

REVIEW

Can experimental paradigms and animal models be used to discover clinically effective medications for alcoholism?

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Abstract

Evaluating medications in animal laboratory paradigms can reveal whether the compound is effective in an established alcoholism model, at clinically relevant doses and exposure conditions, when administered orally (or transdermally) and without serious limiting side effects. Positive outcomes constitute a possible discovery for relevance to alcoholism and, under favorable marketing conditions, encourage further development. Medication testing using animal models of alcoholism might also guide clinical testing by discriminating clinically effective from clinically ineffective compounds. This ability rests on whether there are tests or, more reasonably, batteries of tests having this discriminative ability. The present paper examines this possibility. Effects of naltrexone and acamprosate in animal paradigms which model behavioral aspects of alcoholism are reviewed and compared with the effects of compounds which have limited effects in alcoholics. It is not clear at present whether any single paradigm or combination of paradigms differentiates clinically effective from clinically limited compounds. Steps are suggested to improve the use of preclinical laboratory tests to predict which compounds are likely to be effective medications for reducing drinking and sustaining abstinence in human alcoholics.

Introduction

Medication tests in animal behavioral paradigms have the potential to guide decisions about whether lengthier, more expensive, clinical tests in human alcoholics are warranted. This depends, however, on the availability of laboratory paradigms which adequately model aspects of alcoholism and which have the ability to identify agents that subsequently alleviate targeted features of alcoholism in a significant number of patients.

Alcoholism is heterogeneous in its etiology and expression. It is a dynamic disorder, with many potential therapeutic targets. A single laboratory paradigm does not capture adequately the spectrum of clinical conditions associated with alcoholism. Yet many models used successfully as basic research tools are being adapted to assess the dose effects, time-course, specificity and toxicity of test medications. Ideally, these tests would also allow us to predict the medication's effects in more expensive clinical trials. The ability of animal behavioral paradigms used widely in alcoholism research to discriminate clinically effective from

clinically ineffective medications is considered in the present review.

Can animal laboratory behavioral paradigms identify clinically effective medications for alcoholism?

Clinically effective and ineffective reference compounds

Evaluating the validity of pre-clinical medication testing paradigms requires assessing the concordance between pre-clinical and outpatient studies. Several drugs have demonstrated clinical efficacy for alcoholism under some conditions. Of these, naltrexone and acamprosate are approved by the United States Food and Drug Administration to treat alcoholism through their purported ability to reduce the desire to drink. Both drugs have been tested in a variety of non-human laboratory paradigms.

Naltrexone and acamprosate effects can be compared to the effects of medications having limited therapeutic efficacy. There are no formal criteria for concluding that a medication

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is clinically ineffective for alcoholism. Even medications such as naltrexone, which show clinical efficacy in the preponderance of double-blind, placebo-controlled trials, have failed to show efficacy in some studies (see Fuller & Gordis, 2001; Krystal et al., 2001). Because clinical trials are expensive, an early failure to show clinical efficacy is unlikely to be followed by further extensive clinical testing. Therefore, concluding that a medication is ineffective in the absence of extensive testing is risky, and such medications may improve adjunct symptoms associated with alcohol abuse without influencing drinking and relapse. Nevertheless, for the purpose of the present review, it is necessary to identify medications for which there is little evidence for clinical efficacy for alcohol drinking, craving and relapse.

Serotonin-specific reuptake inhibitors (SSRIs) have been tested sufficiently often to conclude that they are not therapeutically effective for non-depressed alcoholics (Garbutt et al., 1999; Torrens et al., 2005). In addition, alcoholics have reduced dopamine D2 receptor levels in striatal regions of the brain, which has been hypothesized to predispose alcohol use as compensation for decreased dopaminergic neuronal activation (Volkow et al., 1996). Nevertheless, the D2 receptor agonist bromocriptine has failed consistently to perform better than placebo on alcohol use measures in clinical studies (see Kosten et al., 2002), even in individuals having the D2 receptor A1 allele (see Goldman, 1995; Lawford et al. 1995). The 5-HT₂ antagonist ritanseron activates midbrain dopamine neurons by blocking serotonergic inhibition (Ugedo et al., 1989). Ritanserin failed to reduce craving, drinking and relapse more than placebo in a large multi-center clinical trial (Johnson et al., 1996; Wiesbeck et al., 1999) and failed to reduce drinking in a smaller double-blind, placebo-controlled study of heavy drinkers (Naranjo et al., 1995).

Paradigms and models of alcohol drinking, craving and relapse

Virtually all medical knowledge and treatment involves work with animal models. Several laboratory paradigms which model facets of alcoholism are used to study the behavioral effects of ethanol in mice, rats and monkeys. Some are *isomorphic models*, in that they resemble the symptoms and clinical outcome of human alcoholism, although these conditions are produced artificially. There are no truly *homologous models* of alcoholism, however, in that the disease state in animals never fully emulates human alcoholism in etiology, symptoms and prognosis, particularly in cognitive and psychosocial domains. For evaluating medications, however, paradigms need only be *predictive models* which generate significant discriminative or predictive information, but which do not necessarily resemble etiology or symptoms of human alcoholism.

The most widely used method of assessing alcohol drinking is simply to measure home cage consumption with food and water present. Operant self-administration requires that animals emit a specified behavior, typically a bar press, to obtain brief access to an ethanol solution. Operant studies assess the motivation to procure alcohol, and reveal medication effects on within-session ingestion patterns. When operant response requirements are low, as is usually the case, medication effects tend to be similar to those in voluntary drinking paradigms.

Several well-developed laboratory paradigms have been validated as models for dimensions of alcoholism using these methods. They include models of inherited susceptibility to heavy drinking, dependence-driven heavy drinking, alcohol craving and relapse to drinking. Each paradigm models different facets of alcoholism, although none can be said to model one facet exclusively or completely. To be useful for evaluating medications, collectively they must be able to reliably predict clinical efficacy. Each model will be discussed briefly before reviewing the effects of clinically effective and clinically ineffective medications.

Heavy drinking. Laboratory animals do not typically drink ethanol in quantities sufficient to produce blood concentrations exceeding 80 mg/dl, the legal limit of intoxication in most regions of the United States. Rodent models of heavy voluntary alcohol drinking have been developed through selective breeding and through inducing ethanol dependence. Selective breeding increases the frequencies of alleles affecting alcohol preference and intake and models genetic susceptibility to alcohol abuse and alcoholism. The selectively bred alcohol preferring rat strains most commonly used for testing lead compounds are the AA, P, HAD and sP lines.

Alcohol drinking increases substantially following an extended history of intoxication and withdrawal. Dependent Wistar rats self-administer ethanol during the first 12 hours post-withdrawal if they have learned to associate alcohol with the alleviation of acute withdrawal symptoms (Roberts et al., 1996; Schulteis et al., 1996). Repeated cycles of ethanol vapor exposure and withdrawal elevate drinking and operant self-administration which persists months after abstinence symptoms subside in some protocols (Rimondini et al., 2002). The neuroadaptively driven transition to a persistent state of high alcohol drinking emulates the clinical indications of alcoholism in that patients are most vulnerable to relapse long after acute withdrawal.

Alcohol deprivation. When a period of alcohol access is followed by forced alcohol abstinence and then access to ethanol is reintroduced, a transitory period of increased drinking is observed for a day or two. This 'alcohol deprivation effect' is strengthened and prolonged by repeated deprivations. When given access to ethanol concentrations higher than normally preferred, alcohol-preferring P rats drink approximately 5 g/kg ethanol in 2 hours, and achieve a mean BAC of 180 mg/dl (Rodd-Henricks et al., 2001). The paradigm giving rise to the alcohol deprivation effect resembles some dependence-induced drinking paradigms in its cycles of ethanol exposure and abstinence. However, physiological dependence is not required for enhanced drinking and may involve distinct biological substrates. The alcohol deprivation paradigm models drinking binges by human alcohol abusers following a period of abstinence (i.e. relapse).

Reinstatement. Animals trained to bar-press for alcohol will eventually cease bar-pressing if alcohol solutions are no longer presented. The reinstatement paradigm measures the ability of environmental or pharmacological stimuli to revive bar-pressing (i.e. alcohol-seeking) when alcohol is no longer

available. This paradigm does not model conditions leading to alcohol abstinence in humans, but rather models conditions which precipitate craving such as acute alcohol action, the presence of cues predictive of alcohol availability and stressors. The mechanisms by which drugs prevent reinstatement of alcohol seeking differ from those that reduce drinking. For example, the nitric oxide synthase inhibitor L-NAME did not reduce ethanol self-administration, yet significantly reduced reinstatement of alcohol seeking induced by cues associated with ethanol reinforcement (Liu & Weiss, 2004).

Conditioned place preference. In the conditioned place preference procedure, animals learn to associate the effects of alcohol with a physically distinct location in a test box. In subsequent trials, the animal's preference for this location is tested. Like cue-induced reinstatement tests, testing medication effects on conditioned place preferences occur in the absence of ethanol's pharmacological actions. Although not considered to be a model of craving, conditioned place preference paradigm may have relevance to the environments which elicit alcohol craving. Place preference conditioning measures ethanol's motivational effects which may involve processes distinct from those involved in the motivation to self-administer ethanol (Risinger et al., 2002). Using the place preference paradigm to test medications is limited to mice. Rats typically show conditioned place *aversions* and many mouse strains, including some having a high propensity to drink alcohol, do not learn robust place conditioning to ethanol.

Locomotor stimulation and sensitization. Acute ethanol administration stimulates locomotion in some species. Ethanol-stimulated locomotion increases progressively with repeated, intermittent administration. This sensitization is enhanced further when ethanol effects are paired with a particular testing environment (Quadros et al., 2003). Ethanol stimulates locomotor activity through the mesolimbic dopamine (DA) system; sensitization occurs through DA, NMDA, and GABAergic mechanisms. The sensitization of alcohol's motor effects is purported to involve neuroadaptation important to the early stages of addiction, yielding an additional screening target.

Medication effects in alcohol paradigms and models *Voluntary alcohol drinking and self-administration:* *clinically effective medications*

Naltrexone. Naltrexone administration consistently reduced ethanol drinking by mice and rats under scheduled access and operant self-administration conditions (Lê et al., 1993; Stromberg et al., 1998a, b, 2001, 2002a, b; Quintanilla & Tampier, 2000; Goodwin et al., 2001). These effects are not always selective to ethanol, however. In monkeys, naltrexone doses which reduced alcohol self-administration also reduced self-administration of an orange-flavored drink (Shelton & Grant, 2001), water (Williams & Woods, 1999), sucrose (Williams et al., 1998), food and PCP (Carroll et al., 2000). Continuous ethanol access paradigms are less sensitive to naltrexone's effects. Daily naltrexone administration did

not significantly reduce drinking under continuous-access conditions at doses which reduced alcohol drinking under scheduled-access conditions (Goodwin et al., 2001).

Effects of repeated naltrexone dosing are inconsistent. Some report loss of efficacy with repeated opiate antagonist dosing (Gardell et al., 1997; Boyle et al., 1998; Overstreet et al., 1999; Shelton & Grant, 2001), although other studies showed no evidence for tolerance (Gardell et al., 1996; Reid et al., 1996). In other reports, repeated naltrexone administration progressively decreased alcohol drinking (Sinclair, 1989; Stromberg et al., 1998b; Bienkowski et al., 1999). This latter observation suggests that the subject learns that ethanol is no longer reinforcing through repeated drinking experiences in the presence of opioid receptor blockade and, therefore, drinks less (see Sinclair, 2001). Effects of repeated or chronic naltrexone administration on continuous-access ethanol drinking are also inconsistent. Some report transient reductions (Cowen et al., 1999; Middaugh & Bandy, 2000), progressive reductions (Parkes & Sinclair, 2000) and even increased drinking (Phillips et al., 1997).

Acamprosate. Acamprosate, like naltrexone, reduced alcohol drinking under limited access conditions (Olive et al., 2002). Acamprosate's effects were greatly diminished under continuous access conditions (Stromberg et al., 2001) unless rats screened for high ethanol preference were tested (Boismare et al., 1984; Daoust et al., 1987). Acamprosate administration minimally affected operant behavior maintained by ethanol delivery in Wistar rats with a limited history of alcohol exposure (Stromberg et al., 2001; Heyser et al., 2003). Conditions under which acamprosate administration reduced alcohol self-administration more robustly are discussed below.

A modified paradigm measures the motivation to work for alcohol as well as ethanol drinking under limited access conditions (Samson et al., 1998). In this paradigm, animals are required to bar-press at the beginning of the experimental session (appetitive component) to obtain access to a drinking tube for the remainder of the session (consumatory component). Administering naltrexone or acamprosate reduced ethanol drinking in the consumatory component without significantly changing the appetitive bar pressing (Czachowski et al., 2001; Sharpe & Samson, 2001). The clinical and predictive significance of an apparently selective effect on ethanol consumption, rather than the motivation to procure alcohol, remains unclear.

Clinically ineffective medications

SSRIs. SSRI administration reduced ethanol drinking and self-administration in laboratory animals. Although SSRIs may decrease alcohol drinking by reducing ingestive behavior more generally (Gill & Amit, 1989), they also selectively reduce ethanol's reinforcing effects (Lamb & Järbe, 2001; Ginsburg et al., 2005).

Bromocriptine. Bromocriptine effects on voluntary ethanol drinking and self-administration are mixed. Daily bromocriptine injections reduced ethanol drinking and preference in C57 mice (Ng & George, 1994) and reduced operant ethanol self-administration in Wistar rats (Weiss et al., 1990;

Cohen et al., 1998). Two studies report that bromocriptine *increased* ethanol drinking by rats when available continuously, although baseline drinking levels appeared to have been modest (Naeger & Martinez, 1990; Nadal et al., 1996).

Ritanserlin. Ritanserlin was reported to decrease ethanol drinking by rats under continuous access conditions (Meert & Janssen, 1991; Panocka & Massi, 1992; Lin & Hubbard, 1994) and produced non-specific reductions in operant self-administration (Wilson et al., 1998; Gallate & McGregor, 1999). Other studies report no effect of ritanserlin administration on ethanol preference or intake (Myers & Lankford, 1993; Svensson et al., 1993).

Further considerations. Chronic or repeated naltrexone administration has inconsistent effects on voluntary ethanol drinking with some reporting transient reductions in drinking over time, whereas others report tolerance, or even increased drinking. Contrary to studies in animals, repeated administration of long-acting naltrexone appears not to lose its efficacy in humans (Kranzler et al., 1998; Johnson et al., 2004). The progressive reduction in drinking either within-session (Hyytiä & Sinclair, 1993) or across sessions is consistent with naltrexone's purported therapeutic action, which requires that alcohol's effects be experienced under the influence of naltrexone (Sinclair, 2001).

As with the clinically effective medications, clinically ineffective medications had inconsistent effects on voluntary alcohol drinking and self-administration. Based on these findings, voluntary moderate-drinking paradigms do not appear to be capable of distinguishing clinically effective from ineffective compounds.

Heavy drinking models: clinically effective medications

Naltrexone. Naltrexone or naloxone reduced ethanol ingestion in AA rats (Hyytiä & Sinclair, 1993), P rats (Badia-Elder et al., 1999) and HAD rats (Froehlich et al., 1990). Naloxone administration decreased alcohol drinking and preference by dependent rats in an alternating drinking paradigm (Marfaing-Jallat et al., 1983). Naltrexone and naloxone reduce alcohol drinking regardless of whether heavy drinking models are used. To my knowledge, naltrexone effects have not been compared directly between selectively bred rats and the appropriate control strain, or between alcohol-dependent and naive animals. One report suggested that alcohol drinking suppressed by naloxone administration was more persistent in alcohol-preferring AA rats than in Long-Evans rats (Sinclair, 1989).

Acamprosate. Robust reductions of ethanol drinking following acamprosate administration were initially observed in rats which had been chronically exposed to ethanol. For example, acamprosate administration selectively reduced ethanol drinking which had been increased by chronic ethanol intoxication, whereas acamprosate was largely ineffective when tested in animals which had no prior exposure to ethanol (Le Magnen et al., 1987). Similarly, chronic acamprosate treatment initiated during ethanol vapor exposure periods reduced subsequent ethanol drinking (Gewiss et al., 1991). Seven

weeks of intermittent ethanol vapor exposure doubled alcohol drinking when ethanol solutions were introduced following a 2-week alcohol-free period. Daily acamprosate administration initiated during the alcohol-free period completely prevented the increased ethanol drinking, but did not reduce moderate drinking levels in rats having no history of ethanol dependence (Rimondini et al., 2002).

Acute acamprosate administration was recently shown to reduce voluntary alcohol drinking and operant self-administration by alcohol preferring fawn-hooded, iP and AA rats (Cowen et al., in press). Acamprosate became less effective with repeated injections, however. Under these conditions, acamprosate's efficacy was associated with modulation of the mesolimbic dopamine system.

Clinically ineffective medications

SSRIs. Fluoxetine reduced alcohol drinking by P rats (Murphy et al., 1985), cAA rats (Maurel et al., 1999) and HAD rats (Rezvani et al., 2000), without altering food or water intake, and reduced ethanol drinking by sP rats (Ciccocioppo et al., 1997) with varying degrees of specificity. To my knowledge, effects of SSRIs on dependence-induced drinking have not been published.

Bromocriptine. Bromocriptine selectively reduced operant ethanol self-administration and preference in P rats (Weiss et al., 1990) and drinking under continuous access conditions by selectively bred, alcohol-preferring UChB rats (Mardones & Quintanilla, 1996).

Ritanserlin. Although ritanserlin diminished drinking by non-dependent Wistar and Sprague-Dawley rats, ritanserlin had no effect on ethanol drinking in selectively-bred alcohol-preferring cAA rats (Maurel et al., 1999) nor in alcohol-preferring Marchigian sP rats (Panocka et al., 1993).

Further considerations. It might seem reasonable that a less severe indication should be more responsive to treatment than a more pronounced and persistent manifestation of that indication. Using this reasoning, an effective medication should more effectively reduce moderate drinking by a non-dependent animal than heavier drinking by dependent or genetically predisposed animals. This assumption is likely to be wrong, however. As dependence progresses, genes, systems and circuitry are recruited to maintain homeostasis. These may be distinct from those which regulate moderate drinking.

For example, acamprosate's therapeutic efficacy is hypothesized to depend, in part, on its ability to restore a hyper-glutamatergic resulting from prolonged alcohol use. Acamprosate is predicted to be less effective in animals with normal glutamatergic function. Other agents for which there has been little or no testing in human alcoholics also show increased efficacy in heavy drinking models, presumably because they act upon relevant dysregulated systems. Thus, the CRF receptor antagonist d-Phe-CRF reduced ethanol-reinforced level pressing only in rats with a history of ethanol dependence (Valdez et al., 2002). Similarly, a NPY Y2 receptor antagonist was more effective at reducing ethanol

self-administration by Wistar rats with a history of alcohol dependence than rats with no such history (Rimondini et al., 2005). Nociceptin, the endogenous ligand of the opioid-like orphan receptor NOP, reduced ethanol drinking by genetically selected Marchigian Sardinian alcohol-preferring rats, but had no effect on ethanol drinking by Wistar rats (Ciccocioppo et al., 2004; Fedeli et al., 2004). It is interesting that the clinically ineffective medication ritanserin appears to have the opposite effect, reducing drinking by rats that are not predisposed to heavy drinking. These findings suggest that a medication effect selective to heavy drinking models might predict a positive clinical outcome. This prediction remains to be confirmed through clinical testing of CRF, NPY and NOP drugs, and further testing of clinically ineffective compounds under conditions directly comparing post-dependent, or genetically predisposed animals with appropriate control animals.

Alcohol deprivation: clinically effective medications

Naltrexone. Acute naltrexone administration was more effective in reducing drinking following alcohol reinstatement than on baseline drinking in monkeys (Kornet et al., 1991) and in rats (Hölter & Spanagel, 1999). In the latter study, intermittent naltrexone treatment moderately attenuated the alcohol deprivation effect when administered following reinstatement. Chronic naltrexone administration during the alcohol-free period prevented elevated alcohol drinking following reinstatement (Heyser et al., 2003).

Acamprosate. Acamprosate administration (alone or in combination with naltrexone) during alcohol-free periods prevented elevated alcohol drinking following reinstatement (Heyser et al., 2003). Repeated acamprosate administration during the first 48 hours of reinstatement reduced drinking in a dose-dependent manner (Spanagel et al., 1996a).

Further considerations. Clinically effective medications seem to have more robust effects on drinking following a period of abstinence than on baseline drinking. Thus, the alcohol deprivation paradigm, like the heavy drinking models, might be more likely to detect clinically effective medications. Medication effects during abstinence may be compared with those after reintroducing ethanol solutions to elucidate possible therapeutic mechanisms. Examining repeated deprivation and reinstatement periods would reveal whether treatment completely eliminates or merely delays the escalation of drinking with each reinstatement. Clinically ineffective compounds await testing in this model.

Reinstatement of alcohol seeking: clinically effective medications

Naltrexone. Naltrexone administration reduced reinstatement of alcohol-seeking precipitated by ethanol injections, and by cues associated with ethanol administration, but not by stress (Bienkowski, 1999; Katner et al., 1999; Lê et al., 1999; Ciccocioppo et al., 2002; Liu & Weiss, 2002). Naltrexone's ability to attenuate cue-induced reinstatement was signifi-

cantly reduced in rats having a history of repeated ethanol vapor exposure and withdrawal (Ciccocioppo et al., 2003).

Acamprosate. Like naltrexone, acamprosate administration selectively reduced alcohol seeking evoked by environmental cues predictive of alcohol availability (Bachteler et al., 2005). It is unclear whether this effect is due to functional NMDA receptor antagonism, as the NMDA antagonist neramexane had no effect on cue-induced reinstatement.

Clinically ineffective medications

SSRIs. Both naltrexone and fluoxetine decrease alcohol self-administration. However, only naltrexone selectively blocked reinstatement precipitated by a priming injection of ethanol, but not stress-induced reinstatement, whereas fluoxetine blocked stress-induced reinstatement, while having less consistent effects on alcohol-induced reinstatement (Lê et al., 1999).

Further considerations. The isomorphism with conditions which precipitate human relapse, the selective medication effects on precipitating events, and the ability to dissociate naltrexone and acamprosate effects from SSRIs make the reinstatement paradigm attractive for medications testing. Many additional drug classes have been tested in the reinstatement paradigm including dopamine antagonists, CRF antagonists, nociceptin and metabotropic and ionotropic glutamate receptor agonists and antagonists. The success to which outcomes using the reinstatement paradigm predict whether specific clinical indications such as craving or relapse are amenable to treatment by medications acting at these receptors is currently unknown.

Conditioned place preference: clinically effective medications

Naltrexone. Naltrexone reduced the expression of place conditioning in two studies (Middaugh & Bandy, 2000; Kuzmin et al., 2003). In other studies, naloxone administration had little effect on the initial expression of place preference conditioning, but reduced the maintenance of a conditioned preference following repeated tests (Cunningham et al., 1995, 1998). Testing medications on repeated preference tests may prove to be the most sensitive use of the conditioned place preference paradigm for medications testing. Opiate receptor blockade does not appear to affect the acquisition of conditioned place preferences to ethanol.

Acamprosate. In contrast to opiate antagonists, acamprosate dose-dependently reduced the development of conditioned place preferences to ethanol and cocaine, but not to morphine (McGeehan & Olive, 2003).

Clinically ineffective medications. The place-conditioning paradigm may prove to be useful for evaluating test medications if testing procedures sensitive to clinically effective medications are developed further. Unlike in self-administration paradigms, SSRIs do not affect place conditioning to ethanol (Risinger, 1997). Neither bromocriptine nor ritanserin effects on place

conditioning to ethanol appear in the literature, although place preferences conditioned to bromocriptine administration have been reported (Hoffman et al., 1988).

Locomotor stimulation and sensitization: clinically effective medications

Naltrexone. Naltrexone pretreatment reliably blocked ethanol-induced locomotion in mice, as well as the development of locomotor sensitization (Kiianmaa et al., 1983; Camarini et al., 2000; Sanchis-Segura et al., 2004).

Acamprosate. Acamprosate administration attenuated the expression of sensitization to ethanol-induced stimulation in mice bred selectively for ethanol preference (Chester et al., 2001). To my knowledge, the effects of acamprosate administration on the locomotor-stimulant effects of alcohol prior to sensitization have not been reported.

Clinically ineffective medications

SSRIs. A variety of SSRIs failed to block alcohol-induced locomotion (Durcan et al., 1988).

Bromocriptine. Bromocriptine blocked the locomotor stimulatory effects of 0.5 g/kg ethanol at doses which did not affect locomotion when administered alone (Uzbay & Kayir, 2003). The inhibitory effect of bromocriptine on ethanol-induced locomotion was explained as being mediated by presynaptic D₂ autoreceptors.

Further considerations. Testing locomotion is relatively simple, requiring limited investment in time, equipment or training. Although the locomotor sensitization paradigm is often used to study the enhanced incentive salience hypothesized to contribute to stimulant and opioid abuse, its validity as a model of alcoholism is less clear. Locomotor sensitization to ethanol is an unreliable phenomenon in alcohol-preferring mice such as the C57 strain. Nevertheless, ethanol locomotor sensitization may correlate with selection for ethanol pre-

ference in HAP and LAP mice (Grahame et al., 2000). Further confirmation of this relationship would recommend testing medication effects on locomotor sensitization in these mice.

Drug discrimination. Animals or humans trained to discriminate the effects of alcohol from placebo show a remarkable ability to distinguish these effects from those of drugs possessing different pharmacological properties. Ethanol's discriminative stimulus effects are largely mediated by GABA_A and NMDA receptors (Grant & Colombo, 1993). It may seem intuitive that blocking the predominant subjective effects of ethanol would reduce alcohol drinking. The relationship between ethanol's discriminative stimulus effects and its reinforcing effects are complex, however.

Naltrexone significantly reduced discriminative stimulus effects associated with ethanol's excitatory phase (6 minutes post-injection), but was ineffective in antagonizing cues associated with ethanol's sedative effects (Shippenberg & Altshuler, 1985). Other studies failed to show an effect of naltrexone on ethanol discrimination under similar conditions, however (Altshuler et al., 1981; Middaugh et al., 1999). Similarly, acamprosate administration did not affect ethanol discrimination (Spanagel et al., 1996b). The ability to antagonize ethanol's discriminative stimulus effects does not appear to be a necessary property of a clinically effective medication for alcoholism.

Summary and future directions

Table 1 summarizes the effects of the Food and Drug Administration (FDA) approved medications naltrexone and acamprosate and the clinically ineffective SSRIs, bromocriptine and ritanserin. Two additional clinically promising compounds, baclofen and ondansetron, are also shown. It is clear that no single paradigm clearly differentiates clinically effective from clinically ineffective compounds.

Several paradigms—voluntary drinking (moderate and high drinking), alcohol deprivation, cue-induced reinstatement, conditioned place preference, locomotor sensitization—are sensitive to the effects of naltrexone (or naloxone) and acamprosate. Voluntary drinking and operant self-administration tests also yield false positives in that they are reduced by SSRIs, bromocriptine and ritanserin under some conditions.

Table 1. Medication Effects in Animal Behavioral Paradigms

	NAL	ACM	BAC	ODN	SSRIs	BRO	RIT
Limited Access	+	+		+	+		
Continuous Access	<i>mixed</i>	<i>weak</i>	<i>mixed</i>		+	<i>mixed</i>	<i>mixed</i>
Self-administration	+	—	+	—	+	+	+
Selectively Bred: Drinking	+	+	+		+	+	—
Dependence Induced Drinking		+					
Alcohol Deprivation	+	+	+				
Reinstatement							
Priming	+				—		
Cue	+	+			—		
Stress	—				+		
Conditioned Place Preference	+	+	—		—		
Locomotion	+		+		—	+	
Sensitization	+	+	+		—		
Discrimination	—	—		—	—		

NAL = Naltrexone/Naloxone, ACM = Acamprosate, BAC = Baclofen, ODN = Ondansetron; SSRI = Serotonin Specific Reuptake Inhibitor; BRO = Bromocriptine; RIT = Ritanserin

Focusing on relevant phenotypes

Voluntary ethanol drinking, reinforcement, and preference conditioning in animals with limited alcohol experience are widely used to study the neurobiological basis of alcohol drinking and the brain systems underlying ethanol motivation and reward. They have limited value as pre-clinical medication evaluations, however. For example, demonstrating changes in drinking following medication administration provides virtually no information as to the medication's potential clinical efficacy.

When these paradigms focus on features of addiction, such as the excessive, compulsive and persistent ingestion patterns, they are more likely to model biological targets relevant to an alcoholism medication's therapeutic efficacy. Some paradigms have been discussed previously, such as the heavy drinking, alcohol deprivation, reinstatement paradigms and model characteristics of addiction. Further testing of compounds possessing or lacking clinical efficacy in these paradigms would reveal whether these models are more informative medication screens than those which do not model addiction. This could be achieved by comparing medication effects in susceptibility or dependence models of heavy alcohol drinking with effects in non-susceptible animals having no history of dependence, and determining whether clinically effective drugs selectively reduce drinking in the heavy drinking models. Although existing alcohol drinking paradigms model aspects of addiction to ensure sufficient mechanistic diversity, it will also be important to adapt emerging paradigms which model the transition to addiction-like behavior (e.g. Deroche-Gamonet et al., 2004; Vanderschuren & Everitt, 2004).

Test batteries to reveal effective medications

Alcoholism and alcohol dependence are not unitary disorders as reflected by ongoing classifications by patient subtypes (e.g. Type I, Type II, late-onset, early-onset). The variety of clinical indications associated with alcoholism could be modified through many of the diverse pathways and systems affected by alcohol. Given this complexity, no single pre-clinical test is expected to discriminate clinically effective from clinically ineffective compounds. In fact, there may be no definitive pattern of hits on a battery of screens associated with clinical efficacy overall. Rather, there is likely to be conditionally interrelated clusters of responses and non-responses on a battery of protocols which predict clinical efficacy for specific clinical indications, in specific patient subtypes, through specific mechanisms of action. A compound possessing any of these profiles, or approximations of these profiles, would be predicted to have clinical efficacy. The challenge remains to construct such test batteries.

Conversely, it will be important to identify test responses which predict lack of clinical efficacy. Ultimately, if clinically ineffective neurochemical profiles have been identified either through clinical trials or pre-clinical behavioral tests, compounds having these characteristics should be excluded from behavioral screening.

Rapid preliminary assays

Many paradigms, particularly those having a higher degree of isomorphism with human alcoholism, require considerable

time and equipment. Pre-clinical assessments are most valuable when they provide information rapidly and inexpensively. It is possible to identify rapid, inexpensive tests which yield the same information as the more elaborate models, although they may be less obvious models of alcoholism. For example, if medications which block stress-induced reinstatement of alcohol-seeking also block simpler stress responses, it would not be necessary to use the stress-induced reinstatement paradigm in preliminary drug discovery assays. Moreover, acamprosate, naloxone and baclofen (Broadbent & Harless, 1999) reduced ethanol-induced locomotor sensitization. Testing the effects of acamprosate and other medications purported to have clinical efficacy on ethanol-stimulated locomotion would reveal whether this simple paradigm has discriminative properties which recommend this procedure as a primary screen.

Another emerging opportunity is to screen compounds in an array of genetically modified mice having a common target phenotype such as elevated alcohol consumption. NPY (Thiele et al., 1998), ENT1 (Choi et al., 2004), Per2 (Spanagel et al., 2005), the adenosine A2A receptor (Naassila et al., 2002) and delta-opioid receptor (Roberts et al., 2001) are among the targeted gene mutations which elevate alcohol drinking. Identifying response profiles in genetically modified mice which predict effects in the more elaborate alcoholism models (and, ultimately, in the clinic) will facilitate using these models in a primary screen. In addition to assessing efficacy, this approach would also confirm a compound's mechanism of therapeutic action, and possibly reveal new mechanisms.

Ongoing validation

Validating pre-clinical tests requires an ongoing assessment of their concordance with outpatient studies. As test protocols are implemented, methods for assessing the predictive contribution of each test and a combination of tests could be developed. Coordinated efforts to improve the tests such that, in the composite, they reliably predict subsequent clinical efficacy will facilitate our ability to screen medications. Progress toward validating these screens would be facilitated by testing compounds with purported clinical efficacy such as naltrexone, acamprosate, topiramate, baclofen and ondansetron in alcoholism models. Their effects would then be compared to one another, and to those of clinically ineffective compounds. The resulting patterns of responses could then be related to their specific clinical actions. This effort would be augmented by testing medications currently approved for human use but which have not been tested in alcoholics. Those showing positive responses in the preclinical battery—positive responses being somewhat subjective at this stage—would be administered in early Phase II clinical trials for alcoholism. Discontinuing tests and screens having little predictive value, and introducing and evaluating new tests and screens would strengthen pre-clinical test development over time.

Conclusion

Despite their widespread use as basic research tools, experimental paradigms and animal models of alcoholism have not fulfilled their potential usefulness for discovering clinically

effective medications for alcoholism. Tests using optimal combinations of informative alcoholism models have not been widely implemented. Clinical studies revealing effective medications and their phenotypic targets are badly needed to support further model development. Coordinated efforts to develop and implement medication screening programs for alcoholism are currently possible, and are likely to contribute to the discovery of new medications for alcoholism and alcohol use disorders over the next decade.

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