

Ethanol Withdrawal and Hyperalgesia

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Abstract: Hyperalgesia has been observed during ethanol withdrawal, comparable to the hyperalgesia observed during withdrawal from opioids. To determine the extent of this phenomenon and its potential mechanisms, both behavioral and *in vitro* studies are examined, and the roles of GABA_A, glutamate and other receptors in mediating the acute and chronic antinociceptive effects of ethanol are reviewed. Hyperalgesia during ethanol withdrawal is a robust phenomenon that has been observed in various strains of mice and rats, with different methods of exposure to ethanol, and with a variety of nociceptive assays. GABA receptors play an important role in mediating the antinociceptive effects of ethanol, but too little research has examined the role of glutamate receptors to make any conclusion about their importance. Adenosine receptors, calcium channels, and protein kinase C appear to play central roles in mediating tolerance to antinociceptive effects of ethanol and mediating the hyperalgesia seen during withdrawal. Although some key pathways have been identified, further mechanistic work is necessary to fully characterize the mechanisms for the development of hyperalgesia following chronic exposure to ethanol. An understanding of how the hyperalgesia may fit in with other manifestations of ethanol withdrawal may be an important variable in determining treatment outcome. Clinical research is essential to determine the significance of the hyperalgesia to the severity of withdrawal and to relapse.

Keywords: Chronic ethanol exposure, tolerance, withdrawal, hyperalgesia, GABA receptor, NMDA receptor, adenosine receptors, calcium channels.

INTRODUCTION

Seizures, agitation, hyperreactivity, and anxiety have long been considered hallmark signs of ethanol withdrawal. More recently, hyperalgesia has become a recognized sign of alcohol withdrawal [1]. Although hyperalgesia during opioid withdrawal has been well characterized, hyperalgesia during withdrawal from ethanol and other compounds has only been recently studied. An increasing number of studies have examined hyperalgesia during withdrawal, so it is of interest to address whether withdrawal hyperalgesia is a minor, isolated phenomenon or whether it widely occurs during withdrawal from ethanol and other drugs of abuse. The purpose of this review is to summarize the current literature on the hyperalgesia seen during ethanol withdrawal, to draw conclusions regarding its prevalence and mechanisms, and to identify empirical questions which need testing in order to make valid conclusions about the importance of hyperalgesia to the severity of withdrawal and likelihood of relapse.

There are a number of different methods for assessing antinociception and hyperalgesia, so it is of interest to establish whether hyperalgesia is observed using more than one method. Nociceptive tests can involve different sensory modalities, such as temperature (heat or cold), blunt or acute pressure, or chemical substances. Some tests assess responses to acute high intensity stimuli, whereas other tests assess responses to chronic/inflammatory events. Some of the nociceptive responses are mediated by spinal reflex, others supraspinally. Three of the most common tests are the various tail- or paw-withdrawal assays, the hot plate assay,

and the formalin test. The tail-flick assay is a spinally-mediated test of acute, high intensity nociception. The tail or paw of the test subject is placed into a beam of intense light, hot (or cold) water, or increasing pressure. Latency to remove the limb from the stimulus is the primary measurement. The hot plate assay is a supraspinally-mediated test of acute, high intensity nociception. In this test, the subject is placed on a hot surface and latency to lick paws or vocalize is measured. The formalin test measures nociceptive responses produced by inflammation rather than by acute stimuli. Formalin, a pro-inflammatory compound, is injected into paw and nociceptive responses are scored over time.

The earliest studies of ethanol-withdrawal hyperalgesia used the tail-flick assay in male rats [2, 3]. A major question regarding the significance of these findings is whether they can be extended to other, non-thermal, non-spinal assays, to other species, and most importantly, to human alcohol abusers. Once it has been determined that hyperalgesia is an important component of withdrawal, it becomes important to determine the mechanisms that produce the hyperalgesia, what they can tell us about withdrawal in general, and how the hyperalgesia can be treated during ethanol withdrawal treatment. Before we can examine withdrawal in depth, we need to understand the effects of ethanol when administered both acutely and chronically. The first sections of this review will look at the antinociceptive effects of acute and chronic doses of ethanol. The following sections will then examine potential mechanisms for ethanol-withdrawal hyperalgesia.

ACUTE ANTINOCICEPTIVE EFFECTS OF ETHANOL

Description

Acute administration of ethanol produced a modest degree of antinociception in a number of assays, such as tail-

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deflection [4], tail-flick [3, 5-7], foot-shock [8], the formalin test [9], and the hot plate [7, 10]. Ethanol does not produce enough antinociception to be a useful analgesic. However, it does produce a large enough effect to reverse the hyperalgesia produced by withdrawal [2, 3, 11], and this reversal may serve as negative reinforcement for further drinking. Receptors that may mediate the antinociceptive effects of ethanol include GABA_A and NMDA glutamate receptors, which are well known to mediate many of the effects of ethanol. Other possibilities include major receptors involved in pain systems, including receptors for opioids and biogenic amines such as dopamine, norepinephrine, serotonin, and adenosine.

Mechanisms

GABA_A receptors. The antinociceptive effects of ethanol are blocked by the GABA_A receptor benzodiazepine site antagonist flumazenil [2], and ethanol enhanced the antinociceptive effects of benzodiazepines but not of barbiturates [12, 13]. From these data, it might be assumed that GABA_A receptors (or at least the benzodiazepine site) play an important role in mediation of the antinociceptive effects of ethanol. However, diazepam (benzodiazepine site agonist) produced no effect in the tail-flick assay, and a large dose of diazepam (10 mg/kg) in combination with ethanol produced no change in the ethanol dose-effect curve [2]. These findings suggest that activation of the benzodiazepine site may not be necessary for ethanol to produce antinociception.

NMDA receptors. To date, there has been only one study of the role of NMDA receptors in the antinociceptive effects of ethanol. The antinociceptive effects of ethanol in the hotplate test were partially blocked by the NMDA channel blocker dizocilpine [10]. Naloxone (opioid antagonist) also partially blocked the antinociceptive effects of ethanol, and dizocilpine together with naloxone fully blocked ethanol antinociception. The result is surprising, as ethanol also blocks NMDA receptors [14], and dizocilpine typically produces similar effects to ethanol [14, 15]. The study also reported that mice bred for high levels of stress-induced analgesia showed higher levels of antinociception following ethanol administration, and mice bred for low levels of stress-induced analgesia showed little antinociception following ethanol. Naloxone and dizocilpine produced similar effects in both the wild-type and high-stress mice.

Dizocilpine produced antinociceptive effects in spinally-mediated assays such as tail-flick, but little or no antinociception in supraspinal assays such as hotplate [16-18], whereas ethanol produced antinociception in both types of assays [7, 10]. It is possible that dizocilpine would block the antinociceptive effects of ethanol in supraspinally-mediated assays, but not in spinally-mediated assays. Another possibility is that dizocilpine produces different effects at different doses. Dizocilpine does produce a biphasic dose-effect curve for its locomotor activity effects [19]. Given that only a single, small dose of dizocilpine was tested in the hotplate test [10], it is possible that testing the full dose-effect range of dizocilpine could reveal different interaction effects with ethanol at different doses.

Opioid receptors. The effects of opioid antagonists on ethanol-induced analgesia are mixed. One published study reported that naloxone had no effect on ethanol-induced antinociception [6], which agrees with our own findings with

naltrexone (unpublished data). Other studies have reported that naloxone partially reversed ethanol-induced antinociception [4, 7, 10]. Naloxone clearly produced a partial reversal of ethanol's antinociceptive effects in the hotplate test [7, 10], which is a supraspinally-mediated assay. A very small or no reversal of the antinociceptive effects of ethanol was observed in the tail-flick and tail-deflection assays [4, 6], which are spinally-mediated assays. These findings suggest that opioid receptors may contribute to the supraspinal effects of ethanol, but not to the spinal effect.

The one finding that does not fit with this hypothesis is the tail-flick data from the Campbell study, which contains a number of incongruent findings when compared to the other tail-flick studies. Greater than 90% mean peak effect was reported following ethanol (2 g/kg, ip) using a high intensity thermal stimulus (2-4 s baseline tail-flick latency) [7], yet other studies using the same dose of ethanol reported only 60-70% mean peak effect using a low intensity setting (6-8 s baseline latency) [3, 6], and no antinociception was observed following ethanol administration at the high intensity setting [3]. In addition, the blockade of ethanol antinociception was not dose-dependent [7], which suggests non-pharmacological factors may have been operative. Careful research will be necessary to resolve the very different observations between these experiments. In support of a role for opioid receptors in ethanol-induced antinociception, exposure to the cold-water swim potentiated the antinociceptive effects of ethanol [20]. This finding is similar to the observation that mice bred for high swim-stress analgesia show enhanced amounts of ethanol antinociception [10]. Taken together, these findings suggest that opioid receptors contribute at least to the supraspinal aspects of the antinociceptive effects of ethanol.

Adenosine receptors. Adenosine receptors are important contributors to antinociception [21, 22]. Adenosine receptor agonists are highly efficacious analgesics and antagonists produce hyperalgesia [22, 23]. However, a non-hyperalgesic dose of theophylline (non-selective adenosine receptor antagonist) which fully blocked the antinociception produced by 2-CADO (non-selective adenosine agonist) did not block the antinociceptive effects of ethanol [11, 24], which suggests that adenosine receptors do not mediate the antinociceptive effects of acute doses of ethanol.

Summary of acute effects. The mechanism for the antinociceptive effects of acute doses of ethanol has not been well characterized. This is not surprising, as ethanol is not known for possessing major analgesic properties, and indeed produces only small antinociceptive effects at doses that produce significant intoxication. However, understanding the antinociceptive effects of acute doses of ethanol will help us to better understand the antinociceptive mechanisms of chronic ethanol and the development of tolerance. GABA_A and NMDA receptors likely both contribute to the antinociceptive effects of ethanol, although the relative roles of these two receptors are not clear. Very little research has examined the role of NMDA receptors, which is surprising, given its well-known role in mediating many of the effects of ethanol. Opioid receptors may be important in mediating supraspinal effects of ethanol. To date, the effects of dopamine, norepinephrine and serotonin receptors, all of which are important mediators of pain sensation [21], on ethanol-induced antinociception have not been investigated.

ANTINOCICEPTION DURING CHRONIC EXPOSURE TO ETHANOL

Description

Chronic administration of ethanol produces antinociception followed by the formation of tolerance to its antinociceptive effects [2, 3, 25-27]. The antinociceptive effects are small and last a few days, typically peaking within 2 to 4 days. After this point, continued administration of ethanol results in decreasing levels of antinociception, and by days 8-10, nociceptive responses return to baseline [2, 3, 25, 26]. Very long exposure to ethanol (10 weeks) in male rats leads to neuropathy and resultant nociception [28]. These findings indicate that although initial exposure to ethanol produces modest relief of pain, continued exposure triggers mechanisms that continuously decrease the ability of ethanol to relieve pain and can eventually even increase sensitivity to painful stimuli. Whether these mechanisms are wholly subsumed by tolerance mechanisms, or whether other mechanisms also contribute (especially to the allodynia and neuropathy) remains to be fully characterized.

Mechanisms

Even less is known about the mechanism of the antinociceptive effects of chronically administered ethanol than about the effects of acute doses of ethanol. Unlike the antinociceptive effects of acute ethanol, the antinociceptive effects of chronic ethanol are of interest not only to their contribution to the abuse liability of ethanol, but also impact the effects of both prescribed medications and other abused compounds in those who abuse ethanol. Because of this, increasing our understanding of the mechanisms for the antinociceptive effects of ethanol alone as well as mechanisms by which it potentially interacts with the effects of opioids and other prescription drugs (whether used legally or not) is of critical importance. Unfortunately, there has been very little research in this important area.

Mechanisms of the antinociceptive effects of chronic doses of ethanol may or may not be related to those of acute doses of ethanol. Flumazenil, co-administered with chronic ethanol prevented the antinociceptive effects of ethanol [2]. This is not surprising as flumazenil blocked the antinociceptive effects of acute doses of ethanol [2]. However, the dihydropyridine L-type calcium channel blocker nitrendipine also blocked the antinociceptive effects of chronic ethanol when co-administered with the chronic ethanol [26]. These findings were surprising, as L-type calcium channel blockers increased the antinociceptive effects of acute doses of morphine (but not the mu-selective opioid agonist fentanyl) and nicotine, as well as the general anesthetic effects of ethanol [29-33], even though they typically produce little or no antinociception on their own [29, 30, 32, 34-36]. These findings suggest that the mechanism for the antinociceptive effects of acute doses of ethanol may not be the same as that for chronic ethanol, such that some factors may affect the antinociceptive effects of acute ethanol differently than those of chronic ethanol. For example, the differences seen in the effects of the calcium channel blockers may be due to ethanol-induced changes in density of the L-type calcium channels. This issue is discussed in more detail in the section on ethanol withdrawal.

HYPERALGESIA DURING ETHANOL WITHDRAWAL

During ethanol withdrawal, marked hyperalgesia is seen across species, ages and assays. The phenomenon is robust and occurs under a wide range of conditions. Preliminary data that hyperalgesia is also seen during ethanol withdrawal in humans has been reported [37]. Three major research areas have developed: hyperalgesia during ethanol withdrawal in adults, the effects of early exposure to ethanol using neonatal rats, and hyperexcitability of the pain pathways in spinal cord sections.

Characterization of Ethanol-Withdrawal Hyperalgesia

Adult rats and mice. The most common procedure has been to expose Long-Evans rats to an ethanol-containing liquid diet (6.5%) for 10 days and then to test hyperalgesia at various time points following withdrawal of the ethanol diet. Tail-flick hyperalgesia was seen by 3 hr, peaked between 6 to 12 hr, and was gone by 36 hr following withdrawal of the ethanol diet [3]. It should be noted that the effect size was incorrectly calculated in this first paper. Correct calculations reveal an effect size more than double of what was initially reported; from about 15% to nearly 40% of control [2, 3, 11, 24]. Similarly, an increased response to the formalin test was observed 12 hr after withdrawal in Wistar rats exposed to an ethanol-containing liquid diet for 10 days [25].

Variations of these procedures have also resulted in hyperalgesia. In one study, mice were exposed to 2 g/kg of ethanol intragastrically for 7 days, and hyperalgesia was observed 24 hr after the last exposure to ethanol [38]. Another study incorporated a binge model, exposing Sprague-Dawley rats to 6.5% ethanol in liquid diet in cycles of 4 days on ethanol and 3 days off, and testing hyperalgesia using the paw-pressure test [39]. Under these procedures, hyperalgesia developed slowly, not peaking until 48 hr after withdrawal, and lasted for days. The hyperalgesia became more intense each cycle for 4 cycles and then stabilized. Paw-withdrawal latencies did not return to baseline levels even after 2-3 weeks. Curiously, the hyperalgesia developed most rapidly following the lowest concentration of ethanol, although levels of hyperalgesia were comparable by 18 days. In contrast, when rats were administered three 10-day exposures to ethanol with 2 weeks between each exposure, no difference in hyperalgesia was seen over the three exposures (unpublished data). There are a number of procedural differences between these two studies, including strain of rat, hyperalgesia assay, number of days on ethanol, and number of days between exposures, each of which may contribute to the difference in the results. These findings are of interest, as ethanol is often consumed in binge patterns. Identifying the parameters that contribute to the differences in the degree and length of withdrawal hyperalgesia seen in the different binge patterns will be of clinical value.

Neonatal rats. In these studies, male and female Sprague-Dawley rats were given either one large dose of ethanol or repeated doses of ethanol for 4-5 days at various ages. Hyperalgesia was measured at a variety of times, up to several days after exposure. The different ages chosen reflect different interests. Post-natal days 1-6 are comparable to end of pregnancy and infancy in humans. Post-natal day 7 (P7) is comparable to the age of a full-term infant, so treatment days

of P4 to P7 are a model of the effects of 3rd trimester drinking on offspring. Weaning occurs after post-natal day 21, which then serves as a model of early toddler age [40]. Ethanol exposures are timed to these developmental critical periods, to characterize the changing effects of ethanol across these different periods.

A single large dose of ethanol on day P7 produced hyperalgesia to mechanical (Von Frey filaments) and thermal stimuli, and an enhanced response during Phase II of the formalin test [40, 41]. In contrast, these studies reported that a single large dose of ethanol on day P21 produced hyperalgesia to mechanical stimuli but not thermal stimuli, and an enhanced response was seen during the quiescent phase of the formalin test.

When ethanol was administered on days P3 to P7, hyperalgesia (paw withdrawal) was seen at 6 to 8 hours after withdrawal of ethanol and baseline returned to normal within 12 hours [41]. The rats were then administered formalin in one paw on day P11. Ethanol-exposed rats were much more sensitive to the formalin. In a separate study, rats exposed to ethanol on days P3 to P7 were not tested until day P20. There was no difference between ethanol and saline treated rats at the control measurement taken on P20, which indicates that there was no ethanol withdrawal hyperalgesia observed two weeks after the exposure to ethanol. This second study tested a model of post-operative pain in children exposed to ethanol in utero [42]. Rats were exposed to chronic ethanol from P4 to P7. An incision in a paw was made on P21. Paw withdrawal latencies were measured on P21 and then once a week for the next 3 weeks. Ethanol exposure did not alter the hyperalgesia produced by incision of the hind paw.

When ethanol was administered on days P9 to P13, hyperalgesia was seen with the tail-flick test on days P14 and P28 [43]. Similarly, ethanol exposure on days P17 to P21 resulted in prolonged hyperalgesia as measured by the paw withdrawal test. The effect lasted at least 12 hours and was gone by day P25 [41]. These rats were administered formalin on day P25. Rats exposed to ethanol at this age did not show an increased response to the formalin.

Reversal of withdrawal. Administration of ethanol during the withdrawal phase reverses the hyperalgesia [2, 3, 11]. This reduction in hyperalgesia (and other symptoms of withdrawal) may provide motivation to continue drinking. Of interest is the finding that although ethanol will reverse the hyperalgesia at doses that do not produce antinociception in non-dependent rats, large doses of ethanol produce no antinociception when administered during withdrawal [2, 3, 11]. This suggests that there is a profound tolerance to the antinociceptive effects of ethanol that lasts at least 12 hr after withdrawal of ethanol. How long this tolerance lasts has not been characterized. This finding also suggests that the mechanisms for the reversal of the hyperalgesia and for the antinociceptive effects are not the same. These mechanisms will be discussed in detail later in this paper.

In vitro studies. Hyperresponsiveness of motor neurons in rat spinal cord slices has been observed following exposure to ethanol [44, 45]. The slices were exposed to a high concentration of ethanol (100 mM) for 20 min. Withdrawal of the ethanol produced hyperexcitability as measured by

excitatory post-synaptic potentials for 20 min (peak at 10 min). This study did not directly test hyperalgesia; however, sensitization of spinal nociceptive pathways is observed during hyperalgesia induced by a number of procedures, including opioid withdrawal [46, 47]. These studies provide evidence that ethanol hyperalgesia is a robust and common phenomenon that has similarities to the hyperalgesia produced by withdrawal from opioids.

Summary. Hyperalgesia appears to be a phenomenon consistently observed during ethanol withdrawal, as it has been reported using several different assays in adult and juvenile rats, in mice, as well as in spinal cord slices. Further, the hyperalgesia appears to be of large enough magnitude that it could serve as motivation for relapse. The significance of the hyperalgesia is obvious during withdrawal in adults, but it may also be a significant and unrecognized factor in fetal alcohol syndrome. The findings in juvenile rats indicate that exposure to ethanol produces very different effects dependent upon the age when exposed. Prenatal exposure to ethanol produced increased sensitivity as early as day 11, but the increase in sensitivity was gone by day 20 [41]. In humans, this would correspond to increased pain sensitivity at birth, lasting through much of their first year or two of life. This may indicate that any hyperalgesia seen in fetal alcohol syndrome infants may last throughout infancy, however this awaits experimental confirmation.

Mechanisms

GABA_A receptors. As described above, the benzodiazepine site antagonist flumazenil fully blocked the antinociceptive effects of acute doses of ethanol in non-dependent rats [2]. When co-administered with chronic ethanol, flumazenil blocked the antinociception seen during the first days of ethanol exposure and also prevented ethanol-withdrawal hyperalgesia [2]. The classical benzodiazepine diazepam, when administered during ethanol withdrawal, reversed the withdrawal-induced hyperalgesia [2]. However, flumazenil failed to block the ability of ethanol or diazepam to reverse ethanol withdrawal hyperalgesia [2, 11]. This finding questions the role of GABA_A receptors in the hyperalgesia, particularly as a fairly high dose of diazepam (3.2 mg/kg) was required to reverse the hyperalgesia [2]. Typically, smaller doses of analgesics are required to reverse hyperalgesia [48]. In addition, acute administration of flumazenil during withdrawal did not reverse tolerance to the antinociceptive effects of ethanol [2], and co-administration of flumazenil with subchronic ethanol did not prevent the development of tolerance to the antinociceptive effects of ethanol [11]. These findings indicate that although GABA_A receptors are important in mediating the antinociceptive effects of ethanol, and may have some role in mediation of ethanol-withdrawal hyperalgesia, they are not important in the development of tolerance.

NMDA and other glutamate receptors. Even though glutamate receptors are a major site for ethanol's effects, compounds active at glutamate receptors have not been tested in studies of the antinociceptive effects of chronic ethanol or ethanol withdrawal in intact animals. However, the effects of NMDA and AMPA glutamate receptors in spinal cord slices during acute withdrawal have been studied. Following administration of a high concentration of ethanol

(100 mM for 20 min) excitatory post-synaptic potentials in some motor neurons increased markedly [44, 45]. This hyperexcitability lasted for 20 min (peak at 10 min). Pharmacological testing revealed that neurons responsive to AMPA showed little hyperexcitability; however, 82% of NMDA-responsive neurons showed ethanol-withdrawal induced hyperexcitability [45]. These findings suggest that NMDA receptors in spinal cord are involved during ethanol-withdrawal hyperexcitability. This hypothesis has not been tested in intact organisms, but spinal NMDA receptors are known to play an important role in the hyperalgesia produced by morphine withdrawal. Recurrent opioid withdrawal produces increases in spinal glutamate levels [49]. Both morphine-withdrawal hyperalgesia and tolerance to morphine are mediated by NMDA receptors [50, 51]. Just because NMDA receptors are important in mediating tolerance and withdrawal to opioids does not indicate that NMDA receptors play the same role in tolerance and withdrawal to ethanol, but does suggest that further study of the role of NMDA receptors in the mediation of ethanol-withdrawal hyperalgesia is warranted.

Calcium channels. As mentioned previously, the calcium channel blocker nitrendipine (co-administered with the ethanol) dose-dependently blocked the ability of chronic ethanol to produce antinociception, but also dose-dependently attenuated the hyperalgesia seen during ethanol withdrawal [11, 26]. It was not possible to test whether higher doses of nitrendipine would fully block the hyperalgesia, as such doses reduce ethanol consumption in rats [52], which would confound the results. That is, a complete suppression of the hyperalgesia could be due to pharmacological actions of nitrendipine or to the reduced amount of ethanol consumed. The prevention of ethanol-withdrawal hyperalgesia by coadministration of nitrendipine is not surprising, as subchronic administration of ethanol produces an upregulation of L-type calcium channels, and dihydropyridine L-type calcium channel blockers (such as nitrendipine) block this upregulation [53, 54].

In contrast, nitrendipine administered during ethanol withdrawal was not able to reverse the hyperalgesia [11]. One other study is in agreement that coadministration of calcium channel blockers with chronic ethanol resulted in decreased ratings of ethanol withdrawal severity, but acute administration of calcium channel blockers during ethanol withdrawal did not [55]. On the other hand, seizures during ethanol withdrawal were reduced by L-type calcium channel blockers both following co-administration with chronic ethanol and following administration during withdrawal [56, 57].

Even though subchronic nitrendipine prevented the hyperalgesia, it did not prevent the development of tolerance to the antinociceptive effects of ethanol during withdrawal [26]. This finding is surprising, as dihydropyridines blocked the development of tolerance in both *in vitro* and behavioral tests [58-60]. It is possible that the difference is due to the localization of the effects; the *in vitro* assays tested hippocampal slices and the behavioral tests were for ataxia and anesthesia, both of which are supraspinally mediated. These findings are in accord with the possibility that dihydropyridines can prevent the development of ethanol tolerance in the brain but not in spinal cord. However, when nitrendipine was administered during ethanol withdrawal, it fully reversed the

tolerance to ethanol, such that ethanol produced maximal antinociceptive effects [26].

These findings suggest that L-type calcium channels are important in mediating the development of hyperalgesia during ethanol withdrawal, but are not directly involved once the hyperalgesia is established. The role of supraspinal mechanisms remains to be tested. The role of L-type calcium channels in tolerance to the antinociceptive effects of ethanol appears to be opposite, with L-type calcium channels not being involved with development of tolerance, but important in reversing the tolerance once established. Clarification of role of L-type calcium channels and of the brain and/or spinal cord regions involved is still needed.

Taken together, such findings suggest that calcium channel blockers may be a good pharmacological treatment during the early phases of alcohol abuse treatment as they reduce hyperalgesia (and other withdrawal signs) and suppressed ethanol consumption [26, 52, 56, 57].

Adenosine receptors. The non-selective adenosine antagonist theophylline, when coadministered with the chronic ethanol, dose-dependently prevented the development of hyperalgesia during ethanol withdrawal [24]. When administered only during ethanol withdrawal, theophylline markedly exacerbated the hyperalgesia and induced seizures. Similarly, adenosine agonists reversed signs of ethanol withdrawal, including anxiety and seizures [61-64]. These findings suggest that adenosine receptors play an important role in ethanol withdrawal.

Co-administration of theophylline with chronic ethanol also partially prevented the development of tolerance to the antinociceptive effects of ethanol during withdrawal. Testing higher doses of theophylline was prevented by adverse effects [61]; however, adenosine receptors have been shown to play an important role in the development of tolerance to ethanol. Chronic exposure to ethanol reduced adenosine transport and receptor-stimulated cAMP production [65-67], and co-administration of theophylline with ethanol up-regulated adenosine A1 receptors [68], which suggests that theophylline may at least partially counteract the effects of ethanol on adenosine transport. Support for the idea that adenosine receptors are important in development of tolerance to the antinociceptive effects of ethanol was provided by an experiment in which the antinociceptive effects of the adenosine agonist 2-chloroadenosine were markedly reduced during ethanol withdrawal. This effect was completely reversed by co-administration of theophylline with the chronic ethanol [24], which further supports an important role of adenosine receptors.

When theophylline was administered during ethanol withdrawal, it completely prevented ethanol from producing any antinociceptive effects, and blocked ethanol from reversing the hyperalgesia [11]. This finding further supports an important role of adenosine receptors in ethanol withdrawal and tolerance. Taken together, these findings suggest that adenosine receptors play an important role in the development of tolerance to ethanol, as well as in mediation of withdrawal signs.

Analgesics: Non-steroidal anti-inflammatory drugs and opioids. Very little research has been conducted on the effects of analgesic compounds on the antinociceptive ef-

fects of ethanol, none of which examined the effects of opioids on ethanol withdrawal hyperalgesia. However, one study has examined the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on ethanol withdrawal hyperalgesia in mice [38]. The NSAIDs were co-administered with ethanol for 7 days. The COX-2 selective NSAIDs (nimesulide and rofecoxib) were effective at preventing ethanol withdrawal hyperalgesia, but naproxen, which is preferential for COX-1 was not. The receptor mechanism and location (brain, spinal, etc.) for this effect is unknown, but the findings are in agreement with work that has shown similar effects of NSAIDs at preventing hyperalgesia induced by withdrawal from morphine [69, 70].

Signal transduction pathways. A number of the studies described previously examined the roles of various signaling pathways in the mediation of ethanol-withdrawal hyperalgesia. One study which studied hyper-responsiveness in spinal cord motor neurons reported that a protein kinase A inhibitor (Rp-cAMP) did not block the hyper-responsiveness induced by ethanol withdrawal [45]. In contrast, the tyrosine kinase inhibitor genistein did block hyper-responsiveness in this study. The protein kinase A and tyrosine kinase pathways have not been studied in other ethanol withdrawal experiments, so it is not possible at the present to judge their importance in the formation of ethanol-withdrawal hyperalgesia.

In contrast, protein kinase C (PKC) has been studied in more detail, as it is an important mediator of mechanical hyperalgesia [71], and plays an important role in withdrawal from opioids [72]. PKC inhibitors (GF-109203X and chelerythrine chloride) did block the ethanol-withdrawal hyper-responsiveness mediated by NMDA receptors in spinal cord motor neurons [44, 45]. PKC was found to be critical in mediating ethanol-withdrawal hyperalgesia in juvenile rats [40] and in the binge-model with adult rats [39], and was also found to be important in mediating the hyperalgesia induced by long-term chronic exposure to ethanol [28]. Two different isozymes of PKC have been examined. PKC γ was found to translocate from the nucleus to the cytoplasm in response to ethanol, and the PKC γ inhibitor γ V5-3 blocked both this translocation and the ethanol withdrawal hyperresponsiveness [44]. Further, PKC γ was found to be active in attenuating ethanol-withdrawal hyperalgesia in both 7- and 21-day old rats, whereas PKC ϵ was involved only in the 7-day old rats [40]. Blockade of PKC ϵ did block ethanol-withdrawal hyperalgesia in the binge model with adult rats [39] as well as the hyperalgesia seen following long-term exposure to ethanol [28]. In these last two studies, the effects of PKC ϵ were found to be localized in spinal cord or dorsal-root ganglia.

Summary of mechanisms. Compounds active at GABA_A receptors can prevent the development of ethanol-withdrawal hyperalgesia, but cannot reverse the hyperalgesia once it has developed. This suggests the GABA_A receptor is involved in initiating events that lead to hyperalgesia, but is not a direct/final cause of the hyperalgesia. Currently the roles of NMDA and opioid receptors are not clear. Calcium channels and adenosine receptors are involved with development of tolerance to ethanol. Not surprisingly, both play significant roles in the hyperalgesia, perhaps due to the up-regulation of L-type calcium channels and downregulation of adenosine

transport and cAMP produced by chronic exposure to ethanol. Finally, signaling pathways are also involved. PKC is important in mediating the effects of ethanol withdrawal hyperalgesia, and tyrosine kinase may be involved as well.

Many of the reports provide evidence that the spinal cord may be the primary region undergoing neuroplasticity during the development of hyperalgesia during ethanol withdrawal. To date, little is known about the involvement of supraspinal mechanisms. Further, it is not known what events activate the PKC and tyrosine kinase signaling pathways. The connection between those receptors that seem to initiate the development of hyperalgesia and the signal transduction pathways, which mediate the events, will need to be clarified.

THE GENERALITY OF WITHDRAWAL HYPERALGESIA

As described in the preceding sections, ethanol withdrawal hyperalgesia has been observed in different species, different administration procedures, and different antinociceptive assays. The phenomenon appears to be robust and widely replicable. Two other issues of generality remain. The first is whether the phenomenon is seen in both sexes, and the second is whether hyperalgesia is seen during withdrawal from other compounds.

Sex Differences

To date, the effects of ethanol on antinociception have been investigated only in male rats. Many of the studies of ethanol withdrawal hyperalgesia in neonatal and preweaning rats used both males and females, but grouped the data together, as there was no hormonal or behavioral differences between the sexes at those ages [40-43]. However, it is important to note that males and females do indeed show different sensitivities to various antinociceptive compounds, including dizocilpine [73, 74] and alphaxalone [75]. Further, male and female rats show different responses to ethanol withdrawal. Rats trained to discriminate the anxiogenic compounds pentylenetetrazol (a GABA_A antagonist) or m-chlorophenylpiperazine (a 5-HT_{1B/2C} agonist) from saline spontaneously respond on the drug-appropriate lever during ethanol withdrawal [76, 77]. In both models, female rats show lower levels of drug-appropriate responding during ethanol withdrawal [76, 78, 79]. In addition, males showed more anxiety-like behaviors during ethanol withdrawal in the elevated plus maze [80]. Other investigators have reported that female rats are less susceptible to seizures during ethanol withdrawal than males [81, 82].

Taken together, these findings suggest that males and females may indeed show a different response to the antinociceptive effects of ethanol and to hyperalgesia during withdrawal. We have conducted some preliminary studies on sex differences in the antinociceptive effects of ethanol using a radiant-heat tail-flick assay in adult Long-Evans rats. These data suggest that female rats are markedly less sensitive to the antinociceptive effects of ethanol (data not shown). These effects were not due to sex differences in pharmacokinetics, as blood ethanol concentrations for the acute and chronic doses of ethanol are the same or higher in the females [78, 83, 84]. Characterization of the sex differences in response to the nociceptive effects of ethanol and the mechanism for the sex differences will be of importance to under-

standing the generality and limits of the phenomenon of ethanol withdrawal hyperalgesia.

Withdrawal from Other Drugs

Withdrawal hyperalgesia is not limited to ethanol. It is, of course, widely observed during opioid withdrawal in both human and non-human animals [85, 86]. Although it is well beyond the scope of this review to describe the literature of opioid-related hyperalgesia, some similarities between the mechanisms of opioid-withdrawal hyperalgesia and ethanol-withdrawal hyperalgesia have been noted. Both NMDA receptors and PKC are important for the development of hyperalgesia for both opioids [50, 72] and ethanol [28, 39, 40, 44, 45]. As described previously, the mechanisms for the antinociceptive effects of ethanol appear to be different from those that produce tolerance and signs of withdrawal, including hyperalgesia. There appears to be a similar separation of mechanism for the antinociceptive effects of opioids and for opioid tolerance withdrawal [70, 87].

It is also of interest that hyperalgesia is seen not only during withdrawal from highly efficacious analgesics such as the opioids but from other compounds as well, including ethanol, nicotine, and adenosine agonists. Hyperalgesia has been observed during nicotine withdrawal in the mouse using tail-flick, hot plate and plantar stimulation assays [88-90] and in the rat using the jaw-opening reflex test [91]. Similarly, in rats which received repeated administration of the adenosine A1 agonist (N6-cyclopentyladenosine), hyperalgesia was observed during withdrawal precipitated by an adenosine receptor antagonist [92]. Not surprisingly, all of these compounds have antinociceptive effects. These findings raise the question of the generality of the phenomenon of hyperalgesia during withdrawal. Will it be observed only in compounds with antinociceptive effects, or is it an aspect of withdrawal in general? Whether this last possibility is plausible requires empirical testing. The issue is not trivial. If withdrawal from drugs generally produces an increased sensitivity to noxious stimulation, then treatment needs to be adapted to deal with the change in sensitivity.

IMPLICATIONS FOR TREATMENT

Patients undergoing detoxification from opioids report increased pain [93, 94], and non-dependent humans show hyperalgesia during withdrawal from an acute dose of morphine or hydromorphone [85]. The decreased tolerance to pain seems to last for long periods of time, as at least one study has reported that former addicts under methadone treatment are less tolerant to pain [95]. No published studies have examined whether hyperalgesia occurs in the alcoholic population, although preliminary data suggesting that humans show hyperalgesia during withdrawal have been presented [37].

The first obvious implication for treatment of ethanol withdrawal and dependence is that pain relief could be an appropriate goal during treatment. The effectiveness of clonidine at relieving withdrawal signs and symptoms may be partly based on its well-known analgesic properties [96, 97]. A second implication is that the effects of pain medications may be altered in those who chronically abuse ethanol and in those who have been dependent. Alterations in the

efficacy of pain medications could lead to over or under use, and could lead to an increased risk of adverse effects.

Animal research has indicated that ethanol withdrawal hyperalgesia can be relieved by administration of ethanol during the withdrawal phase [2, 3, 11]. The ability of ethanol to reverse the increase in sensitivity to noxious stimuli during withdrawal may play a role in relapse. In addition, because tolerance to the antinociceptive effects of ethanol continues through withdrawal, increasing the dose of ethanol for further pain relief would be ineffective, and could possibly be a mechanism for initiation of binge drinking.

SUMMARY

Hyperalgesia has become a recognized sign of alcohol withdrawal in addition to more traditional signs such as seizures and anxiety [1]. With this acceptance comes a need to understand the mechanisms of the hyperalgesia, how it relates to other signs of withdrawal, and its generality to withdrawal from other compounds. Current knowledge of the mechanisms of ethanol withdrawal hyperalgesia can be summarized as follows, although the roles of other receptor systems, signal transduction pathways, neural pathways, and brain regions also need to be investigated:

1. The antinociceptive effects of acute and long-term administration of ethanol are mediated at least in part by GABA_A receptors. No research has currently been conducted on the role of NMDA or other glutamate receptors in the antinociceptive effects of ethanol, so the role of glutamate in these effects is not known.
2. The development of tolerance to the antinociceptive effects of ethanol may be triggered by effects at GABA_A receptors, but adenosine receptors and L-type calcium channels appear to be the primary receptors that mediate development of tolerance to ethanol. The role of NMDA receptors may also be important, but has not been tested.
3. The hyperalgesia seen during ethanol withdrawal seems to be mediated largely by changes in adenosine receptors, although L-type calcium channels contribute to the development of hyperalgesia. Other mechanisms may be involved, such as the PKC signaling pathway, which also plays an important role in mediating development of hyperalgesia.

It is known that much of drug seeking is motivated by escape from the withdrawal syndrome [98-100]. Hyperalgesia is but one aspect of the many behavioral/neurological changes that occur during withdrawal, including increased anxiety, startle, and sympathetic nervous system arousal, decreased appetitive motivation and profound neuroplasticity of the brain reward/reinforcement pathways [80, 100, 101]. The relative contribution of increased sensitivity to noxious, unpleasant, and disrupting stimuli to withdrawal, and how it contributes to the other, better-understood aspects of withdrawal deserves further examination.

Hyperalgesia is not only a clinically significant aspect of withdrawal from ethanol, but may be of importance to withdrawal from other drugs including opioids and nicotine. Hyperalgesia during opioid withdrawal is well known, although a very small amount of research has examined hyperalgesia during nicotine withdrawal. Little attention has been given to

hyperalgesia in the design of treatment for substance abuse and dependence, even though there are obvious ramifications in terms of potential interactions between heavy use of ethanol or nicotine and pain medications. Clinical research on the significance of withdrawal hyperalgesia to detoxification, relapse prevention, and effects of pain medication will be necessary to determine its importance to treatment design.

Key Learning Objectives:

1. Hyperalgesia is a significant component of withdrawal from ethanol and other drugs of abuse, including opioids and nicotine.
2. The antinociceptive effects of acute and long-term administration of ethanol are mediated at least in part by GABA_A receptors.
3. Adenosine receptors and L-type calcium channels appear to be the primary receptors that mediate development of tolerance to ethanol.
4. The hyperalgesia seen during ethanol withdrawal seems to be mediated largely by changes in adenosine receptors, although L-type calcium channels contribute to the development of hyperalgesia. Other mechanisms may be involved, such as the PKC signaling pathway, which also plays an important role in mediating development of hyperalgesia.

Future Research Questions:

1. What is the role of NMDA receptors in mediating the antinociceptive effects of acute and chronic ethanol?
2. What is the role of NMDA receptors in mediating development of tolerance to ethanol?
3. What is the role of biogenic amines and the pain pathways in the antinociceptive effects of ethanol?
4. Which is the main area of the nervous system responsible for the neuroadaptive changes leading to ethanol withdrawal hyperalgesia?
5. Does fetal exposure to ethanol result in persistent changes in pain sensitivity during adulthood?
6. Will treating withdrawal hyperalgesia decrease the likelihood of relapse?

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