Article Addendum

The N-type calcium channel is a novel target for treating alcohol use disorders

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We recently validated the N-type calcium channel as a target for the treatment of alcoholism and anxiety. N-type calcium channels are neuronal presynaptic ion channels that regulate neurotransmitter release at many sites in the brain. Mice lacking N-type calcium channels exhibit reduced ethanol consumption and show resistance to the acute intoxicating effects of ethanol. In wild type rodents, pretreatment with a novel N- and T-type calcium channel blocker, NP078585, reduces the intoxicating and reinforcing effects of ethanol and abolishes stress-induced reinstatement of alcohol seeking. Here we discuss these findings and expand upon their implications for the N-type calcium channel as a target for drug development. An important consideration in the development of drugs to treat any addiction is that the medication itself not be addictive. We attempted, and failed, to generate a conditioned place preference for NP078585, suggesting that NP078585 is not rewarding.

Introduction

The N-type calcium channel is one of a family of voltage gated calcium channels that also includes L-, P/Q-, R- and T-types. All types of voltage gated calcium channel are found in the brain. The channel types are distinguished principally by different pore-forming subunits which confer distinct electrophysiological properties.1,2 N-type calcium channels contain the Ca\textsubscript{v}2.2 subunit.3 The best understood role of N-type calcium channels is in mediating neurotransmitter release.4 Many important G-protein-coupled neuromodulators regulate the release of neurotransmitters by inhibiting N-type calcium channels. These include opioids,5,6 cannabinoids,7 dopamine,8 GABA acting at GABA\textsubscript{A} receptors9,10 and adenosine acting at A1 receptors.11 Significantly, all of these neuromodulators regulate ethanol consumption in rodents.12-14 Some of these modulators are also coupled to P/Q-type channels, but current evidence indicates that G-proteins inhibit N-type calcium channels to a greater extent than P/Q-type channels.15,16

Alcohol Use Disorders

Alcoholism is a major health problem throughout the world. In the United States alone, alcohol use disorders affect about 14 million people, costing over $180 billion a year due to lost wages, legal and medical costs.17,18 Treatment options are currently limited and 40–70% of patients return to excessive drinking within a year of treatment.19 To date, no receptor has been found for alcohol, although numerous targets have been directly and indirectly implicated.20-22 A wide range of drugs have been proposed as novel treatments for alcohol use disorders; drugs acting on the opioid, glutamatergic, dopaminergic, serotonergic, cholinergic, corticotropin-release factor, endocannabinoid and neurokinin systems have all had some positive effects in clinical and preclinical tests,13,23-27 as have various anticonvulsants whose mode of action is not completely understood, including topiramate, gabapentin, lamotrigine and amlodipine.28 One thing that almost all these systems and drugs have in common is that they act, at least in part, by inhibiting N-type voltage-gated calcium channels.

Stress and anxiety are among the most important risk factors for developing alcohol use disorders. Anxious individuals are more likely to develop alcoholism and vice versa29 and stress is a significant trigger for relapse in otherwise abstinent alcoholics.30 Alcohol is a potent anxiolytic and it has thus been hypothesized that anxious individuals may drink alcohol to self-medicate their anxiety.31 The sedative-hypnotic effect of benzodiazepines, the class of drugs most commonly used clinically to treat anxiety, is significantly potentiated by ethanol making them unsuitable for the long-term treatment of anxious alcoholics. Naltrexone, perhaps the most commonly used medication for alcoholism,27 appears to be less effective in subgroups of patients who show greater risk for anxiety.32 There have been few clinical trials aimed at the treatment of alcoholism and co-morbid anxiety disorders,33 even though...
40–70% of patients with alcohol use disorders exhibit significant co-morbid anxiety symptoms.34

N-type Calcium Channels and Ethanol

We have previously shown that chronic ethanol treatment increases the abundance of N-type calcium channels, suggesting that these channels are involved in the neuroadaptive changes caused by long-term ethanol exposure.35 We subsequently showed that this effect was specific for certain splice variants of the channel's alpha-1 subunit.36 We also showed that mice lacking N-type calcium channels exhibit reduced ethanol consumption and show resistance to the acute intoxicating effects of ethanol.37 These data suggested that treatment with an N-type calcium channel blocker might reduce ethanol consumption and intoxication.

We tested this hypothesis using the novel N-type calcium channel blocker, NP078585, which inhibits N-type calcium channels in vitro with a K_d of 110 nM. NP078585 also inhibits all T-type (Ca_v3.1-3.3) channels with similar potency38 and shows oral bioavailability, which is an important consideration in the development of medications for human use. As stated above, no receptor for alcohol has been identified and alcohol use disorders are complex with multiple overlapping symptoms. As a result, behavioral studies of alcoholism require the use of multiple paradigms. We first tested the effects of NP078585 on the acute intoxicating effects of ethanol in three paradigms.

1. Loss of the righting reflex, where animals are treated with a high dose (3.2–4.0 g/kg) of ethanol that produces hypnosis and causes them to lose the ability to right themselves when placed on their backs. The time taken for them to regain this “righting reflex” is measured. 2. Rotarod ataxia, where animals are trained to run on a rotating drum. A moderate dose of ethanol (2.0 g/kg) causes them to become ataxic and less able to stay on the rotating drum. 3. Hyperlocomotion. Moderate doses (2 g/kg) of ethanol produce hyperlocomotion in mice. In all these paradigms, pre-treatment with NP078585 reduced or abolished the effects of ethanol without having any effect by itself. The magnitude of the reduction was very large. In the loss of the righting reflex assay we tested NP078585 in mice lacking N-type calcium channels where it was without effect, suggesting that NP078585 is acting through N-type calcium channels.

We also tested NP078585 in animal models that measure motivation to seek and consume ethanol and have predictive validity for human alcoholism.39 NP078585 completely abolished the expression of an ethanol conditioned place preference; this assay measures the motivation of the test subject (mouse) to seek an environment that was previously paired with an intoxicating dose of ethanol (2 g/kg) (see also below). We also investigated the effects of NP078585 on operant self-administration of ethanol by Long-Evans rats. Rats were trained to administer a moderate (-0.47 g/kg) dose of ethanol by lever pressing in 30-minute sessions. When treated with NP078585, this self-administration was not immediately affected. However, the day after NP078585 treatment, when we tested the rats again, their self-administration was reduced. A major problem in alcoholism is relapse, which can be modeled in rodents using “reinstatement” paradigms.40 In a separate experiment we trained rats to self-administer ethanol and then extinguished that response over a 7 week period during which lever pressing did not result in the delivery of ethanol. They were then treated with the pharmacological stressor yohimbine. Stress causes a reinstatement of ethanol seeking; rats will once again press the ethanol lever even when doing so does not result in the delivery of ethanol. Pre-treatment with NP078585 abolished this stress-induced reinstatement. This was perhaps the most exciting result from the study, given the paucity of treatments to treat anxious alcoholics as described above.

Abuse Potential

An important consideration in the development of drugs to treat any addiction is that the medication not be addictive itself. This is especially important for the N-type calcium channel since multiple addictive drugs of abuse act, at least in part, through inhibitory actions at N-type calcium channels. These include opioids,5,6 cannabinoids 7 and dopamine,8 whose abundance is increased by the actions of most addictive psychostimulants. We thus investigated whether NP078585 has any abuse potential by attempting to generate a conditioned place preference for NP078585. The conditioned place preference paradigm is a well-established paradigm that measures the motivation of the subject to enter and spend time in an environment (conditioned stimulus) that was previously paired with administration of a rewarding unconditioned stimulus (e.g., a drug of abuse). Conditioned stimuli that are aversive, such as lithium chloride or social defeat, produce a conditioned place aversion. A conditioned place preference can be generated for almost every drug of abuse and thus the paradigm may be used as a simple measure of the ability for a drug to produce reward and thus, potentially, be abused.41

Results

Place preference was not observed at any dose in either rats or mice (Fig. 1). Data are presented as time spent in the vehicle-paired chamber on test-day subtracted from time spent in the NP078585-paired chamber on test day. This CPP score was calculated for all animals. A one-sample t-test revealed that the mean CPP score was not different from zero at either dose in rats (p = 0.59 for 10 mg/kg, p = 0.44 for 25 mg/kg) or mice (p = 0.68). The same result was obtained when comparing time spent in the vehicle-paired chamber with time spent in the drug-paired chamber on test day by Wilcoxon signed rank test as in previous work, p = 0.62 for 10 mg/kg (rat), p = 0.42 for 25 mg/kg. (rat), p = 0.65 for 25 mg/kg (mouse).

Discussion

Multiple addictive drugs act, at least in part, through inhibitory actions at N-type calcium channels. The conditioned place preference paradigm is a very well-established paradigm that is used as a simple measure of the ability for a drug to produce reward and thus, potentially, be abused.41,48 In this assay NP078585 failed to produce a conditioned place preference in both mice and rats. These results suggest that NP078585 is not rewarding and therefore has low potential for abuse. It is, however, conceptually
N-type calcium channels as therapeutic targets for alcoholism

The N-type calcium channel as a drug target. The best characterized inhibitor of N-type calcium channels is ω-conotoxin GVIA; the established means by which N-type calcium current is distinguished from other calcium current types in vitro. However this peptide toxin shows very poor penetration of the blood-brain barrier and so is not suitable for systemic delivery. Intracerebroventricular delivery causes tremor making it unsuitable for behavioral assays. Nevertheless, a synthetic derivative of ω-conotoxin GVIA, called ziconotide, has been approved for the treatment of chronic intractable pain. Ziconotide also does not penetrate the blood-brain barrier and thus has to be delivered through an intrathecal pump. Abuse has not been reported, and euphoria has not been reported as a side effect, supporting the idea that N-type calcium channel blocking medications have limited abuse potential. The N-type calcium channel is being actively targeted by medications development programs for the treatment of pain. If these programs are successful there is a real possibility that N-type calcium channel blockers could be tested as a treatment for alcoholism in human trials.

Mechanistic interpretation. We did not observe any locomotor, sedating or ataxic effects of NP078585 alone. This observation, that NP078585 alone is without effect but significantly attenuates the behavioral effects of ethanol, suggests that ethanol may activate the N-type calcium channel and that this activation is required for ethanol to produce its effects. This activation may be through direct or indirect effects of ethanol at the channel. Ethanol has repeatedly been demonstrated to increase release at GABAergic synapses. Given the presynaptic location of N-type channels and their demonstrated role in regulating GABA release at a number of different synapses, it is possible that the effect of blocking N-type channels may be to inhibit ethanol-induced increases in GABA release and so reduce several behavioral effects of ethanol. Testing this hypothesis is the subject of ongoing studies.

Materials and Methods

Long-Evans rats (Harlan) were housed individually and allowed four days to acclimate to the animal facility. We used Long-Evans rats because they are able to quickly (three or fewer conditioning sessions) learn a robust conditioned place preference, as has been demonstrated for amphetamine, morphine, estradiol, methylenidate, and pentobarbital. Over the next week they were individually handled 3 times. The last two of those sessions were accompanied by a saline injection. CPP apparatus (ENV-013, Med-Associates, Georgia, VT) consisted of three chambers. Two conditioning chambers (28 x 21 x 21 cm) joined by a central gray access chamber. One conditioning chamber had white walls and a grid floor while the other had black walls and a rod floor. Both chambers were dimly lit. Rats were acclimated to the apparatus in a 30-minute baseline session in which they were allowed free access to all three chambers.

Animals were then conditioned in 6 consecutive daily sessions in which they received either vehicle (PEG-400) or NP078585 (10 or 25 mg/kg) on one day in one chamber (black or white) and then the other treatment in the alternate chamber the next day. Animals were injected with either vehicle or NP078585 and placed immediately in the conditioning chamber for 30 minutes before being returned to the home cage. Equal numbers of rats were conditioned to the black and white chambers. Equal numbers of rats received vehicle on either days 1, 3 and 5 or days 2, 4 and 6. N = 12 for each dose of NP078585.

On the day immediately following the final conditioning session, animals were tested for the expression of a conditioned place preference by allowing them 30 min unlimited access to all three chambers. Time spent in each chamber was recorded. Data were analyzed by subtracting time spent in the vehicle-paired chamber on test day from time spent in the NP078585-paired chamber. Data shown are time spent in the vehicle-paired chamber on test-day subtracted from time spent in the NP078585-paired chamber. A one-sample t-test indicated that this value was not significantly different from zero, indicating that the rats had no preference or aversion for the NP078585-paired chamber.

Figure 1. NP078585 does not support the development of a conditioned place preference. Animals were conditioned in 6–8 consecutive daily sessions. Animals were injected with either vehicle or NP078585 and placed immediately in one conditioning chamber for 30 minutes. The following day they received the alternate treatment in the opposite chamber. On the day immediately following the final conditioning session, animals were tested for the expression of a conditioned place preference by being allowed 30 min unlimited access to both vehicle and NP078585 conditioned chambers. Data shown are time spent in the vehicle-paired chamber on test-day subtracted from time spent in the NP078585-paired chamber. One-sample t-test indicated that this value was not significantly different from zero, indicating that the rats had no preference or aversion for the NP078585-paired chamber.

difficult to prove a negative. We failed to generate a place preference for NP078585 in two species and at multiple doses but we would not conclusively rule out the possibility that a place preference could be generated by altering one or more of the following parameters; dose, number of conditioning trials, length of conditioning trials, degree of pre-exposure to NP078585, vehicle, strain of rat/mouse or species. Nevertheless, we have failed to generate a place preference using doses of NP078585 that are biologically active and reach brain concentrations sufficient to inhibit N-type calcium channels, using experimental parameters that produce a place preference for almost all drugs of abuse (see materials and methods). Two notable exceptions include cannabinoids, where initial experience appears aversive but a place preference can be generated after pre-exposure to the drugs in the home cage, and ethanol, where place preference requires the use of very short (5 min) conditioning sessions and even then appears to only work well in mice. The N-type calcium channel as a drug target. The best characterized inhibitor of N-type calcium channels is ω-conotoxin GVIA; the established means by which N-type calcium current is distinguished from other calcium current types in vitro. However this peptide toxin shows very poor penetration of the blood-brain barrier and so is not suitable for systemic delivery. Intracerebroventricular delivery causes tremor making it unsuitable for behavioral assays. Nevertheless, a synthetic derivative of ω-conotoxin GVIA, called ziconotide, has been approved for the treatment of chronic intractable pain. Ziconotide also does not penetrate the blood-brain barrier and thus has to be delivered through an intrathecal pump. Abuse has not been reported, and euphoria has not been reported as a side effect, supporting the idea that N-type calcium channel blocking medications have limited abuse potential. The N-type calcium channel is being actively targeted by medications development programs for the treatment of pain. If these programs are successful there is a real possibility that N-type calcium channel blockers could be tested as a treatment for alcoholism in human trials.

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chamber to generate a CPP score. A one-sample t-test was then used to determine whether this value was significantly different from zero. Data were also analyzed by comparing time spent in the drug-treated side to time spent in the vehicle-treated side on test day.

We also repeated the experiment using DBA/2 mice (Taconic) (n = 10). Conditions were as described for the rat experiment except that we used eight conditioning sessions (four vehicle and four NP078585) and mouse open-field chambers (ENV-515, Med Associates) equipped with 2-chamber place preference inserts (ENV-517, Med Associates). One chamber consisted of a mesh floor with white walls while the other chamber had a rod floor with black walls. Chambers were separated by a manual guillotine door that was closed during training and open during habituation and test sessions. For the mouse study we used a dose (25 mg/kg i.p.) that we found to be effective in our alcohol studies.38

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