorly understood. The major reason is that, unlike other addictive drugs (eg, morphine, cocaine or nicotine) that have specific molecular targets, EtOH affects much wider neuronal functions. These functions include phospholipid membranes, various ion channels and receptors, synaptic and network functions, and intracellular signaling molecules. The major targets in the brain that mediate EtOH's effects remain unclear. This knowledge gap results in a therapeutic barrier in the treatment of alcoholism. Interestingly, alcohol and nicotine are often co-abused, which suggests that neuronal nicotinic acetylcholine receptors (nAChRs), the molecular targets for nicotine, may also contribute to alcohol's abusive properties. Here, we briefly summarize recent lines of evidence showing how EtOH modulates nAChRs in the mesolimbic pathway, which provides a perspective that nAChRs are important targets mediating alcohol abuse.

Keywords: alcoholism; abused substance; ethanol reward and dependence; smoking; nicotine; nicotinic acetylcholine receptor; mesolimbic dopamine system

Introduction

Alcoholism is a serious public health problem and has been identified as the third major cause of preventable mortality in the world[1]. Approximately 2 billion people worldwide consume alcohol, with 76.3 million who have diagnosable alcohol use disorders (AUDs). Alcohol is currently responsible for the deaths of 4% of the world's adult population (about 2.5 million deaths each year), and this number will increase by 2020 unless effective action is taken[2]. Economically, in 2005, more than $200 billion of the total United States. healthcare cost was attributable directly to the productivity impacts of alcohol, such as lost wages, which were significantly higher than cancer ($196 billion) or obesity ($133 billion)[3]. Thus, there is an urgent need to reduce the global rate of AUDs. Unfortunately, attempts to combat alcohol abuse have been severely confounded.

Alcohol is the most commonly abused substance by humans. Ethanol (EtOH) is the intoxicating agent in alcoholic drinks that can lead to abuse and dependence[4]. Alcohol use has been ascribed both positive and negative effects. While alcohol at low doses has been shown to provide cardiovascular protection[5], binge drinking is associated with higher incidents of cardiovascular disease and associated mortality[6]. Projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), by way of the medial forebrain bundle, make up a vital component of the mesolimbic pathway[7]. The rewarding effects of EtOH have been linked to the mesolimbic dopamine (DA) system[7], wherein an increase in DA in the NAc is thought to be vital for reward signaling. This system has been connected to the rewarding effects of many abused drugs. However, unlike other addictive drugs (eg, morphine, cocaine or nicotine) which have specific molecular targets, EtOH affects much wider neuronal functions including phospholipid membranes, various ion channels and receptors, synaptic and network functions, and intracellular signaling molecules[8]. Although it has been extensively investigated

(especially in GABA$_A$ receptors[9]), the major target mediating EtOH reward signaling and the precise mechanisms of EtOH reward and dependence are still poorly understood[10]. This gap in knowledge results in a therapeutic barrier in the treatment of alcoholism.

It is well known that alcohol and nicotine are often co-abused. The number of alcoholics who also smoke has been reported to be as high as 96%[11], suggesting that neuronal nicotinic acetylcholine receptors (nAChRs), the molecular targets of nicotine, may contribute to the abusive properties of alcohol. Mounting genetic, pre-clinical, and clinical evidence demonstrates that EtOH directly and indirectly modulates nAChR function in the mesolimbic pathway, which may underlie alcohol reward and dependence. These lines of evidence also build the rationale that nAChRs are likely important targets which mediate alcohol abuse. However, a consensus is yet to emerge as to which nAChR subtype critically mediates EtOH's central effects.

Impact of nAChRs in EtOH reward and dependence

nAChRs are ligand-gated ion channels expressed in a variety of compositions with two subtypes, α and β. Nine types of α subunits (α2–α10) are known to be expressed in vertebrates, as well as three β subunit types (β2–β4)[12]. The pentameric structure of each individual nAChR determines the variety of ion that is able to pass through the receptor's channel[12]. For example, the α4β2 receptor mostly permits the passage of sodium through its pore while the α7 receptor permits sodium passage and allows relatively high calcium permeability[12]. The known subunits found in the human brain are thought to be α3-α7, β2, and β4, although not all are presently known[12, 13]. The most common nicotinic pentamers in the human brain consist of heteromeric α4 and β2 subunits or α7 subunits[14]. These pentamers could be joined as α4$_{(2)}$β2$_{(3)}$, α4$_{(3)}$β2$_{(2)}$, or homomeric α7 receptors. nAChR α6 subunits are not widely expressed in the brain, but are prevalent in midbrain DAergic regions associated with pleasure, reward, and mood control[15], suggesting that α6*-nAChRs play critical roles in nicotine dependence and in the ability to modulate mood and emotion attributed to nicotine[16].

The concept that nAChRs are important targets in the mediation of EtOH reward and dependence is built on the pharmacological blockade of EtOH reward and dependence in a variety of alcoholic animal models by a nAChR antagonist mecamylamine. For instance, systemic mecamylamine significantly reduces EtOH-mediated extracellular DA release in the NAc[17], and reduces EtOH consumption in rats[18]. Local injection of mecamylamine into the VTA reduces rat operant responding for EtOH and EtOH-associated cues, as well as consumption during relapse[19, 20]. Mecamylamine delivered systemically reduces EtOH consumption in C57Bl/6J mice in restricted access EtOH consumption "drinking in the dark" (DID) paradigm[21], a model of binge drinking, as well as in the two-bottle choice consumption assay[22]. More recently, it has been demonstrated that mecamylamine blocks EtOH-mediated activation of VTA DAergic neurons in mouse mid-

brain slices[23]. Mecamylamine also blocks the ability of EtOH condition place preference in mice[24]. Since mecamylamine is a non-specific nAChRs blocker, it usually blocks all nAChR subtypes except α7-nAChRs at the doses used in these studies. Thus, the effects of nAChR subtype special antagonists on EtOH-induced reward, dependence and consumption have been examined. Unfortunately, neither the α4β2 nAChR antagonist dihydro-β-erythroidine (DHβE) nor the α7 nAChR antagonist methyllycaconitine (MLA) reduce EtOH-mediated DA release in the NAc, EtOH intake or consumption[20, 25]. On the other hand, the nAChR antagonist α-conotoxin MII, which blocks α3β2*, β3* and α6* subtypes, inhibits EtOH consumption, operant responding, and DA release in the NAc of rats[20, 26]. This pharmacological data suggest that both α4β2 and α7 nAChR subtypes may not be critical for ethanol reward and consumption behavior, while α6* and/or α3* nAChR subtypes are likely important targets for these EtOH-induced behavioral alterations.

Further studies using nAChR subunit knock out (KO) mice show that nAChR β2, α5*, α6*, or α7 KO mice exhibit similar EtOH consumption behavior to WT mice[27-29]. Interestingly, in α6 and α5, but not β3, KO mice, high doses of EtOH-induced sedation was enhanced[28, 29]. In addition, α4 KO mice show significantly less acute EtOH consumption to high (20%) but not low (2%) concentrations of EtOH[30]. Considering nAChR subunit compensation in a nAChR KO mouse background, these results collected from nAChR KO mice will need to be verified using shRNAs to knock-down nAChR subunits in discreet brain regions, and these data must also be interpreted with pharmacological evidence.

Finally, recent accumulating evidence suggests that common genes may influence the development of alcohol and nicotine behaviors individually, and contribute to both disorders in humans[31]. For example, the mammalian genes that code for the α6 and β3 subunits of the nAChRs (Chrna6 and Chrnb3, respectively) are located adjacent to one another on human and mouse chromosome 8. These two subunits have gained special attention for their expression in the VTA, their mRNA increase in the VTA after acute exposure to EtOH[30], and their roles in regulating EtOH-induced increase in DA release[26]. More importantly, human genetic studies have shown that variation in these genes is associated with alcohol phenotypes[32]. These lines of evidence suggest that the α6* and β3* nAChRs may modulate alcohol behaviors. Collectively, mounting lines of evidence suggest that various nAChR subtypes are involved in alcohol reward and dependence[33]. Although having very limited investigation, the α6* nAChRs in the VTA have attracted special attention[33] (Figure 1).

Cellular mechanisms of nAChR-mediated EtOH reward and dependence

Neuronal nAChRs mediate cholinergic modulations in brain function through both pre- and post-synaptic mechanisms. Most nAChRs in the central nervous system are located on presynaptic terminals/boutons[34, 35], where they modulate various neurotransmitter releases[36], including acetylcholine
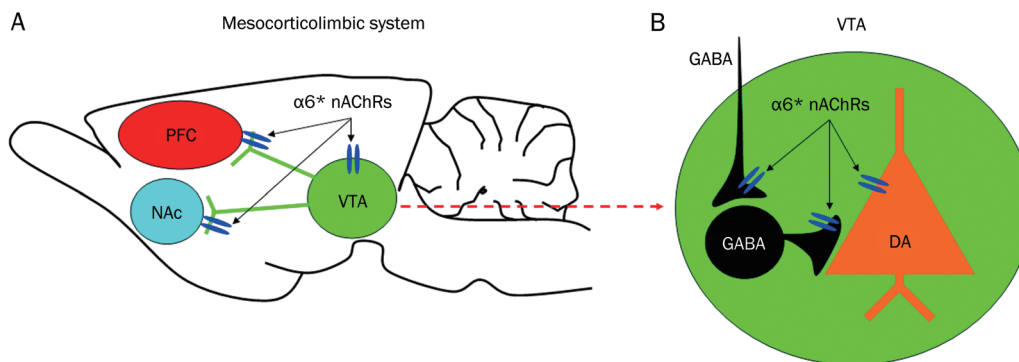
Figure 1. α6* nAChRs in the mesocorticolimbic dopaminergic system. (A) The structure of mesocorticolimbic circuitry, and the distributions of α6* nAChRs. (B) Within the VTA, α6* nAChRs express on DA neuronal somato, and GABAergic terminals/boutons synapsing on DA neurons and GABA neurons.

(ACh) itself when nAChRs are activated (or desensitized). nAChRs are also expressed on neuronal somatodendritic regions, where they presumably modulate neuronal excitability directly. Therefore, EtOH reward and dependence through nAChRs involved these pre- and post-synaptic mechanisms. On one hand, EtOH alters cholinergic modulations in neurotransmitter releases in the mesolimbic pathway. On the other hand, EtOH directly modulates nAChR expression, up or down regulation, and functions such as allosteric modulation, stabilization, desensitization or internalization[37]. Through these mechanisms, EtOH enhances mesolimbic DA signaling and consequently triggers reward and dependence. For example, systemic EtOH-induced DA release in the rat NAc. This DA release was completely abolished by nAChR antagonist, mecamylamine[17]. Interestingly, only perfused mecamylamine in the VTA, but not in the NAc, prevented the accumbal DA overflow after systemic EtOH[38]. The voluntary EtOH self-administration demonstrated an increase in DAergic and cholinergic neurotransmission[39], suggesting that VTA nAChRs may play an important role in mediating the mesolimbic activating and reinforcing properties of EtOH[40]. During *in vitro* preparations, EtOH potently modulates nAChRs at low concentrations (100 μmol/L–10 mmol/L), suggesting nAChRs as potential targets for EtOH action[41]. In *Xenopus* oocytes, acute EtOH (75 mmol/L) potentiated ACh-induced current of α2β4, α4β4, α2β2, and α4β2 nAChRs, while lower concentrations of EtOH (20–50 mmol/L) inhibited nicotine-induced current of α7 nAChRs and all concentrations of EtOH tested have no effect on α3β2 or α3β4 nAChRs[42]. In cultured cortical neurons, EtOH potentiated non-α7 nAChR- but inhibited α7 nAChR-mediated currents[43]. In brain slices[44] or isolated neurons[45], EtOH excited VTA DAergic neurons and increased neuronal firing rate. Taken together, EtOH directly and/or indirectly modulates nAChR functions, which in turn alters mesolimbic function, and leads to reward and dependence.

nAChR-associated ligands as a new therapeutic strategy to treat alcoholism

There have been three FDA approved medications for treating alcoholism; (1) Disulfiram, approved in 1954, is an acetaldehyde dehydrogenase inhibitor which improves alcohol symptoms such as headache, nausea, vomiting, weakness, mental confusion, or anxiety[46]. (2) Naltrexone, available since 1994, is a competitive opioid receptor antagonist that works by decreasing the euphoric effects produced by alcohol[47]. (3) Acamprosate is a partial agonist of NMDA receptors and an antagonist of metabotropic glutamate receptors and is thought to act as an anti-craving medication by inhibiting glutamate signaling[48]. Unfortunately, only 20%–30% of treated patients respond positively to these drugs[49] and some of these drugs have shown serious negative side effects. Thus, there is an urgent need to develop new drugs for the treatment of alcoholism. Recently, nAChR-associated ligands have been shown as potential candidates for this purpose. For example, pharmacotherapeutic targeting nAChRs such as cytisine, sazetidine A, varenicline, lobeline mecamylamine, PF-4575180 and CP-601932 are the new strategies to treat alcohol dependence including reducing voluntary alcohol consumption or modulating alcohol drinking behavior in animal models and humans[50].

In conclusion, alcoholism is a complex disorder which alters many brain functions. EtOH induces an array of neuronal functions. The action of EtOH on nAChRs in the mesolimbic pathway marks these receptors as important for EtOH reward and dependence. Current evidence establishes nAChRs as new and promising pharmacological targets in the development of new drugs for the treatment of alcoholism.

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