Alcohol Withdrawal Seizures

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CHAPTER 13

Alcohol Withdrawal Seizures

PROSPER N’GOUEMO AND MICHAEL A. ROGAWSKI

Ethyl alcohol (ethanol) is a central nervous system (CNS) depressant that exerts diverse behavioral actions. At low blood concentrations, alcohol produces euphoria and behavioral excitation, and at concentrations greater than 0.08 g/dl (17 mM), it significantly impairs motor skills. Concentrations of 0.15 to 0.30 g/dl induce acute intoxication, which manifests as drowsiness, ataxia, slurred speech, stupor, and coma. The acute effects of alcohol on brain function are believed to result largely from its actions on ligand-gated and voltage-gated ion channels, resulting in alterations in neuronal signaling (Crews et al., 1996; Deitrich and Erwin, 1996; Nevo and Hamon, 1995). Chronic alcohol consumption leads to the development of tolerance and physical dependence, which may result from compensatory changes in neuronal signaling that balance the acute effects of alcohol. Abrupt cessation of chronic alcohol consumption unmasks the compensatory physiologic change, leading to a cluster of neurologic signs and symptoms known as alcohol withdrawal syndrome. In humans alcohol withdrawal syndrome includes blackouts, tremors, muscular rigidity, delirium tremens, and seizures (Hillbom et al., 2003; Kosten and O’Connor, 2003). These various manifestations of alcohol withdrawal may reflect an involvement of different neuronal networks and cellular mechanisms (Schmidt and Sander, 2000). The most prominent and dramatic aspects of alcohol withdrawal syndrome in humans are alcohol withdrawal seizures, which can be life threatening (Hillbom et al., 2003; Mattson, 1983; Peinikeroinen et al., 1992; Ripley, 1990). Alcohol withdrawal seizures are usually generalized tonic-clonic ("grand mal") seizures, although 60% of subjects experience multiple seizure types (Victor and Brausch, 1967). These additional seizure types include partial seizures and partial seizures progressing to generalized tonic seizures (Freedland and McMicken, 1993a). Alcohol withdrawal seizure has been included in the International League Against Epilepsy Classification of Epilepsies and Epileptic Syndromes as a condition with epileptic seizures that does not require a diagnosis of epilepsy (Engel, 2001). These seizures typically occur 6 to 48 hours after discontinuation of alcohol consumption, but they may also occur up to 7 days after the last drink, particularly if the patient has been abusing other psychoactive agents, such as benzodiazepines or barbiturates.

Generalized tonic-clonic seizures are the most severe type of alcohol withdrawal seizures in humans. Convenient rodent models that mimic human alcohol withdrawal-related tonic-clonic seizures have been developed, thus providing a substrate to study the underlying seizure mechanisms and for the development of treatment approaches. In these models, the following tonic-clonic seizure types can be observed during a finite period (typically 1 to 3 days) after the cessation of alcohol intake: (1) spontaneous seizures, (2) auditory-evoked seizures ("audiogenic seizures," or AGS), and (3) handling-induced convulsions (HIC).

This chapter describes procedures for inducing alcohol dependence, precipitating withdrawal, and eliciting and scoring the resultant alcohol withdrawal seizures. In these models rats or mice are exposed to alcohol by intragastric intubation, inhalation, or feeding in a nutritionally complete liquid diet for periods of 2 to 21 days. (The use of a seizure-sensitive mouse strain that exhibits an increase in seizure severity after a single dose of alcohol is briefly discussed in the section entitled Selection of a Model.) Each of the models is associated with highly reproducible alterations in seizure susceptibility so that they are suitable for basic neurobiological, pharmacologic, and genetic studies. In
addition, the models can be of value in evaluating potential therapies for alcohol withdrawal seizures. This chapter also reviews the present understanding of the brain mechanisms underlying withdrawal seizures and considers briefly seizures related to withdrawal of other CNS depressants.

**METHODS FOR INDUCTION OF ALCOHOL DEPENDENCE AND FOR MONITORING WITHDRAWAL SEIZURES**

**Species, Strain, Gender, and Age Considerations**

As in humans, an alcohol withdrawal syndrome that includes generalized tonic-clonic seizures has been observed in the mouse, rat, cat, dog, monkey, and chimpanzee (Ellis and Pick, 1970; Essig et al., 1969; Freund, 1969; Guerrero-Figueroa et al., 1970; Majchrowicz, 1975; Pieper et al., 1972). In all species, the signs of alcohol withdrawal last for 1 to 3 days, after which behavior returns to normal and there is no enhanced seizure susceptibility. Rodents are the most common species used in laboratory studies of alcohol withdrawal seizures. Here we describe methods for the induction of alcohol dependence and for inducing and scoring withdrawal seizures in the rodent species that are most frequently used in laboratory experiments. The intragastric intubation method was originally developed in studies with rats and is mainly applied in this species. In contrast, inhalation methods are most commonly used in mice. The liquid diet procedure is applied in both mice and rats. The experimental methods can be adapted for use with other species.

Among rodents, there are substantial differences among strains in the severity of withdrawal seizures (Crabbe, 2002; Metten and Crabbe, 1994). For example, DBA/2 inbred mice exhibit more severe acute alcohol withdrawal seizures than do C57BL/6 mice (Roberts et al., 1992). As discussed in the section entitled Metabolic Changes Following Alcohol Withdrawal, there has been an extensive effort to identify the specific genes that influence alcohol withdrawal seizure severity and other behavioral components of alcohol dependence and withdrawal. Consideration of strain differences is critical when selecting animals for experimental studies, and in the future, as specific susceptibility loci are identified, the specific genotype will also likely be a factor of relevance.

There are significant sex differences in the incidence of ethanol abuse and alcoholism, with nearly 20% of adult men affected, in contrast to only about 5 to 6% of adult women (Devaud et al., 1999). Alcohol withdrawal seizures seem to affect predominantly men (Essardan Daryanani et al., 1994; Pilke et al., 1984). These differences have been attributed mainly to societal influences, although neurobiological factors could also play a role. Both male and female rodents exhibit withdrawal seizures in alcohol-dependence models. However, there may be small sex differences in susceptibility. For example, male mice may experience more severe seizures than females do (Buck et al., 2002).

Adult animals are typically used in studies of alcohol withdrawal seizures. Interestingly, however, mice that have been bred for susceptibility to ethanol withdrawal seizures may also show enhanced susceptibility to AGS, but only at young ages (Feller et al., 1994) corresponding with the developmental sensitivity of mouse strains such as DBA/2, which are only susceptible to audiogenic seizures from postnatal days 20 to 80.

**Procedures**

**Methods for Alcohol Administration**

**Intragastric Intubation**

Induction of alcohol dependence in rats by intragastric intubation was first described by Majchrowicz (1975). A key advantage of this approach over other methods of alcohol administration is that dosing is highly reliable, so when the treatment is discontinued, all animals exhibit the major signs of withdrawal. In addition, this method allows physical dependence to be produced in a relatively short time. In the model, 50 mM ethanol (95%) is administered in ISOMIL Soy Infant Formula Concentrate diluted 1:1 with water (Riaz and Faingold, 1994). A priming dose of 5 g/kg of ethanol is administered to each animal, and subsequent doses are determined by the degree of intoxication exhibited. Dosing occurs at 8-hour intervals for 4 days, with total daily doses in the range of 9 to 15 g/kg/day. The dosages are adjusted so that the animals exhibit mild to moderate ataxia (Table 1). Ethanol is withdrawn after the second dose on the

<table>
<thead>
<tr>
<th>TABLE 1 Behavioral Rating Scale for Alcohol Intoxication</th>
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<tbody>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td>Neutral</td>
</tr>
<tr>
<td>Sedation</td>
</tr>
<tr>
<td>Ataxia 1</td>
</tr>
<tr>
<td>Ataxia 2</td>
</tr>
<tr>
<td>Ataxia 3</td>
</tr>
<tr>
<td>Loss of righting reflex</td>
</tr>
<tr>
<td>Coma</td>
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</table>

Adapted from Majchrowicz (1975).
fourth day. Control animals are maintained under similar conditions but are fed the ISOMIL diet without ethanol. This method can be associated with significant mortality (9–23%; N’Gouemo et al., 1996).

Inhalation

Alcohol intoxication is initiated by administration of a loading dose of ethanol (1.6 g/kg; 8%, wt/vol). Some investigators administer the alcohol dehydrogenase inhibitor pyrazole to enhance and stabilize the blood ethanol concentrations (Goldstein and Pal, 1971). Animals are then placed in a closed inhalation chamber as illustrated in Figure 1, and alcohol dependence is induced by continuous exposure to alcohol vapors for 2 to 7 days. Air is continuously delivered to the chamber at a rate of 10 liters per minute to provide for the respiratory need of the animals. Ethanol (95%) is evaporated into the air so that the experimental chamber holding the animal receives air with an ethanol concentration of 7 to 35 mg/liter. Control chambers are similarly configured, but with the absence of ethanol vapor.

Liquid Diet

Alcohol can also be chronically administered in a liquid diet. Rats have an aversion to alcohol. However, high alcohol intake can be induced with a nutritionally complete liquid diet used as the only food source, such as the Lieber-DeCarli diet (Dyets, Inc., Bethlehem, PA) (Lieber and DeCarli, 1982). Control animals receive a similar diet in which an isocaloric amount of maltose is substituted for ethanol. The ethanol concentration is typically 6 to 7% (vol/vol) and is administered for 4 to 21 days. At the end of the alcohol exposure period, animals are switched to a regular diet. Mice can be treated with a similar diet, but the ethanol concentration may be lower (4.5 to 6.5%) and the exposure period is typically 2 to 6 days. Mice can also be “kindled” by repeated withdrawal (periods of 1 to 2 days of

![FIGURE 1 Schematic representation of a system for administration of ethanol by inhalation modified after Ruwe et al. (1986). Ethanol (95%) is delivered by a solvent metering pump into a 250-ml vaporization chamber. The airtight vaporization chamber is maintained at 37°C by a water bath. Air is delivered into the vaporization chamber with an air pump to provide a flow rate of 2.5 to 4 liters per minute. Animals are exposed to ethanol vapor concentrations of 7 to 35 mg/liter of air in a Plexiglas experimental chamber. A food tray and water bottle are securely affixed to the experimental chamber, giving the animal free access to food and water. The sample port allows air within the experimental chamber to be sampled for determination of ethanol concentrations. (See color insert.)](image-url)
abstinence during the chronic ethanol feeding), which potentiates the severity of withdrawal seizures (Becker and Hale, 1993; Pinel, 1980; Ripley et al., 2002).

**Blood Sampling and Measurement of Blood Alcohol Concentrations**

Blood alcohol concentrations are typically measured during intoxication, at the onset of withdrawal symptoms, and during the fully developed withdrawal syndrome. Blood samples are usually collected from the tail vein in mice or by intracardiac sampling in deeply anesthetized rats using large-bore (21-gauge) needles to prevent hemolysis. Blood samples are stored in tubes containing heparin or the anti-coagulant potassium oxalate and sodium fluoride (Becton Dickinson Vacutainer Systems, Rutherford, NJ). Blood ethyl alcohol concentrations are measured using gas chromatography (Brown and Long, 1988) or determined in the plasma using the alcohol dehydrogenase method, which requires a spectrophotometer to measure the absorbance at 340 nm (Pointe Scientific, Canton, MI).

**Seizure Monitoring Following Alcohol Withdrawal**

**Spontaneous Seizures**

On administration of the last dose of ethanol, the animals are placed in a Plexiglas observation chamber located in a sound-attenuated room (Gonzalez et al., 1989). The behavior of each animal is recorded on videotape at 30 frames per second for about 84 hours from the time of ethanol discontinuation. The videotapes are scanned at high speed to identify segments with evidence of seizure-like behavior and then at slower speeds for scoring. The incidence and severity of the following behaviors are recorded: (1) myoclonic jerks of the head or trunk; (2) jumping episodes; (3) generalized tonic-clonic seizures consisting of loss of upright posture and repetitive extension and retraction of the limbs and trunk; and (4) rigid, tonic extension of the limbs. Myoclonic jerks occur most frequently during the first 6 hours following alcohol withdrawal. The incidence of generalized tonic-clonic seizures is maximal between 24 and 60 hours after alcohol withdrawal; tonic seizures are most frequent at 60 to 84 hours following withdrawal.

**Audiogenic Seizures**

Mice and rats subjected to alcohol withdrawal are susceptible to AGS between 10 and 26 hours following the last doses of alcohol (Freund, 1969; Riaz and Faingold, 1994). AGSs are induced in a sound-attenuating chamber using an electric bell that produces a sound volume of about 122 dB at the middle of the acoustic chamber. The bell tone is presented either until a seizure is triggered or for 60 seconds. The animal's behavior is recorded on videotape for subsequent review. The incidence and type of seizures (wild running, bouncing clonus, tonic) are noted, and the overall severity is scored according to the scale of Jobe et al. (1973) (Table 2) and also Chapter 20. Audiogenic seizures can be measured repeatedly in the same animal with high interrater reliability.

**Handling-Induced Convulsions**

Mice are observed for seizure activity in response to various degrees of stimulation. Animals that exhibit spontaneous seizures or a tonic-clonic seizure within seconds of being handled during removal from their home cages receive the highest seizure scores. If no seizure occurs immediately with routine handling, animals are picked up by the tail and observed for an additional 2 seconds. If they still fail to exhibit a seizure, they are gently spun by the tail through a 180- to 360-degree arc. A score is assigned as defined in Table 3 based on the degree of stimulation required to elicit the seizure (handling alone, tail lift, or spinning) and the type of seizure (clonic, tonic, tonic-clonic) (Becker et al., 1997; Crabbe and Kosobud, 1990; Goldstein and Pal, 1971). HIC can be measured repeatedly in the same animal with high interrater reliability.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Behavioral Rating Scale for Audiogenic Seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>Description of Behavior</td>
</tr>
<tr>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td>1</td>
<td>Running only; no convulsion</td>
</tr>
<tr>
<td>2</td>
<td>Two running phases separated by a refractory period; generalized clonus involving forelimbs and hindlimbs</td>
</tr>
<tr>
<td>3</td>
<td>One running phase; generalized clonus involving forelimbs and hindlimbs</td>
</tr>
<tr>
<td>4</td>
<td>Two running phases separated by a refractory period; tonic flexion of neck, trunk and forelimbs with clonus of hindlimbs</td>
</tr>
<tr>
<td>5</td>
<td>One running phase and no refractory period; tonic flexion of neck, trunk and forelimbs with clonus of hindlimbs</td>
</tr>
<tr>
<td>6</td>
<td>Two running phases separated by a refractory period; tonic flexion of neck, trunk and forelimbs with hindlimbs in partial tonic extension (i.e., tonic extension of thighs and legs with clonus of feet)</td>
</tr>
<tr>
<td>7</td>
<td>One running phase and no refractory period; tonic flexion of neck, trunk and forelimbs with hindlimbs in partial tonic extension (i.e., tonic extension of thighs and legs with clonus of feet)</td>
</tr>
<tr>
<td>8</td>
<td>Two running phases separated by a refractory period; tonic flexion of neck, trunk and forelimbs with hindlimbs in complete tonic extension (maximal convulsion)</td>
</tr>
<tr>
<td>9</td>
<td>One running phase and no refractory period; tonic flexion of neck, trunk and forelimbs with hindlimbs in complete tonic extension (maximal convulsion)</td>
</tr>
</tbody>
</table>

From Jobe et al. (1973).
TABLE 3  Behavioral Rating Scale for Handling-Induced Convulsion

<table>
<thead>
<tr>
<th>Score</th>
<th>Description of Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No convulsion or facial grimace on tail lift, or after gentle 180- to 360-degree spin</td>
</tr>
<tr>
<td>1</td>
<td>Facial grimace after 180- to 360-degree spin</td>
</tr>
<tr>
<td>2</td>
<td>Tonic convulsion after 180- to 360-degree spin</td>
</tr>
<tr>
<td>3</td>
<td>Tonic-clonic convulsion after 180- to 360-degree spin</td>
</tr>
<tr>
<td>4</td>
<td>Tonic convulsion on tail lift</td>
</tr>
<tr>
<td>5</td>
<td>Tonic-clonic convulsion on tail lift, onset delayed by 1-2 s</td>
</tr>
<tr>
<td>6</td>
<td>Severe tonic-clonic convulsion on tail lift, no delay in onset</td>
</tr>
<tr>
<td>7</td>
<td>Severe tonic-clonic convulsion before tail lift</td>
</tr>
</tbody>
</table>

Adapted from Becker et al. (1997) and Crabbe and Kosobud (1990).

BEHAVIORAL FEATURES AND BRAIN MECHANISMS

Spontaneous Seizures

Spontaneous seizures are observed between 24 to 60 hours following cessation of alcohol consumption, and their incidence reaches a peak between hour 36 and 48 of the withdrawal period (Clemmesen et al., 1988; Gonzalez et al., 1989). The behaviors observed include myoclonic jerks, facial and forelimb clonic seizures, rearing, falling, tonic-clonic seizures, and tonic seizures. The neuronal networks that generate these behaviors are as yet unknown. However, the similarity to amygdala kindled seizures suggests that they may originate in the limbic system. Interestingly, studies with 2-deoxyglucose autoradiography have implicated amygdala neurons in the generation of spontaneous seizures associated with alcohol withdrawal (Clemmesen et al., 1988).

Handling-Induced Convulsions

Handling-induced convulsions are characterized by the occurrence of tonic-clonic seizures with routine handling as well as tonic or tonic-clonic seizures on tail lift or after spinning. The network for HIC is still poorly understood. Seizures elicited after spinning suggest an initial involvement of the vestibular system. The pontine reticular formation and periaqueductal gray are thought to play important roles in the expression of alcohol withdrawal-related clonic and tonic audiogenic seizures (see subsequent discussion). It is likely that these brainstem loci may also play a similar role in HIC.

Audiogenic Seizures

The behavioral features of AGS following alcohol withdrawal consist of wild running, bouncing clonus, and tonic seizures. Wild running is considered a model of partial seizures, whereas bouncing clonus represents generalized tonic-clonic seizures. The neuronal networks for AGS associated with alcohol withdrawal appear to be located in the brainstem, and a large body of evidence suggests that inferior colliculus (IC) neurons are critical in the initiation of these seizures (Faingold et al., 1998). Bilateral lesions of the IC, but not of the medial geniculate body, the first efferent synaptic target of IC neurons, block AGS susceptibility following alcohol withdrawal (Frey et al., 1986). These findings suggest that alcohol withdrawal seizure activity diverges from the classic auditory pathway at the levels of the IC and that these seizures are not of auditory nature per se. Consistent with this idea, epileptic activity was observed in hippocampal neurons, but with significant delay after the onset of auditory-evoked tonic-clonic seizures following alcohol withdrawal, suggesting an involvement of limbic structures in the network for AGS (Hunter et al., 1973). The IC plays an important role in the processing of auditory information and is the gateway to sensorimotor integration for the auditory system (Atkin, 1986). The IC can be subdivided into three main subnuclei: the central nucleus, dorsal cortex, and external cortex. All three subnuclei of the IC have been implicated in alcohol withdrawal seizures, and the IC external cortex appears to be important for the convergence of the outflow from the IC to the neuronal network responsible for producing motor seizures, which include the superior colliculus, periaqueductal gray and reticular formation (Faingold et al., 1998).

SELECTION OF AN EXPERIMENTAL MODEL

There are various tradeoffs in the choice between spontaneous seizures or seizures elicited by audiogenic or handling stimulation as the endpoint in studies of alcohol withdrawal seizures. Spontaneous seizures may have greater face validity to human alcohol withdrawal seizures because human alcohol withdrawal seizures occur paroxysmally without an apparent eliciting stimulus. Spontaneous alcohol withdrawal seizures in rodents are observed over a time course similar to that in humans. However, spontaneous seizures in experimental animals are mainly myoclonic seizures, whereas alcohol withdrawal seizures in humans are mostly generalized tonic-clonic seizures, as are vestibular or auditory-evoked seizures in animals. Such reflex seizures may have significantly different underlying pathophysiological mechanisms from alcohol withdrawal seizures in humans, which are not elicited by audiogenic or vestibular stimulation. (It is notable that some features of the alcohol withdrawal syndrome in humans are, in fact, triggered by external stimuli, including visual, auditory, or tactile
h hallucinations.) From a practical point of view, monitoring spontaneous seizures following alcohol withdrawal is technically more difficult than assessing seizures that are induced under the control of the experimenter; so reflex seizures are used more often in pharmacologic and physiologic studies.

In some mouse strains, mild HIC scores are elicited in some animals, even in the absence of alcohol. In these sensitive mouse strains, it may be possible to obtain potentiation of HIC scores after a single dose of alcohol. Withdrawal from alcohol in these strains is associated with higher HIC scores. A test paradigm using a single dose of ethanol has been used extensively in genetic studies to select for mice that are more or less withdrawal-seizure prone (Metten and Crabbe, 1994; 1999). Figure 2 schematically illustrates the results of a typical experiment with mice that are withdrawal-seizure prone (WSR) or withdrawal-seizure resistant (WSP). At baseline, WSP mice exhibit slightly greater HIC scores than WSR mice. A single hypnotic dose of ethanol (4 g/kg) is administered at zero time, and HIC susceptibility is monitored for the subsequent 12 hours. Both strains show a reduction in HIC score at 2 hours because of the anticonvulsant effects of alcohol. As blood alcohol levels fall and the animals are in a state of alcohol withdrawal, there is an increase in HIC score, which peaks at about 6 hours; WSP mice exhibit greater HIC scores than do WSR mice.

**SINGLE-NEURON FIRING DURING CELLULAR ELECTROPHYSIOLOGY OF ALCOHOL WITHDRAWAL SEIZURES**

**Audiogenic Seizures**

During alcohol withdrawal-related AGS, the cortical electroencephalogram typically shows no sign of paroxysmal activity compatible with the idea that the seizures are mediated largely in the brainstem (Hunter et al., 1973; Maxson and Sze, 1976). Nevertheless, epileptiform activity has been observed in the hippocampus, but with a significant delay after the onset of AGS, suggesting a role in the propagation rather than in the initial generation of the seizures (Hunter et al., 1973).

**Inferior Colliculus**

Acute alcohol intoxication suppresses spontaneously and acoustically evoked neuronal firing in the IC central nucleus, whereas alcohol withdrawal is accompanied by significant increases of these responses (Faingold and Riaz, 1995). Electrophysiologic studies have revealed that, at the transition to seizure, the IC central nucleus exhibits sustained increases in firing that persist during wild running, the initial phase of AGS (Chakravarty and Faingold, 1998). It has therefore been suggested that the IC central nucleus plays a role in the initiation of these seizures. The IC external nucleus is also a target of alcohol. Indeed IC external nucleus responses were suppressed during both acute alcohol intoxication and alcohol withdrawal (Chakravarty and Faingold, 1998). However, during alcohol withdrawal, IC external cortex neuronal firing is increased before the onset of AGS and persists during the wild running phase. The IC external cortex is believed to amplify and propagate neuronal activity originating in the IC central nucleus. Increased neuronal activity in these systems is then transmitted to nuclei responsible for the generation of convulsive motor behaviors.

**Superior Colliculus**

Spontaneous and acoustically evoked responses are suppressed during acute alcohol intoxication and alcohol...
withdrawal in the deep layers of the superior colliculus (SC) (Yang et al., 2001a). However, these neurons exhibit tonic neuronal firing before the onset and during wild running in alcohol withdrawal-related AGS. The tonic firing preceding the onset of wild running suggests that SC neurons may play a role in the generation of this component of AGS.

**Periaqueductal Gray**

Acute alcohol intoxication suppresses spontaneous and acoustically evoked periaqueductal gray (PAG) neuronal responses (Yang et al., 2003). During alcohol withdrawal, a significant increase in spontaneous and acoustically evoked PAG responses is observed. PAG neurons exhibit burst firing during wild running and tonic repetitive firing during the tonic-clonic phase of alcohol withdrawal-related AGS. The bursting activity preceding tonic-clonic seizures suggests that PAG may play a role in the generation of these seizures.

**Pontine Reticular Formation**

Spontaneous and acoustically-evoked pontine reticular formation (PRF) neuronal firing is suppressed by acute alcohol intoxication, whereas alcohol withdrawal associated with enhanced seizure susceptibility is accompanied by a significant increase in spontaneous and acoustically evoked PRF responses (Faingold and Riaz, 1994). The neuronal firing during alcohol withdrawal-related sound induced seizures has not yet been characterized. Nevertheless, studies using the genetically epilepsy-prone rat, which exhibits an inherited susceptibility to AGS, have found that PRF neurons exhibit tonic firing during the tonic phase of AGS and this pattern of neuronal activity persists during the postictal depression following the seizures (Faingold and Randall, 1995). Such a pattern of neuronal firing could also occur in PRF neurons during alcohol withdrawal seizures.

**Synaptic Pathways Generating Audiogenic Seizures**

The evidence to date indicates that IC neurons are critical in the initiation of alcohol withdrawal-related AGS. It is hypothesized that seizure activity propagates from the IC to deep layers of the SC, a major output of the IC, to trigger the wild running phase of the AGS. The deep layers of the SC send projections directly to the spinal cord via the PRF and the periaqueductal gray, which is thought to trigger clonic seizures, and the PRF is implicated in generation of the tonic phase of AGS activity (Faingold, 2004).

**Handling-Induced Convulsions**

The cellular electrophysiologic correlates of alcohol withdrawal-related HIC have not yet been described. Nevertheless, brief spindle episodes (BSEs) are observed in hippocampal neurons in animals that exhibit such HIC (Veatch and Becker, 2002). Although the incidence of BSE peaks earlier than HIC in these animals, the discharges could play a role in the seizures. The electrographic patterns of alcohol withdrawal-related BSEs resemble those observed in models of generalized absence seizures (Danover et al., 1998; Sned, 1995). However, because alcohol withdrawal-related HIC are tonic-clonic seizures, there are likely important differences in the underlying mechanisms.

**NEUROPATHOLOGICAL EFFECTS OF ALCOHOL WITHDRAWAL**

In humans, alcohol withdrawal seizures have been associated with ventricular and sulcal enlargement as well as significantly smaller volume of temporal lobe white matter and hippocampal sclerosis (Essardas-Daryanani et al., 1994). In animal models, there is evidence that alcohol intoxication can lead to selective damage to specific brain regions, including the hippocampus (Ikonomidou et al., 2000; Walker et al., 1980). Withdrawal from long-term alcohol consumption can aggravate alcohol-induced neurodegeneration. Indeed alcohol withdrawal is associated with augmented loss of CA1 and CA3 pyramidal neurons, mossy fiber-CA3 synapses, and dentate gyrus granule cells (Cadete-Leite et al., 1989; Paula-Barbosa et al., 1993; Scorza et al., 2003). The mechanisms underlying alcohol withdrawal-induced neurodegeneration are not completely understood. However, alcohol withdrawal, but not alcohol intoxication, is thought to be associated with significant increases in free intracellular calcium in hippocampal neurons (Prendergast et al., 2004). Thus alcohol withdrawal-induced neurotoxicity may result, in part, from enhanced calcium signaling. Indeed there is evidence for increased synaptic activation of calcium channels in alcohol withdrawal seizures (Whittington et al., 1993, 1995). Another key mediator of calcium entry into neurons is the N-methyl-D-aspartate (NMDA) receptor. There is an increasing body of data supporting the view that NMDA receptor function is enhanced in alcohol withdrawal (Krystal et al., 2003). This may result from enhanced activity in glutamatergic systems (Hoffman and Tabakoff, 1996), and, in fact, there is evidence of increased brain extracellular glutamate concentrations following ethanol withdrawal (Rossetti and Carboni, 1995). Moreover, whereas acute ethanol inhibits NMDA receptors, prolonged ethanol exposure may result in a compensatory upregulation of NMDA receptor function (Carpenter-Hyland et al., 2004; Floyd et al., 2003; Gulya et al., 1991; Snell et al., 1996). Overall the excessive activation of NMDA receptors could be a major contributor to the neuropathology of alcohol withdrawal.
METABOLIC CHANGES FOLLOWING ALCOHOL WITHDRAWAL

Alcohol intoxication decreases local cerebral glucose utilization (LCGU) in many areas of the brain, including the limbic system, cerebellum, and motor system (Eckardt et al., 1992). The most striking effects on LCGU are observed in the IC (Grunwald et al., 1993), in accordance with other evidence indicating that this brain site is a major target of alcohol. Alcohol withdrawal is associated with increased brain glucose uptake, oxygen consumption, and blood flow (Eckardt et al., 1992; Hemmingsen et al., 1979; Newman et al., 1985). Significant increases in LCGU with alcohol withdrawal have been reported in motor systems, the auditory system (including IC), and the mammillary bodies-anterior thalamic-cingulate cortex pathway (Eckardt et al., 1992), although one study reported decreases with acute withdrawal in most limbic regions and no changes in cerebellum and subcortical structures (Clemmesen et al., 1988).

Animals that had experienced spontaneous withdrawal seizures exhibited relatively greater reductions in glucose utilization in the amygdala, whereas those with previous withdrawal-related audiogenic seizures had greater reductions in the auditory system, including the IC.

GENETICS OF ALCOHOL WITHDRAWAL SEIZURES

Alcohol-associated seizures tend to run in families. For example, it has been reported that the incidence of seizures in first-degree relatives of individuals who experienced alcohol associated seizures is 2.45-fold that of unaffected individuals, whereas no excess incidence was observed in family members of persons experiencing posttraumatic seizures (Schaumann et al., 1994). An increased incidence of seizures in relatives was found whether the proband had alcohol-related seizures (spontaneously occurring seizures in association with chronic alcohol abuse) or alcohol withdrawal seizures. Thus some people without a history of epilepsy may have inherited genetic susceptibilities that make seizures more likely to occur in the setting of alcohol abuse.

One gene implicated in susceptibility to alcohol withdrawal seizures is the dopamine transporter (DAT) gene (SLC6A3), which has a variable-number tandem repeat (VNTR) in exon 15 encoding the 3' untranslated region of its messenger RNA (mRNA) (Gorwood et al., 2003; Vandenbergh et al., 1992). In a study of alcoholics who reported histories of withdrawal seizures or delirium, Sander et al. (1997) found an increased prevalence of the nine-repeat (A9) allele compared with nonalcoholic controls. Two issues must be considered in assessing the significance of this finding. First, the presence of the marker does not influence coding of the DAT protein and is therefore unlikely to have functional relevance itself. However, it has been noted that the repeat is close to the coding region and could be in linkage disequilibrium with a vulnerability-causing mutation that alters gene expression or protein structure. Precisely how alterations in DAT would influence seizure susceptibility is unknown. Alternatively the marker could be linked to other susceptibility alleles within a haplotype. The second issue is that the candidate gene approach, such as used in the Sander et al. study, is inherently susceptible to false positives. False associations can occur because of the large number of potential candidates and the low a priori probability that any particular candidate is a true susceptibility gene. Moreover, there is evidence of ethnic stratification of the DAT VNTR alleles so that uncontrolled differences in ethnicity between the affected and control populations could increase the risk of false positives, particularly in a study with a small sample size as was the case in Sander and colleagues (Uhl, 2004).

Although the association of the DAT A9 allele with susceptibility to alcohol withdrawal seizures in humans requires confirmation, studies in animals do indicate the existence of inherited factors that predispose to the symptoms of alcohol withdrawal, including seizures. Gor-Maslak et al. (1991) concluded that an aggregate set of genetic markers accounts for up to 62% of the variability in withdrawal syndrome severity. However, candidate gene studies have been disappointing, with no consistent results linking genes for serotonin, y-aminobutyric acid (GABA), endorphin, and dopamine receptors and the serotonin transporter with susceptibility to the withdrawal syndrome (Schmidt and Sander, 2000). The available evidence suggests that multiple genes are involved in various components of the syndrome, each of them contributing only modestly to withdrawal vulnerability. Nevertheless, in the mouse, there is good evidence that allelic variation in Mpdz influences the liability of alcohol withdrawal seizures (Fehr et al., 2004). Mpdz encodes the multiple PDZ domain protein (MPDZ). Mpdz haplotypes in standard mouse strains encode the three distinct protein variants MPDZ1-3. Recently it was reported that MPDZ status cosegregates with withdrawal convulsion severity in lines of mice selectively bred for phenotypic differences in severity of acute withdrawal from alcohol. MPDZ1 strains have significantly less severe acute alcohol withdrawal seizures than strains that express MPDZ2 or the closely related MPDZ3. Severity of pentobarbital withdrawal seizures is similarly correlated with MPDZ status (Fehr et al., 2002). Many ion channels and transporters possess PDZ-binding domains that are important in their trafficking and targeting to synapses. Although the MPDZ allelic variants could participate in such targeting, their precise functional roles are poorly understood and the differences among the variants that
would confer differences in seizure susceptibility have not yet been defined.

CELLULAR AND MOLECULAR MECHANISMS

Although alcohol is the most widely used psychoactive agent, the pharmacologic basis of its intoxicating effects is incompletely understood. Similarly the molecular mechanisms underlying alcohol dependence and withdrawal are obscure. Nevertheless, it is well recognized that alcohol affects the functional activity of many receptors and ion channels, including NMDA (Lovinger et al., 1989, 1990), kainate (Carta et al., 2003), serotonin (Lovinger and White, 1991), GABA_A (Davies, 2003) and glycine (Mihic et al., 1997) receptors, and G protein-coupled inwardly rectifying potassium channels (Kobayashi et al., 1999) and calcium channels (Walter and Messing, 1999). In most cases, the effects of alcohol on these targets occur at high concentrations. However, the effects of alcohol on certain GABA_A receptor isoforms occur with concentrations within the intoxicating range. Acute alcohol potentiates these GABA_A receptor isoforms and therefore enhances GABA-mediated inhibition through allosteric modulation of the receptors. Recent evidence indicates that GABA_A receptors containing δ-subunits are preferentially affected (Wei et al., 2004). Such δ-subunit-containing receptors appear to be located extrasynaptically, where they sense ambient GABA in the extrasynaptic environment and mediate tonic inhibition. The role of GABA_A receptor-mediated tonic inhibition is not fully defined. However, the extrasynaptic δ-containing receptors that are responsible for tonic inhibition appear to be an important target of alcohol at intoxicating concentrations (Hanchar et al., 2004). It is interesting to speculate that these extrasynaptic receptors may be activated by spillover of GABA when GABAergic interneurons are intensely activated, such as occurs during a seizure discharge. The ability of alcohol to potentiate extrasynaptic GABA receptors could therefore contribute to the anticonvulsant activity of ethanol, including its protective activity against alcohol withdrawal seizures.

Alcohol tolerance and dependence have been linked to changes in the function of GABA_A receptors that are possibly related to alterations in subunit assembly (Devaux et al., 1997; Kang et al., 1996, 1998; Mahmoudi et al., 1997; Matthews et al., 1998; Mhatre et al., 1993; Morrow et al., 1990). In addition to effects on GABA_A receptor isoforms that are located postsynaptically, there is emerging evidence that alcohol also enhances GABA-mediated inhibition via a presynaptic mechanism that involves GABA release from interneurons (Carta et al., 2004). It is hypothesized that there is a compensatory downregulation of GABA_A receptors during chronic exposure to alcohol. When alcohol is withdrawn and its potentiating effects are no longer present, the reduced functional activity of GABA_A receptors would predispose to seizures. Indeed a loss of GABA-mediated inhibition has been associated with enhanced susceptibility to alcohol withdrawal seizures (Faingold et al., 2000; N’Gouemo et al., 1996).

Alcohol exposure can also influence excitatory neurotransmission (Dodd et al., 2000). Acute alcohol exposure inhibits NMDA and kainate receptors, whereas chronic alcohol exposure results in upregulation of these receptors (Carta et al., 2002; Costa et al., 2000; Lovinger et al., 1990; Peoples et al., 1997). The ability of ethanol to inhibit NMDA responses appears to be dependent on NR1/NR2A and NR1/NR2B NMDA receptor isoforms (Wirkner et al., 1999). Abrupt cessation of alcohol exposure may result in brain hyperexcitability because inhibition of the upregulated NMDA and kainate receptors is uncovered (Whittington et al., 1995). There is evidence of upregulation of the NR1 and NR2A subunits of the NMDA receptor (Gulya et al., 1991; Snell et al., 1996; Trevisan et al., 1994) and the GluR6/7 kainate receptors (Carta et al., 2002).

Alcohol also produces important effects on ion channels mediating intrinsic neuronal excitability, particularly calcium channels. Electrophysiologic studies suggest that increased neuronal firing is critically important for alcohol withdrawal seizures (Chakravarty and Faingold, 1998). However, the shape of action potentials is not significantly altered in brain neurons following alcohol withdrawal (Evans et al., 2000; Yang et al., 2002), indicating that there are not likely to be changes in the voltage-gated sodium and potassium channels that are responsible for action potentials. On the other hand, there is some evidence that ethanol selectively inhibits N-, P-, and Q-type calcium channels that mediate neurotransmitter release (Maldve et al., 2004; Newton et al., 2004). Moreover, increased L- and P-type calcium channel-current density has been observed following alcohol withdrawal associated with enhanced seizure susceptibility (N’Gouemo and Morad, 2003; Perez-Velazquez et al., 1994).

As previously noted, neurons within the central nucleus of the IC represent an important target of alcohol and seem to play a critical role in the initiation of alcohol withdrawal-related AGS. Although it is plausible that alcohol withdrawal could be associated with changes in synaptic function and intrinsic excitability within this subnucleus (Evans et al., 2000; Yang et al., 2002), experimental support is not yet available.

TESTING PHARMACOLOGICAL AGENTS IN ANIMAL MODELS OF ALCOHOL WITHDRAWAL SEIZURES

Because alcohol withdrawal-related AGS and HIC can be elicited at the will of the experimenter during a defined
period after cessation of alcohol intake, the anticonvulsant properties of pharmacologic agents can be easily studied (unlike the situation with spontaneous seizure models, where seizures occur unpredictably, requiring sophisticated monitoring systems and less robust trial designs). A limitation of these models is that AGS or HIC do not occur in every animal. However, the incidence (and severity) of AGS can be increased with intermittent ethanol administration (C.L. Faingold, personal communication). The peak incidence of AGS occurs at about 24 hours following alcohol withdrawal. Thus pharmacologic agents are typically tested between hour 20 and 28 following alcohol withdrawal, which encompasses a period of high seizure likelihood. Pharmacologic substances are typically administered 30 to 60 minutes before the time at which the AGS is elicited, but the choice of interval for any specific substance must be based on its pharmacokinetic characteristics. If the time of peak blood-brain levels is not known, a time course study should be carried out.

Table 4 compares the potencies of various anticonvulsant substances for protection against alcohol withdrawal seizures (AGS in rats and HIC in mice) and in conventional seizure models used for the evaluation of antiepileptic drugs (White et al., 2002). The values in the diverse models are not directly comparable because of significant experimental differences. However, it is apparent that there is a rough concordance in the effectiveness of substances for protection against seizures in the alcohol withdrawal and non-alcohol–related models, with the possible exception of the sodium channel–blocking anticonvulsant drugs carbamazepine and phenytoin (Rogawski and Lüscher, 2004), which may be less effective against alcohol withdrawal seizures than in conventional seizure models. This corresponds with the lack of effectiveness of these drugs for many forms of generalized seizures (although the drugs are generally believed to be useful for generalized tonic-clonic seizures).

### SEIZURES RELATED TO WITHDRAWAL OF OTHER CENTRAL NERVOUS SYSTEM DEPRESSANTS

Drugs that increase GABA,–mediated inhibition (e.g., benzodiazepines, barbiturates) are commonly prescribed for their anxiolytic, sedative, hypnotic, muscle relaxant, and anticonvulsant properties. Prolonged administration of benzodiazepines and barbiturates can result in tolerance and physical dependence. The spectrum of behavioral signs and symptoms that occur following withdrawal from barbiturates and benzodiazepines—including hyperexcitability, tremors, and seizures—is similar to that occurring following alcohol withdrawal (Busto et al., 1986; Hillbom et al., 2003). In addition, genetic characteristics that influence susceptibility to alcohol withdrawal also affect susceptibility to barbiturate and benzodiazepine withdrawal (Metten and Crabbe, 1994, 1999). Furthermore, there is cross-tolerance among benzodiazepines, ethanol, and barbiturates, and ben-

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**TABLE 4 Comparison of the Potencies of Anticonvulsant Substances for Protection Against Alcohol Withdrawal Seizures and in Conventional Seizure Models**

<table>
<thead>
<tr>
<th>Substance</th>
<th>AGS (rat)</th>
<th>HIC (mice)</th>
<th>AGS (mice)</th>
<th>PTZ (mice)</th>
<th>MES (mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>150'</td>
<td>NE^b</td>
<td>11.2'</td>
<td>&gt;50'</td>
<td>7.8'</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>−100'</td>
<td>20'</td>
<td>0.04–0.12'</td>
<td>0.27'</td>
<td>18.7'</td>
</tr>
<tr>
<td>Diazepam</td>
<td>NE^a</td>
<td>0.1'</td>
<td>0.047'</td>
<td>1.17'</td>
<td>0.95'</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>−50 (mice)^a</td>
<td>91.1'</td>
<td>0.1'</td>
<td>47.5'</td>
<td>78.2'</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>50'</td>
<td>NE^a</td>
<td>3.9'</td>
<td>&gt;50'</td>
<td>5.6'</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>300'</td>
<td>300'</td>
<td>55–300'</td>
<td>220'</td>
<td>263'</td>
</tr>
</tbody>
</table>

ED50, median effective dose; HIC, handling-induced convulsions; PTZ, pentylentetrazol seizure test; MES, maximal electroshock seizure test; NE, not effective. Conventional AGS testing is performed in strains of mice that are genetically susceptible to AGS, mainly DBA/2 but also Frings.

^aChu, 1979; ^bGrant et al., 1992; ^cWhite et al., 2002; ^dGreen et al., 1990; ^eOgren, 1986; ^fPilip et al., 1998; ^gLittle et al., 1986; ^hCrabbe, 1992; ^iChapman et al., 1984; ^jSwinyard and Castellion, 1996; ^kMorrisett et al., 1990; ^lGrant et al., 1982; ^mChapman et al., 1989; ^nRogawski et al., 1991; ^oWatson et al., 1997; ^pChu et al., 1981; ^qGessner, 1974; ^rGoldstein, 1979.
zodiazepines and barbiturates can protect against ethanol withdrawal convulsions in humans and rodents (see section on Relevance of Alcohol Withdrawal Seizures in Rodents to the Human Condition for further discussion of benzodiazepines in alcohol withdrawal). These various factors suggest that similar underlying mechanisms mediate the withdrawal syndromes that occur with the different CNS depressants (Kliethermes et al., 2000).

Handling-induced convulsions have been used extensively to study the relationship between seizures that occur on withdrawal of alcohol and other CNS depressants such as benzodiazepines, barbiturates, and also inhalation anesthetics. However, pharmacokinetic factors may require alterations in the specific procedures that are used with certain agents. For example, because of its long half-life, diazepam does not produce the waxing and waning pattern of HIC exacerbation after injection that occurs with alcohol withdrawal as discussed previously and illustrated in Figure 2 (Crabbe, 1992). However, injection of the benzodiazepine receptor antagonist flumazenil precipitates a brief, relatively intense withdrawal reaction that lasts several minutes after the injection (Metten and Crabbe, 1994, 1999). In genetic studies in mice, a single dose of diazepam (20 mg/kg) is administered, followed by flumazenil (10 mg/kg) at an interval of 60 minutes. Withdrawal HICs are scored 1, 3, 5, 8, and 12 minutes later. In contrast to diazepam, zolpidem, a benzodiazepine that is selective for GABA, receptors containing α1 subunits, does not require precipitation by an antagonist (Metten et al., 1998). Withdrawal from barbiturates, such as pentobarbital, is also associated with potentiation of HICs. In studies of pentobarbital withdrawal for genetic studies, HICs are assessed at hourly intervals from 1 to 8 hours following injection (Metten and Crabbe, 1994). Nitrous oxide withdrawal is similarly associated with potentiation of HICs (Belknap et al., 1993). In a typical paradigm used for genetic studies, mice are exposed to a mixture of 75% nitrous oxide and 25% oxygen for 1 hour in an inhalation chamber and then returned to room air. HICs are assessed at baseline; immediately on removal from the inhalation chamber; and 5, 10, 15, 20, 40, and 60 minutes later (Metten et al., 1998).

In addition to withdrawal of alcohol and GABA-potentiating drugs, seizures can also be induced by withdrawal of GABA administered locally in susceptible brain regions, including the cerebral cortex, amygdala, hippocampus, or IC (Brailowsky et al., 1988; Yang et al., 2001b). GABA solutions have been delivered through an indwelling catheter using a subcutaneously implanted osmotic minipump. In experiments examining IC infusion in rats, 1M GABA is delivered bilaterally at the rate of 0.25 μl per hour for 7 days (Yang et al., 2001b). Thirty minutes following abrupt cessation of the GABA infusion, animals exhibited spontaneous seizures (17% of rats tested) and a susceptibility to AGS (39% of rats) that persisted in some animals for as long as 6 months. The sound-induced behaviors following GABA withdrawal consisted of wild running and bouncing clonus, which resemble seizures observed after ethanol withdrawal.

**RELEVANCE OF ALCOHOL WITHDRAWAL SEIZURES IN RODENTS TO THE HUMAN CONDITION**

Although alcohol withdrawal seizures in rodents do not represent a perfect model of human alcohol withdrawal seizures, the available evidence indicates that the animal models are valid in many respects. As noted, most alcohol withdrawal seizures in humans are generalized tonic-clonic seizures. Similarly, the various forms of alcohol withdrawal seizures in rodents represent generalized convulsions. In both humans and rodents, the peak incidence of alcohol withdrawal-related generalized seizures occurs between 20 to 24 hours following cessation of alcohol intake. In addition to exhibiting shared behavioral features, the brain systems underlying alcohol withdrawal seizures in humans and rodents are likely to be similar across species. There is no cortical paroxysmal activity in the electroencephalogram during auditory-evoked tonic-clonic alcohol withdrawal seizures in rodents (Hunter et al., 1973; Maxson and Sze, 1976). Epileptiform activity is also rare in the electroencephalogram recorded between episodes of alcohol withdrawal tonic-clonic seizures in humans (Sand et al., 2002; Touchon et al., 1981). The lack of cortical epileptic activity interictally during alcohol withdrawal suggests that the withdrawal seizures may not be initiated by cortical hyperexcitability but instead result from the abnormal function of subcortical neuronal networks that eventually trigger seizure discharges in the cortex. One neuronal network of interest is the brainstem auditory pathway, which has been implicated in rodent AGS (see previous discussion). Indeed significant abnormalities in auditory-evoked potentials have been reported in humans suffering from alcohol withdrawal seizures, including increased latency to wave V, which is unique to individuals suffering from alcohol withdrawal seizures (Neiman et al., 1991; Touchon et al., 1984). IC neurons are the major source of wave V in brainstem auditory-evoked potentials (Hughes and Fino, 1985), suggesting that abnormalities in the function of IC neurons can contribute to the genesis of alcohol withdrawal seizures in humans, as is believed to be the case in rodents. Indeed IC neurons are not only a component of the neuronal network for alcohol withdrawal seizures, but they are also believed to play an important role in other models of epilepsy and are considered a critical site for the genesis of tonic-clonic seizures whatever the underlying etiology (Faingold, 1999).
Neuronal plasticity mechanisms may play a role in the susceptibility to alcohol withdrawal seizures in humans and rodents. In humans the number of detoxifications, not the absolute amount of alcohol intake, best predicts the likelihood of subsequent alcohol withdrawal seizures (Ballenger and Post, 1978). Similarly studies in rodents have shown that repeated alcohol withdrawal experiences increase the severity and duration of subsequent withdrawal seizures. For example, this was the case in the study of Becker and Hale (1993) in which adult male mice were chronically exposed to ethanol vapor by inhalation. Animals in a multiple withdrawal group experienced three 16-hour exposure periods separated by 8-hour periods of abstinence; a single withdrawal group received a single 16-hour bout of ethanol exposure. The severity of HIC was significantly greater in the multiple withdrawal group than in the single withdrawal group. In additional studies, mice experiencing multiple withdrawal episodes were found to have greater susceptibility to chemoconvulsant-induced seizures (Becker et al., 1998). Furthermore, in rats, multiple withdrawal episodes from chronic alcohol treatment facilitate the rate of the development of IC kindling while at the same time inhibiting the evolution of amygdala and hippocampal kindling (Gonzalez et al., 2001; McCown and Breese, 1990). This observation provides further support for the concept that brainstem systems encompassing the IC are critical to the initiation of alcohol withdrawal seizures, whereas the forebrain mechanisms mediating “limbic” seizures (the equivalent of complex partial seizures in humans) do not play a major role, at least in triggering these seizures. This conclusion is consistent with observations from studies of cerebral glucose metabolism (see previous section entitled Metabolic Changes Following Alcohol Withdrawal). In chronic alcohol abusers, it seems likely that kindling-like effects of multiple detoxifications lead to hyperexcitability in IC neurons, which further predispose to withdrawal seizures (Duka et al., 2004).

Overall the various lines of evidence discussed in this section support the view that the neural mechanisms mediating alcohol-withdrawal tonic-clonic seizures in humans and rodents are similar. Do the animal models represent appropriate test systems for the evaluation of agents useful in the treatment of alcohol withdrawal seizures in humans? The available data suggest that the models can be applied for identification of agents useful in preventing alcohol withdrawal seizures, but there could be limitations, as highlighted by what appears to be poor concordance between the efficacy of benzodiazepines in the models and their use in clinical practice. In the United States, benzodiazepines are considered the drugs of choice to treat alcohol withdrawal and to prevent the occurrence of seizures (D’Onofrio et al., 1999; Mayo-Smith, 1977). In Europe, carbamazepine, chlormethiazole, and valproate are often used. Benzodiazepines have been shown to be protective in some animal models of alcohol withdrawal seizures (Becker and Veatch, 2002; Mhatre et al., 2001), although they may not exhibit high potency (see Table 4). In fact, benzodiazepines generally have low potency in models of tonic seizures, such as the maximal electroshock test (see Table 4). In animal models, benzodiazepines are modestly effective in preventing the increased withdrawal severity that occurs with repeated withdrawals (Ulrichsen et al., 1995), although the drugs can also produce a paradoxical worsening (Becker and Veatch, 2002), and not all studies have yielded positive results (Mhatre et al., 2001), indicating that caution is warranted in using benzodiazepines for alcohol detoxification. Alcohol withdrawal has been associated with alterations in the subunit composition of GABA\textsubscript{A} receptors, including an increase in the expression of the \( \alpha \)4 subunit that confers benzodiazepine insensitivity (Cagetti et al., 2003; Devaud et al., 1997; Sanna et al., 2003). Clinical experience demonstrates that benzodiazepines do reduce the risk of recurrent seizures in patients who present with an alcohol withdrawal seizure (D’Onofrio et al., 1999), so that in practice there is not complete benzodiazepine resistance. However, GABA\textsubscript{A} receptor modulators other than benzodiazepines that would not be expected to lose activity might be superior therapeutic agents. In fact, chlormethiazole is a positive modulator of GABA\textsubscript{A} receptors, which, in contrast to benzodiazepines, has high efficacy in enhancing GABA\textsubscript{A} receptors containing \( \alpha \)4 subunits (Usala et al., 2003). Chlormethiazole has been shown to protect transiently against alcohol withdrawal seizures in mice withdrawn from exposure to inhaled ethanol (Green et al., 1990) and, in Central Europe, the drug represents the standard of care for the acute treatment of alcohol withdrawal (Majumdar, 1990; Morgan, 1995). It is interesting to speculate that chlormethiazole might be superior to benzodiazepines in the treatment of alcohol withdrawal as a result of its activity as a modulator of benzodiazepine-insensitive GABA\textsubscript{A} receptor isoforms.

Carbamazepine may decrease the craving for alcohol after withdrawal, but there is little evidence that it prevents seizures and delirium. In fact, carbamazepine was inactive in blocking alcohol withdrawal-related HIC in mice (Grant et al., 1992), and only very high doses were able to suppress withdrawal-related AGS in rats (Chu, 1979). Interestingly, in humans, phenytoin is not effective in protecting against the recurrence of alcohol withdrawal seizures (Rathlev et al., 1994). The animal model therefore shows a good correspondence with clinical experience. Valproate also has some protective activity against alcohol withdrawal-related HIC in mice (Goldstein, 1979), and topiramate may also protect against enhanced seizure susceptibility in ethanol-dependent rats (Cagetti et al., 2004). There is increasing interest in the potential of gabapentin as a treatment for alcohol withdrawal, inasmuch as encouraging results have been produced in several small clinical studies (Bonnet et al., 1999; Bozikas et al., 2002; Myrick et al., 1998; Rustembegovic et
CONCLUSIONS

It is estimated that two million Americans experience the symptoms of alcohol withdrawal each year (Bayard et al., 2004). Generalized tonic-clonic seizures are the most dramatic and dangerous component of the syndrome. In this chapter we have reviewed rodent models of alcohol withdrawal seizures that are commonly used for mechanistic and genetic studies and that can also be applied in the identification of new treatment approaches. In each of these models, withdrawal from alcohol, administered either chronically or in some instances acutely, leads to enhanced seizure susceptibility and occasionally spontaneous seizures. Interestingly the brain substrates that trigger these seizures are largely distinct from those responsible for other clinically important seizure types, and it is likely that the pathophysiologic mechanisms are different. Therefore it is not surprising that pharmacologic agents effective in other seizure types may not be effective in the treatment of alcohol withdrawal seizures. The alcohol withdrawal models provide unique opportunities to gain insights into the specific cellular mechanisms underlying this distinctive seizure syndrome. They also provide opportunities to optimize the therapy of alcohol withdrawal seizures. Indeed newer agents such as clormethiazole, gabapentin, or valproate, which are effective in the models, are gaining acceptance clinically. NMDA receptor antagonists are especially active in animal models of alcohol withdrawal seizures, in accordance with the substantial evidence that alterations in NMDA receptor function play a key pathophysiologic role; whether such agents will have a role in clinical practice will require further study. An important challenge is to develop strategies to interdict the development of enhanced seizure susceptibility that occurs with multiple episodes of detoxification. Determining whether NMDA receptor antagonists or other pharmacologic approaches have such antiepileptogenic actions in repeated episodes of withdrawal will represent an important future application of the animal models.

References


