Neurobiology of Alcohol Withdrawal: Inhibitory and Excitatory Neurotransmitters

Dr. Ertuğrul EŞEL

INTRODUCTION

The acute, toxic effects of alcohol on the brain manifest as changes in behavioral and cognitive functions. These acute effects have been known to emerge as a result of alcohol’s effects on several neurotransmitter, neuropeptide, and neuroendocrine systems, and voltage-gated ion channels.

Since the neurobiology of alcohol withdrawal provides important information about the mechanisms of alcohol dependence, it has become a major area of interest in recent years. The symptoms of alcohol withdrawal syndrome are typically recognized within 6-24 h of alcohol cessation. Most of the symptoms observed during the withdrawal period seem to be related to the adaptive changes (plasticity or allostasis) in neurotransmitter, neuropeptide, and neuroendocrine systems that result from continuous alcohol intake (Koob, 2003). This is because compensating neuroadaptive changes occur in the brain in response to the balance-imparing effects of chronic alcohol use. As a result of these adaptive changes, we observe decreased central inhibition [due to decreased gamma amino butyric acid (GABA) activity and due to decreased magnesium] and increased excitation [due to increased glutamate, dopamine (DA), and noradrenaline (NE)] during alcohol withdrawal (Nutt 1999). Of all these systems, the GABA and glutamate systems contribute most to the symptoms of alcohol withdrawal.

It has been suggested that the negative effects observed during alcohol withdrawal become worse with each subsequent abstinence attempt,
which is the biggest behavioral trigger (therefore, negative reinforcer) of compulsive alcohol intake (Koob, 2003). Therefore, symptoms that are perceived negatively by the individual and the negative mood state, seen in both acute and protracted alcohol withdrawal syndrome, play an important role in the continuation of alcohol dependence, alcohol cravings, and relapse (McBride et al., 2002). In this paper, we will review the changes in the inhibitory and excitatory systems, which interact with each other closely and are purported to play important roles in the development of alcohol withdrawal symptoms (Figure 1), and when necessary, we will refer to the biological mechanisms involved in the development of alcohol dependence, beginning with the mechanisms underlying alcohol withdrawal.

**GABA system**

The effects of ethanol on the GABA system are thought to be, to some extent, related to its reinforcing effect for long time. Ethanol is the allosteric modulator of GABA<sub>α</sub> receptors. Thus, the decrease in central GABA activity during alcohol withdrawal is a major cause of negative affects resulting from hyperexcitation and an increased reward threshold (Koob, 2003).

GABA has two main receptor subtypes, GABA<sub>α</sub> and GABA<sub>β</sub>. The GABA<sub>α</sub> receptor complex is connected to a chloride channel and regulates the passage of chlorine ions into cells. Stimulation of GABA<sub>α</sub> receptors causes the chloride channel to open in most of the neurons and hyperpolarisation of the cell. This makes the cell less excitable and thus, GABA is an inhibitory neurotransmitter. GABA<sub>β</sub> receptors function in connection with protein G.

Alcohol renders its acute central effects (anxiolytic, sedative, anticonvulsant, and motor coordination impairment) mainly through its agonistic effect on GABA<sub>α</sub> receptors; however, the levels of this effect are not the same on all parts of the brain, or even on all the cells of a particular brain region. It is known that ethanol mainly increases the inhibitory effect of GABA<sub>α</sub> in the cerebral cortex and medial septal neurons, and in certain hippocampal neurons (Faingold et al., 1998).

It is suggested that chronic alcohol intake down-regulates GABA<sub>α</sub> receptors by decreasing gene expression of receptor subunits (Cagetti et al., 2003; Malcolm 2003). Confirming this idea, screening studies of alcohol addicts found reduced numbers of GABA<sub>α</sub>-benzodiazepine (GABA<sub>α</sub>-Bz) receptor complexes, especially in the frontal lobes (Lingford-Hughes et al., 1998). Yet, some authors claim that this reduction might not be due to alcohol intake and might already exist in people with a tendency for alcohol dependence, which in fact, is a trait-marker (Eşel, 2003; Lingford-Hughes et al., 2003). However, the studies we cited showing that the reduction of GABA<sub>α</sub>-Bz receptors is related to the amount of consumed alcohol and the severity of alcohol addiction, suggests that this reduction is a result of alcohol consumption. In both cases, low numbers of GABA<sub>α</sub>-Bz receptor complex is an important factor relating to continued alcohol consumption and addiction development.

Regardless of the underlying reason, a down-regulation of GABA<sub>α</sub> is the reason for insufficient central inhibition during acute alcohol withdrawal, which leads to the symptoms of hyperexcitation (Figure 1). Additionally, animal studies confirm the decreased activity of GABA during ethanol withdrawal. In rats, epileptic seizures can be triggered easily by acoustic stimuli during alcohol withdrawal (Faingold et al., 2000). These seizures are known as acoustic seizures and it has been suggested that the inferior colliculus (IC), where acoustic information is processed, is responsible for these seizures. The reduction in GABAergic neuronal transmission and a rise in glutamatergic transmission in the IC of rats during alcohol withdrawal and acoustic seizures have been demonstrated experimentally (Faingold et al., 2000). GABA levels in the cerebrospinal fluid (CSF) of humans have also been found to be low during alcohol withdrawal (Tsai et al., 1998). Therefore, the idea that hyperexcitation and seizures originate from decreased inhibition made by GABA<sub>α</sub> and increased excitation made by glutamate seems reasonable. The reduction of alcohol withdrawal symptoms with GABA agonists or substances similar to GABA, and worsening of symptoms with GABA antagonists, further validates this idea (Poldrugo and Addolorato, 1999; Jung et al., 2000; Anton, 2001). The alleviation of withdrawal symptoms with the inhibitors of GABA transaminase enzyme (i.e. vigabatrine), which is responsible for the metabolization of GABA, provides additional supporting evidence (Sherif et al., 1997) (Table 1).

It has also been reported that repeated with-
withdrawal periods exacerbate the reduction of GABA receptor function and leads to more severe with-
drawal symptoms with each repeated withdrawal (De Witte et al., 2003). This phenomenon can be
described as kindling. This kindling phenomenon is supposedly related to changes in the GABA_4 subunit over time (Mahmoudi et al., 1997).

The fact that GABA receptor antagonists like baclofen or GABA reuptake pump inhibitors like tiagabine reduce withdrawal symptoms suggests that factors other than GABA receptors can also induce a functional deficit of GABA during withdrawal (Malcolm, 2003). GABA inhibits dopaminergic and glutamatergic receptors through GABA receptors. Baclofen might, in this way, reduce withdrawal symptoms through inhibiting DA and glutamate release. Moreover, since DA is an im-
portant neurotransmitter in the rewarding effect of alcohol, baclofen, acting through the same mecha-
nism, seems to be a promising drug for the treatment of alcohol dependence (Malcolm, 2003). It is also reported that gamma-hydroxy butyric acid is a similar molecule to GABA and substantially reduces alcohol withdrawal symptoms (Korninger et al., 2003).

Decreased GABA function and the increased effect of glutamate during alcohol withdrawal are, in fact, events related to each other. Because GABAergic neurons include glutamate receptors, when interacting with N-methyl-D-aspartate (NMDA) receptors, glutamatergic neurons are exposed to GABAergic effect through glutamate release controlling presynaptic GABA receptors (Fadda and Rossetti, 1998). In recent years, it has been proposed that the acute GABAergic effects of alcohol and some withdrawal symptoms can develop through neurosteroids (Khisti et al., 2002; Kartalci and Eşel, 2004; Finn et al., 2004). It is suggested that some behavioral effects of ethanol, such as hypnotic and anxiolytic effects, are the result of the GABAergic effects of neurosteroids such as 3, 5α-tetrahydro progesterone (allopregnanolone) and 3, 5α tetrahydro DOC, and that acute alcohol intake increases plasma levels of these substanc-
es, while in contrast, allopregnanolone levels are reduced during alcohol withdrawal, which might be related to the depression commonly observed during alcohol withdrawal (Morrow et al., 2001; Khisti et al., 2002).

Thus, it is thought that allopregnanolone for the treatment of alcohol withdrawal might be useful and good results have been obtained in animals (Morrow et al., 2001). Some symptoms of alcohol withdrawal might arise from changes in brain sensitivity to pregnane neurosteroids or from changes in the synthesis of these neurosteroids. Therefore, synthesis of neurosteroids might be a new direc-
tion to target in alcoholism treatment (Finn et al., 2004)

### Glutamate system

Glutamate is the main excitatory neurotransmit-
ter in the brain. With the stimulation of glutamate receptors, almost all neurons become depolarized by glutamate. Glutamate receptors are primarily divided into 2 main categories: metabotropic and ionotropic receptors. Metabotropic receptors function with intracellular secondary messengers through G-proteins. Ionotropic receptors, on the other hand, are ligand-gated ion channels managing rapid changes in sodium, calcium (Ca), and potassium passage. Subtypes of ionotropic receptors are NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptors (AMPA), and universal receptors. NMDA receptors are controlled by 2 components consisting of cell membrane potential and binding of the ligand. While some of the AMPAs and kainat receptors allow Ca to enter the cell, all NMDA receptors are Ca permeable. Thus, glutamate seems to be the initiator and the conductor of adaptive periods leading to long-term changes in cells responsible for learning and mem-

---

**Table 1.** Useful or possibly useful drugs for treating alcohol withdrawal symptoms.

<table>
<thead>
<tr>
<th>Category</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA A agonists</td>
<td>- Benzodiazepines</td>
</tr>
<tr>
<td>Partial GABA agonists</td>
<td>- Abecarnil</td>
</tr>
<tr>
<td>GABA agonists</td>
<td>- Baclofen</td>
</tr>
<tr>
<td>Other GABAergic drugs</td>
<td>- Vigabatrin</td>
</tr>
<tr>
<td>- Tiagabine</td>
<td></td>
</tr>
<tr>
<td>- Gamma-hydroxy butyric acid</td>
<td></td>
</tr>
<tr>
<td>Glutamate antagonists</td>
<td>- Acamprosate</td>
</tr>
<tr>
<td>- Memantine</td>
<td></td>
</tr>
<tr>
<td>- MK-801</td>
<td></td>
</tr>
<tr>
<td>Allopregnanolone</td>
<td></td>
</tr>
<tr>
<td>Ca channel blocker</td>
<td></td>
</tr>
<tr>
<td>NE decreasing drugs</td>
<td></td>
</tr>
</tbody>
</table>

GABA: Gamma amino butyric acid; NE: Norepinephrine; Ca: Calcium
ory functions (Wirkner et al., 1999; Demirci and Eşel, 2004).

The glutamate system is known to play a role in the acute behavioral effects of alcohol, the development of alcohol dependence, alcohol withdrawal syndrome, and also in the familial predisposition to alcohol dependence (Krystal et al., 2003a).

Tolerance that develops with chronic use of alcohol is simply a neuroadaptive process, which attempts to reduce the acute effects of the substance and provide homeostasis. Ethanol also has an inhibitory effect on NMDA receptors found in various neurons of the brain (Faingold et al., 1998). Therefore, it is expected that ethanol inhibits the intracellular Ca increase caused by NMDA receptors. Indeed, this Ca reduction due to alcohol has been shown in cortical neurons, cerebellar granular cells, and mesencephalic neurons (Bhave and Hoffman, 1997). Moreover, ethanol inhibits long-term potentiation (LTP), which is the cellular equivalent of memory, by limiting NMDA channel function (Schummers et al., 1997); but, the area where ethanol blocks the NMDA receptor channel is not precisely known. It is accepted that magnesium blocks the NMDA receptor channel with a rapid, open channel blockage, in a voltage-dependent manner. NMDA receptors, such as MK-801 (dizocilpine), ketamine, and memantine, are similarly open channel blockers. Ethanol seems to inhibit receptors through this mechanism (Wirkner et al., 1999). It has been found that ethanol's acute inhibitory effect on glutamate can be reversed by glycine and protein kinase C (PKC) inhibitors; consequently, ethanol's inhibitory effect on NMDA receptor function might result of its effect on PKC (Faingold et al., 1998).

On the other hand, chronic ethanol use leads to an increase in NMDA receptor function; that is to say, continuous alcohol intake results in a compensating adjustment of these receptors, namely up-regulation (Faingold et al., 1998). Yet, this functional increase seems to derive not from an increase of all NMDA receptors, but from various up-regulations of various NMDA receptor subunits (Nagy et al., 2004).

Another possible mechanism for the increase in NMDA receptor activity might be the increased interaction of NMDA with intracellular messengers. For instance, neuronal activation of the enzyme, nitric oxide synthetase (NOS), is dependent on Ca. It has been claimed that nitric oxide (NO) is an important mediator for the physiological and pathological effects of NMDA receptors.

While increased NMDA activity during alcohol withdrawal results in a parallel increase in NO and contributes to excitotoxicity generated by abstinence, it may also be responsible for some withdrawal symptoms as well. Increased NOS activity has been demonstrated after long-term exposure of animal neuronal cultures to alcohol (Chandler et al., 1997). It is proposed that this might be an adaptive change due to chronic alcohol exposure and the reason for alcoholic memory deficit (Uz-bay and Oglesby, 2001).

Acute alcohol intake not only inhibits NMDA receptors, but also other glutamate receptors, such as AMPA, in addition to kainate receptors and voltage-gated Ca channels. Therefore, with continuous alcohol intake, these non-NMDA pathways are also up-regulated and are partly responsible for the symptoms of hyperexcitation seen during withdrawal (Carta et al., 2002).

Chronic alcohol intake seems to increase synaptic glutamate release in addition to increasing NMDA receptor activity, and, to a degree, non-NMDA receptor activity. Indeed, raised extracellular glutamate levels during alcohol withdrawal have been observed (Gonzales et al., 1996). This might occur as a result of excitation of presynaptic neurons by NO acting as a reverse messenger. Indeed, microdialysis studies in animals showed extracellular glutamate increase in the corpus striatum, nucleus accumbens, and hippocampus during ethanol withdrawal. In humans, an increase in excitatory neurotransmitters in CSF has also been reported (Tsai et al., 1998; Dahchour et al., 1998; Rossetti et al., 1999). In further support of this claim, it has also shown that in rats, NOS inhibitors alleviate seizures and other symptoms of alcohol withdrawal, while L-arginine, a precursor of NO, exacerbates these symptoms (Uzbek et al., 1997, 2000; Goren et al., 2002). Therefore, an increase in glutamate release and in the sensitivity and number of NMDA receptors create a feedback loop through increasing each other’s activity. With the effects of all these mechanisms, NMDA-regulated cationic current (Grove et al., 1998) and intracellular Ca increase (Smother et al., 1997) becomes apparent (Figure 1). For instance, NMDA receptor increase in the neocortex might be related to amnesia caused by ethanol, in the long run, and
its increase in the locus ceruleus might be related to withdrawal symptoms. (De Witte, 2004).

Neuroadaptation, which is recognized in the form of increased glutamate activity and develops as a result of chronic alcohol intake, is responsible for symptoms such as hyperexcitation, anxiety, and epileptic seizures during alcohol withdrawal. It is suggested that excess activity of NMDA during withdrawal is related to tyrosine kinase and PKC intracellular transmission pathways (Li and Kendig, 2003).

Since the increase in NMDA receptor activity is responsible for many significant symptoms of withdrawal, NMDA receptor antagonists might be considered for the treatment of withdrawal symptoms (Table 1). Indeed, these drugs reportedly decrease the magnitude of symptoms and prevent epileptic seizures (Karcz-Kubicha and Liljequist, 1995; Bisaga and Popik, 2000; Nagy et al., 2004). For example, the NMDA receptor antagonist MK-801 was shown to decrease withdrawal seizures (Morgan et al., 1992).

Additionally, the use of NMDA receptor antagonists like memantine and acamprosate, and presynaptic glutamate release inhibitors like lamotrigine are thought to be useful in the treatment of alcohol withdrawal and in the prevention of relapse, since it has been proposed that the adaptive increases of NMDA receptors caused by alcohol contribute to the development of alcohol tolerance and dependence, and also the rewarding effect of alcohol (Bisaga and Popik, 2000; Kotlinska, 2001; Mason, 2003; Nagy et al., 2004). This idea is also supported by Marcon et al. (2003), who reported that the metabotropic glutamate receptor antagonists reduce alcohol intake in animals. In addition to their ability to reduce physical symptoms during withdrawal and late withdrawal periods, glutamate antagonists can also improve mood disorders during alcohol-free periods, and thus, alleviate alcohol cravings.

Furthermore, since the glutamate receptor system is important in memory and learning, it may play an important role in the reinforcing effect of alcohol intake and through learning (or conditioning) of environmental cues in repeating alcohol dependency. Glutamate antagonists can be a reasonable choice for withdrawal treatment by inhibiting the conditioning behavior caused by alcohol (Bisaga and Popik, 2000; Le ve Shaham, 2002).

Glutamate up-regulation in response to chronic alcohol intake seems to be involved in increased sensitization to withdrawal, which is characterized by an increase in withdrawal symptoms and seizure risk (kindling) with each subsequent withdrawal (Gonzales et al., 2001; De Witte et al., 2003). This is because with repeated episodes of withdrawal, GABA<sub>A</sub> receptor function decreases and NMDA receptor functions gradually increase. Indeed, in rats, during repeated withdrawal episodes, excitatory amino acids in the hippocampus, such as glutamate and aspartate, increased (Dahchour and De Witte, 2003). Thus, the prevention of plasticity (or kindling) due to withdrawal with the use of glutamate antagonists will not only be helpful in directly reducing withdrawal symptoms, but will also provide additional benefits such as indirect alleviation of alcohol cravings and future withdrawal symptoms (Anton, 2001; Krystal et al., 2003b).

Alcohol-dependent people experience a period called protracted abstinence, which can continue for months after the acute withdrawal period; it includes symptoms like sleep disorders, depressive mood, and low energy. This protracted abstinence period is known for its importance to relapses (De Soto et al., 1985). Altered glutamate activity is also reported during this period (Krystal et al., 2003b). Long-term alcohol intake increases the number of NMDA receptors in the ventral tegmental area, which results in hyperexcitation in mesolimbic DA pathways and, in the long run, in depolarization blockade. Thus, excess glutamate activity during abstinence and protracted abstinence might cause a gradual decrease in DA release with each subsequent withdrawal, contributing to the depression observed during these periods (Fadda and Rossetti, 1998).

The possible importance of NMDA receptors in the predisposition to alcoholism and a reduced response to glutamate antagonists like ketamine in individuals coming from alcoholic families is reported (Krystal et al., 2003b). Therefore, glutamate receptor alterations in alcoholics might be the result of a combination of congenital predispositional factors and alterations due to chronic alcohol intake (Krystal et al., 2003a).

Degenerative changes in certain areas of the brain due to chronic alcohol intake are known. Generally, chronic alcohol intake causes degeneration in the diencephalon, medial temporal lobe structures, neocortical structures, basal forebrain,
and cerebellum. Chronic alcohol intake causes neuroadaptation in the form of glutamate activity increase, which appears to be responsible for alcohol withdrawal symptoms and for the neurodegeneration that develops at the end of periods of withdrawal (Wirkner et al., 1999; Dahchour and De Witte, 2003). During alcohol withdrawal, together with an increase in CSF excitatory neurotransmitters, indicators of oxidative stress, such as superoxide dismutase and lipid hyperoxidase are reported to increase, resulting in neurotoxicity (Tsai et al., 1998; Thomas and Morrisett, 2000; De Witte et al., 2003). Neuregeneration is claimed to be primarily caused by alcohol withdrawal, but not by chronic alcohol intake where the glutamate system plays the main role (Lundqvist et al., 1995). It is reported that NMDA receptor activity increase is a must for the development of neurotoxicity due to withdrawal, which is usually observed within the first days of withdrawal (Thomas and Morrisett, 2000; Nagy et al., 2003). Therefore, NMDA antagonists can also prevent neuroregeneration due to alcohol withdrawal.

Apart from these, the deprivation phenomenon of the glutamate system might play a role in animals and social drinkers, and is responsible for increased alcohol consumption when alcohol is ingested following a period of withdrawal. Acamprosate and some other NMDA antagonists reportedly reduce this deprivation effect (Le and Shaham, 2002). Furthermore, glutamate receptor antagonists also reduce alcohol use relapse due to the effect of alcohol reminders (the cue-induced relapse to alcohol) (Backstrom and Hyytia, 2004), and for this reason, these drugs might be useful in preventing relapse triggered by these mechanisms.

Voltage-gated Ca channels

It has been known that alcohol not only effects NMDA-dependent Ca transmission, but also voltage-gated (L-type) Ca channels. While acute alcohol intake inhibits passage through voltage-gated Ca channels in the IC, chronic alcohol intake can cause up-regulation of these channels (Ngouerno and Morad, 2003). Therefore, in the treatment of alcohol withdrawal syndrome and in the prevention of withdrawal sensitization, the use of Ca channel inhibitors might be considered useful (Table 1) (Veatch and Gonzales, 2000; Uzbay 2004).

Norepinephrine system

One of the reasons for hyperexcitation in alcohol withdrawal is increased norepinephrine (NE) activity. Excess NE activity in the initial stage of alcohol withdrawal (Linnola, 1998) and, in contrast, decreased adrenergic activity in people who have abstained from alcohol for a long period of time (Borg et al., 1983) is reported. More recent studies indicate increased NE in plasma and CSF during the first few days of withdrawal, which then returns to normal in the following weeks (Kovacs et al., 2002; Patkar et al., 2003).

One month after the cessation of the alcohol use, reduced CSF levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and reduced plasma levels of DA beta-hydroxylase enzyme, which is responsible for converting DA into NE (Heinz et al., 1999; Köhnke et al., 2002) is indicative of the gradual decrease in NE activity that follows the initial period of withdrawal. It is suggested that this might be due to reduced postsynaptic receptor sensitivity (Krystal et al., 1996). This decreased NE activity during prolonged withdrawal might be associated with the frequently seen depression and relapses in alcohol-dependent people (Heinz et al., 1999).

NE activity increase in early stage of withdrawal occurs concurrently with GABA function reduction and glutamate function increase, and might actually be a consequence of these (Figure 1). Increased NE activity in the brain during alcohol withdrawal also affects other organs by way of increased sympathetic activity, and symptoms like palpitations, hypertension, sweating, and tremor are observed. The cause of this functional increase in NE might be hyperexcitation of NE neurons by increased glutamate and loss of auto-inhibition of NE due to a functional decrease in presynaptic α2-adrenoreceptor activity (Nutt, 1999). Neuroendocrinological studies showing decreased growth hormone (GH) response to clonidine during withdrawal confirm the down-regulation of α2 receptors (De Witte et al., 2003).

Adenosine

Adenosine is a purine-based neurotransmitter found in the brain and has GABA-like inhibitory and anxiolytic functions. It has 3 receptors: A1, A2, and A3. All of these function in connection with protein G. Acute alcohol intake inhibits the reuptake pump and thus reduces adenosine reuptake and increases extracellular adenosine (Kaplan et
al., 1999). Symptoms like ataxia caused by alcohol are thought to be due to adenosine increase. It has been shown that some DA receptors in the nucleus accumbens include both D2 and A2 receptors at the same time, which function in synergy. Alcohol-induced adenosine increase activates A2 receptors and results in the activation of Gs proteins, cAMP, and PKA sequentially, and consequently increases cAMP-dependent gene expression. The existence of D2 and A2 receptors in the nucleus accumbens might be the reason for increased cell sensitivity to the effects of alcohol (Mailliard and Diamond, 2004). Thus, adenosine receptor antagonists might have a place in the treatment of alcohol dependence and the prevention of relapse.

Chronic alcohol intake down-regulates A2 receptors and reduces cAMP production (Kaplan et al., 1999). Adenosine levels decrease during alcohol withdrawal and this might be related to the seizures and sleep disorders observed during withdrawal (Landolt and Gillin, 2001; De Witte et al., 2003).

CONCLUSION

Alcohol withdrawal syndrome is an entity that is the result of the brain’s adaptation to long-term alcohol intake and is identified by changes in neurotransmitter, neuropeptide, and hormone systems. During alcohol withdrawal, many inhibitory and excitatory neurotransmitter systems of the brain interact with each other and with other neuropeptide systems in a very complex manner, and this results in symptoms such as increased excitation, anxiety, excess autonomic activity, sleep disorders, depressive mood, and epileptic seizures. Thus, in the treatment of alcohol withdrawal, as in the treatment of other psychiatric disorders, the use of various drugs affecting many neurotransmitter systems appears to be necessary.

REFERENCES


