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Effects of disulfiram and dopamine beta-hydroxylase knockout on cocaine-induced seizures

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Abstract

The antialcoholism drug disulfiram has shown recent promise as a pharmacotherapy for treating cocaine dependence, probably via inhibition of dopamine β -hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine (DA) to norepinephrine (NE). We previously showed that DBH knockout (*Dbh* $-/-$) mice, which lack NE, are susceptible to seizures and are hypersensitive to the psychomotor, rewarding, and aversive effects of cocaine, suggesting that disulfiram might exacerbate cocaine-induced seizures (CIS) by inhibiting DBH. To test this, we examined CIS in wild-type and *Dbh* $-/-$ mice following administration of disulfiram or the selective DBH inhibitor nepicastat. We found that *Dbh* genotype had no effect on CIS probability or frequency, whereas disulfiram, but not nepicastat, increased the probability of having CIS in both wild-type and *Dbh* $-/-$ mice. Both disulfiram and nepicastat increased CIS frequency in wild-type but not *Dbh* $-/-$ mice. There were no genotype or treatment effects on serum cocaine levels, except for an increase in disulfiram-treated *Dbh* $-/-$ mice at the highest dose of cocaine. These results suggest that disulfiram enhances CIS via two distinct mechanisms: it both increases CIS frequency by inhibiting DBH and increases CIS frequency in a DBH-independent manner.

Keywords

norepinephrine; dopamine beta-hydroxylase; disulfiram; nepicastat; seizure; cocaine

1. Introduction

Cocaine is considered the most potent stimulant of natural origin, yet despite a host of negative social, psychological, and medical consequences of the drug's abuse, there are currently no widely used pharmacotherapies for cocaine addiction. Recently, the compound disulfiram, also known as Antabuse (Odyssey Pharmaceuticals, East Hanover, NJ), has shown promise as a candidate pharmacotherapy. Disulfiram has been FDA approved for the treatment of alcohol dependence for over 50 years. Its efficacy is due to the inhibition of the enzyme aldehyde dehydrogenase, which induces an aversive reaction following alcohol consumption by elevating toxic aldehydes in the liver. This "Antabuse reaction" manifests as flushing, headache, nausea, weakness, dizziness, anxiety, vertigo, and ataxia. Interestingly, disulfiram

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has been shown to decrease cocaine intake regardless of concurrent alcohol consumption (Carroll et al., 1998, 2000; George et al., 2000; Petrakis et al., 2000), although the mechanisms by which it does so have not been fully elucidated. Nevertheless, because the combination of disulfiram and cocaine does not result in aldehyde accumulation, aldehyde dehydrogenase inhibition probably cannot account for the efficacy of disulfiram here. Because its major metabolite (diethyldithiocarbamate) is a copper chelator (Johansson, 1989), disulfiram affects enzymatic reactions that require copper as a cofactor. One hypothesis to account for disulfiram's efficacy in reducing cocaine intake is its inhibition of dopamine β -hydroxylase (DBH). DBH is a copper-containing monooxygenase enzyme that converts dopamine (DA) to norepinephrine (NE), thus controlling NE production and consequently the NE/DA ratio in noradrenergic neurons. Alteration of this ratio has been found to alter behavioral responsivity to cocaine in rodents and humans. For example, DBH knockout (*Dbh* $-/-$) mice are hypersensitive to the locomotor, rewarding, and aversive effects of cocaine (Schank et al., 2006). Pharmacological inhibition of DBH with disulfiram, which decreases the NE/DA ratio in the rodent brain (Karamanakos et al., 2001; Bourdlat-Parks et al., 2005), facilitates the development of behavioral sensitization to cocaine (Haile et al., 2003). Furthermore, a common polymorphism in the *Dbh* gene influences both DBH enzymatic activity and cocaine-induced paranoia (Zabetian et al., 2001; Kalayarisi et al., 2007).

Noradrenergic transmission has been implicated in the modulation of seizure activity (reviewed by Weinshenker and Szot, 2002). Enhancement of noradrenergic transmission suppresses seizure activity (Lindvall, et al., 1988; Weinshenker et al., 2001; Kaminski et al., 2005), whereas norepinephrine depletion with 6-hydroxydopamine or disulfiram exacerbates seizures and facilitates seizure kindling (Corcoran, et al., 1974; Callaghan and Schwark, 1979; McIntyre, 1980; Abed, 1994; Amabeoku and Syce, 1997), and *Dbh* $-/-$ mice have increased susceptibility to seizure induced by flurothyl, pentylenetetrazole, kainic acid, and sound (Szot et al., 1999).

Approximately 27% of all drug-related emergency room episodes are related to cocaine abuse (SAMHSA, 1996). Cocaine-induced seizures are a manifestation of the toxicity associated with the drug, and estimates are that 8-12% of patients admitted to emergency departments with cocaine intoxication have seizures (Derlet and Albertson, 1989; Dhuna et al., 1991; Koppel et al., 1996). These seizures can be resistant to common anticonvulsant drugs, such as benzodiazepines and barbiturates, and constitute a major fraction of cocaine-related deaths (Dhuna et al., 1991; Benowitz et al., 1993). In addition, there have been several reports of individuals without a history of epilepsy developing seizures following treatment with therapeutic doses of disulfiram (Liddon and Satran, 1967; Price and Silberfarb, 1976a, 1976b; McConchie et al., 1983; Daniel et al., 1987).

Concurrent use of cocaine and disulfiram is now on the rise, as disulfiram is under evaluation as a pharmacotherapy for cocaine dependence. Because pharmacological or genetic inhibition of DBH increases the sensitivity to seizures and the behavioral effects of cocaine, we sought to examine the effects of DBH and disulfiram on susceptibility to cocaine-induced seizures (CIS). We measured the probability of having a seizure and the frequency of CIS following a high dose of cocaine (60 mg/kg) in both wild-type (*Dbh* $+/+$) and *Dbh* $-/-$ mice. We hypothesized that (1) *Dbh* $-/-$ mice would be hypersensitive to cocaine-induced seizures (CIS) and (2) disulfiram would exacerbate CIS in a *Dbh* genotype-dependent manner. To further examine whether disulfiram affects cocaine responses via a DBH-dependent mechanism, we also tested the selective DBH inhibitor nepicastat (Stanley et al., 1997). To determine whether the effects of these drugs could be attributed to changes in cocaine metabolism, we also measured peak serum cocaine levels.

2. Methods

2.1 Animals and housing

Adult *Dbh* $+/+$ and $-/-$ mice maintained on a mixed 129/SvEv and C57BL/6/J background were developed and generated as previously described (Thomas et al, 1995, 1998). Genotypes were confirmed by PCR. All mice were reared in a specific pathogen-free facility with a 12-h light/dark cycle (lights on at 0700 h, lights off at 1900 h); food and water were available ad libitum. Naïve mice between 3 and 6 months of age were used for all experiments, as were both male and female mice. No sex differences were observed, and results were combined. Experimental protocols were approved by the Emory University IACUC and meet the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2 Cocaine-induced seizures

Mice were given 3 injections of saline, disulfiram (100 mg/kg, i.p.), or the selective DBH inhibitor nepicastat (100 mg/kg, i.p.), with 2 hours between each injection. Two hours following the last injection, all mice were injected with a high dose of cocaine (60 mg/kg, i.p.). This dose was found to induce seizures in ~50% of mice of the same strain during a pilot study. Mice were observed for 30 minutes following cocaine administration, and the latency to first seizure and seizure frequency were recorded. The first seizure and/or ataxia typically occurred within 2-4 minutes postinjection. Seizures were defined as repetitive, rapid periods of jumping, wild-running, tonic-clonic activity, or a loss of the righting reflex. $N = 9-16$ for each treatment group.

2.3 Cocaine metabolism

Mice were given 3 injections of either saline or disulfiram (100 mg/kg, i.p.) each 2 hours apart. Two hours following their last injection, they were injected with cocaine (20 or 60 mg/kg, i.p.). These two doses were chosen because the 20-mg/kg dose supports a conditioned place preference in control mice, but elicits a conditioned place aversion in *Dbh* $-/-$ mice, while the 60-mg/kg dose was the one used to produce seizures in this study. Mice were decapitated 5 minutes later. We chose this time point because cocaine levels peak in mice ~5 minutes after i.p. cocaine administration (Benuck et al., 1987). Trunk blood was collected in microcentrifuge tubes containing 5 μ l each of NaF (132mg/ml) and $(\text{COOK})_2$ (106.7 mg/ml). Blood was centrifuged for 5 minutes at 10K, and serum was isolated and frozen until analysis. Serum cocaine levels were measured by HPLC. $N = 3-8$ for each genotype and treatment group.

2.4 Measurement of serum cocaine levels by HPLC

Cocaine was quantified in mouse serum by extraction with preparatory columns and isocratic HPLC with UV detection. Varian Bond Elut Certify C18 (130 mg) preparatory columns were placed on a vacuum manifold and pretreated with 2 ml of methanol and then 2 ml of 100 mM phosphate buffer (pH 6) at constant pressure (5 psi). Two hundred μ l of calibrators, controls, and samples were mixed by vortexing with 20 μ l of a 10 μ g/ml nalorphine solution (internal standard), 1.8 ml of saline, and 1 ml of 100 mM phosphate buffer (pH 6). Columns were washed with 6 ml of Milli-Q water, then 3 ml of 1 M acetic acid solution, and finally vacuum dried. Next, the columns were washed with 6 ml of methanol, and then cocaine was eluted with 2 ml of dichloromethane:isopropanol (80:20) containing 2% ammonium hydroxide. Final sample eluates were dried to residue with streaming nitrogen; residues were redissolved in 250 μ l of mobile phase, then 100 μ l of each sample was injected into the HPLC system [Waters model 510 pump, Waters 717 sample injector, Waters 2587 UV detector; a Phenomenex C18 column (5 micron, 4.5 mm ID $\times$ 150 mm L)]. The flow rate of the mobile phase was 1.5 ml/min. Cocaine, BE, and nalorphine were detected at a fixed wavelength of 214 nm. The mobile phase contained 8% acetonitrile, 12% methanol, and 80% of a solution of 12 mM KH_2PO_4 (pH 2.5).

Calibrators were spiked mouse serum at concentrations of 100, 500, 1000, 5000, and 10000 ng/ml. Cocaine and benzoylecgonine concentrations were expressed in ng/ml.

2.5 Drugs

All drugs were freshly prepared before being used and injected i.p. in a volume of 10 ml/kg. Cocaine HCl (Sigma Aldrich, St Louis, MO) and nepicastat (Roche Biosciences, Palo Alto, CA) were dissolved in 0.9% NaCl. Disulfiram (Sigma Aldrich, ST Louis, MO) was injected as a suspension in 0.9% NaCl following sonication.

2.6 Statistical analyses

The effect of disulfiram and nepicastat on the probability of an animal exhibiting a cocaine-induced seizure was analyzed using the χ^2 distribution. The effect of the DBH inhibitors on the number of cocaine-induced seizures exhibited in a 30-minute period and serum cocaine levels were analyzed using a 2-way completely randomized design ANOVA with post-hoc Bonferroni comparisons. A p -value of <0.05 was considered significant. Outliers were detected using the Grubb's test. One subject (disulfiram-treated *Dbh* $-/-$) met this criteria and was removed from all statistical analysis.

3. Results

3.1 Disulfiram and cocaine-induced seizures

Dbh genotype alone had no effect on CIS probability; 64.2% of *Dbh* $+/+$ and 66.6% of *Dbh* $-/-$ mice had seizures following cocaine administration (Fig. 1A). Disulfiram pretreatment increased the probability of having at least one cocaine-induced seizure in both *Dbh* $+/+$ and *Dbh* $-/-$ mice with similar magnitude (*Dbh* $+/+$, 64.2% saline vs. 95% disulfiram; *Dbh* $-/-$, 66.6% saline vs. 92.9% disulfiram) (Fig. 1A). This effect was statistically significant in *Dbh* $+/+$ mice, but did not quite reach significance in *Dbh* $-/-$ mice (*Dbh* $+/+$, $\chi^2 = 5.346$, $p=0.02$; *Dbh* $-/-$, $\chi^2 = 3.027$, $p=0.08$).

Dbh genotype alone also had no effect on CIS frequency (Fig. 1B); however, disulfiram increased CIS frequency in *Dbh* $+/+$ but not *Dbh* $-/-$ mice (Fig. 1B). ANOVA revealed a significant effect of pretreatment, with disulfiram significantly increasing the number of seizures during the 30-minute test period following cocaine administration in wild-type (WT) but not knockout (KO) mice ($F=12.58$, $df=1$; $p < 0.001$ for WT). An additional ANOVA was performed only on data from subjects exhibiting at least one cocaine-induced seizure. Analysis of seizure frequency in these animals showed the same effect of disulfiram, as pretreatment with the drug significantly increased seizure frequency only in wild-type mice ($F= 14.13$, $df=1$; $p < 0.001$) (Figure 1C).

3.2 Nepicastat and cocaine-induced seizures

To further determine whether the effects on CIS from disulfiram were mediated by DBH inhibition, we tested the selective DBH inhibitor nepicastat. In contrast to disulfiram, nepicastat pretreatment did not affect the probability of having a cocaine-induced seizure in wild-type mice, and in fact significantly reduced this probability in *Dbh* $-/-$ mice ($\chi^2 = 5.35$; $p < 0.05$) (Fig. 2A).

The pattern of nepicastat effects on CIS frequency was similar to that of disulfiram. Nepicastat pretreatment increased CIS frequency in wild-type but not *Dbh* $-/-$ mice. The effect on *Dbh* $+/+$ mice did not quite reach significance when all mice were included in the analysis ($F=0.3672$, $df= 1$; $p > 0.05$) (Fig. 2B), but did reach significance when data from animals exhibiting at least one seizure were analyzed ($F=1.518$, $df= 1$; $p < 0.05$) (Fig. 2C).

3.3 Cocaine metabolism

To determine whether the disulfiram-related rise in CIS probability and frequency could be attributed to drug effects on cocaine metabolism, we measured peak serum cocaine levels in *Dbh* $+/+$ and $-/-$ mice after administration of 20 or 60 mg/kg of cocaine. We found that cocaine serum levels were unaffected either by *Dbh* genotype or by disulfiram in most cases. The exception was an increase in serum cocaine levels by disulfiram in *Dbh* $-/-$ mice at the high dose of cocaine, and ANOVA revealed a genotype $\times$ treatment interaction ($F=5.312$, $df=1$; $p<0.05$) (Figure 3). These results indicate that changes in cocaine metabolism do not underlie the effects of disulfiram on CIS.

4. Discussion

Cocaine-induced seizures account for approximately 10% of cocaine-related emergency room visits to hospitals and are a common manifestation of cocaine toxicity. While there are no widely accepted pharmacotherapies for cocaine addiction, disulfiram has shown recent promise as a treatment for cocaine dependence. Unfortunately, this treatment may be hazardous to some patients, due to its mechanism of action. Disulfiram inhibits the enzyme DBH, which could lead to increases in seizures and cocaine sensitivity. Therefore, we chose to examine how pharmacological DBH inhibition and DBH genotype affects CIS probability and frequency in an animal model. In order to assess this, we pretreated *Dbh* $+/+$ and $-/-$ mice with disulfiram or nepicastat, a direct, selective DBH inhibitor, prior to administering a high dose of cocaine. We hypothesized that *Dbh* $-/-$ mice would be hypersensitive to CIS and that disulfiram would exacerbate CIS in a *Dbh* genotype-dependent manner. We also assessed changes in serum cocaine levels to determine whether the effects of these drugs could be attributed to changes in cocaine metabolism.

Disulfiram had two distinct effects on CIS; it increased the probability of having a seizure in *Dbh* $+/+$ and *Dbh* $-/-$ mice and increased CIS frequency in *Dbh* $+/+$ mice only. Nepicastat did not increase seizure probability, but increased the frequency of CIS in *Dbh* $+/+$ mice only. These results indicate that pharmacological DBH inhibition is responsible for increasing the frequency of CIS, while disulfiram's ability to raise the probability of CIS is mediated by a DBH-independent mechanism. Given disulfiram's mechanism of action as a copper chelator, the inhibition of cocaine metabolic enzymes, such as cholinesterase and carboxylesterase, could underlie its effects on CIS probability. However, we did not find this to be the case, as disulfiram did not alter serum cocaine concentrations in wild-type mice. Interestingly, nepicastat actually tended to inhibit CIS in *Dbh* $-/-$ mice. Nepicastat does not chelate copper and is a direct, potent inhibitor of DBH, and initial screening did not reveal any other high-affinity targets aside from DBH. It appears that the lack of DBH in *Dbh* $-/-$ mice revealed a secondary anticonvulsant target for nepicastat.

Because pharmacological DBH inhibition increased CIS frequency, *Dbh* $-/-$ mice would be expected to demonstrate more frequent CIS. We did not find this to be the case. One possible explanation is that compensatory changes in monoamine neurotransmitters arise following chronic and complete knockout of DBH and NE function. One likely compensatory candidate is the serotonin (5-HT) system. Serotonergic activity modulates seizure activity in response to cocaine and seems to have proconvulsant effects. Selective 5-HT reuptake inhibitors, such as fluoxetine, citalopram, paroxetine and the tricyclic antidepressant imipramine, all facilitate CIS (O'Dell et al., 1999; Ritz and George, 1997), whereas 5-HT₂ receptor antagonists decrease CIS (O'Dell, et al., 1999, 2000; Ritz and George, 1997; Schechter and Meehan, 1995). Since cocaine is known to inhibit 5-HT transporters (SERT), increases in 5-HT following cocaine administration can lead to accumulation of serotonin in synapses, which in turn can increase seizure activity via 5-HT₂ receptor activation. The serotonergic raphe nuclei receive dense innervation from brainstem noradrenergic nuclei (Baraban and Aghajanian, 1981;

Marcinkiewicz et al., 1989; Fritschy and Grzanna, 1990; Peyron et al., 1996), and activation of α_1 -adrenergic receptors increases tonic excitatory activity in the dorsal raphe nucleus (Baraban and Aghajanian, 1980; Vandermaelen and Aghajanian, 1983; Hertel et al., 1998; Pudovkina et al., 2003; Judge and Gartside, 2006). Therefore, *Dbh* $-/-$ mice should have lower levels of extracellular 5-HT, because they lack the noradrenergic excitatory drive on the serotonergic system. Indeed, when compared with control mice, *Dbh* $-/-$ mice have decreased 5-HT release in the nucleus accumbens following amphetamine treatment (D. Weinshenker and S. Puglisi-Allegra, unpublished observations), as well as in the hippocampus following fluoxetine administration (Cryan et al., 2004). This decreased concentration of the proconvulsant 5-HT in *Dbh* $-/-$ mice may explain their “normal” CIS susceptibility at baseline.

Because disulfiram acts as a copper chelator, this drug is relatively nonspecific and inhibits two cocaine-metabolizing enzymes, cholinesterase and carboxylesterase (Zemaitis and Greene, 1976; Nousiainen and Törrönen, 1984; Savolainen et al., 1984). Disulfiram treatment increased cocaine plasma levels and decreased cocaine clearance in humans following intranasal cocaine administration (McCance-Katz et al., 1998a,b; Hameedi et al., 1995; Baker et al., 2007). Thus, it is possible that the disulfiram-induced increase in CIS was a direct result of decreased cocaine metabolism. However, disulfiram did not alter peak serum cocaine levels in most cases in our study. The single exception was an interaction between disulfiram treatment and genotype; the higher dose of cocaine (60 mg/kg) significantly increased cocaine plasma levels in *Dbh* $-/-$ mice only. The mechanisms underlying this synergy is unclear. One possibility is that NE limits the spread of cocaine through the bloodstream via its vasoconstrictive properties, while cholinesterase and carboxylesterase are responsible for its metabolism. Perhaps at low doses of cocaine, either mechanism alone is sufficient to maintain “normal” peak cocaine serum levels, but at high doses, the impairment of both noradrenergic function and cocaine metabolic enzymes results in increased serum levels. It is not clear why disulfiram did not increase peak serum cocaine levels in wild-type mice. The differences between the effects of disulfiram on serum cocaine levels in humans and rodents may be due to different routes of cocaine administration (intranasal in humans vs. intraperitoneal in mice) or to species differences in cocaine metabolism.

Our results show that acute disulfiram administration increases the probability and frequency of CIS, which may present a clinical problem during cocaine addiction treatment with disulfiram pharmacotherapy. It should be noted that, while CIS have not been reported during cocaine dependence clinical trials examining disulfiram efficacy, there have been several reports of individuals without a history of epilepsy developing seizures following treatment with therapeutic doses of disulfiram (Liddon and Satran, 1967; Price and Silberfarb, 1976a, 1976b; McConchie et al., 1983; Daniel et al., 1987). Thus, clinicians should be cautious when considering disulfiram as a cocaine pharmacotherapy, particularly in patients with a history of epilepsy or cocaine overdose. The more selective DBH inhibitor nepicastat may be a safer alternative to disulfiram for treating cocaine dependence, as it does not increase CIS probability and is in fact anticonvulsant in *Dbh* $-/-$ mice. DBH activity is genetically controlled and highly variable in humans (Weinshilboum, 1978; Zabetian et al., 2001). The haplotype associated with low DBH activity in humans is also associated with more cocaine-induced paranoia (Cubells et al., 2000; Kalayarisi et al., 2007). This increase in one of the aversive properties of cocaine may underlie the effectiveness of DBH inhibition via disulfiram in curbing cocaine intake. Given that the proconvulsant effect of disulfiram on CIS frequency is absent in *Dbh* $-/-$ mice, disulfiram pharmacotherapy might perhaps be safer for cocaine addicts with low DBH activity. Preliminary data suggest that disulfiram is most effective for these individuals (R. Schottenfeld and J. Cubells, personal communication), possibly due to their enhanced aversion to cocaine. Our results indicate that they may also be resilient to disulfiram-induced exacerbation of CIS and possibly other toxic effects of cocaine.

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References

- Baker JR, Jatlow P, McCance-Katz EF. Disulfiram effects on responses to intravenous cocaine administration. *Drug Alcohol Depend* 2007;87:202–9. [PubMed: 16979847]
- Baraban JM, Aghajanian GK. Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography. *Brain Res* 1981;204:1–11. [PubMed: 6166350]
- Baraban JM, Aghajanian GK. Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. *Neuropharmacology* 1980;19:355–63. [PubMed: 6104308]
- Benowitz NL. Clinical pharmacology and toxicology of cocaine. *Pharmacol Toxicol* 1993;72:3–12. [PubMed: 8441738]
- Benuck M, Lajtha A, Reith ME. Pharmacokinetics of systemically administered cocaine and locomotor stimulation in mice. *J Pharmacol Exp Ther* 1987;243:144–9. [PubMed: 3668848]
- Bourdelat-Parks BN, Anderson GM, Donaldson ZR, Weiss JM, Bonsall RW, Emery MS, Liles LC, Weinschenker D. Effects of dopamine beta-hydroxylase genotype and disulfiram inhibition on catecholamine homeostasis in mice. *Psychopharmacology (Berl)* 2005;183:72–80. [PubMed: 16163519]
- Carroll KM, Nich C, Ball SA, McCance E, Rounsaville BJ. Treatment of cocaine and alcohol dependence with psychotherapy and disulfiram. *Addiction* 1998;93:713–27. [PubMed: 9692270]
- Corcoran ME, Fibiger HC, McCaughan JA Jr, Wada JA. Potentiation of amygdaloid kindling and metrazol-induced seizures by 6-hydroxydopamine in rats. *Exp Neurol* 1974;45:118–33. [PubMed: 4153438]
- Cryan JF, O'Leary OF, Jin SH, Friedland JC, Ouyang M, Hirsch BR, Page ME, Dalvi A, Thomas SA, Lucki I. Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. *Proc Natl Acad Sci U S A* 2004;101:8186–91. [PubMed: 15148402]
- Cubells JF, Kranzler HR, McCance-Katz E, Anderson GM, Malison RT, Price LH, Gelernter J. A haplotype at the DBH locus, associated with low plasma dopamine beta-hydroxylase activity, also associates with cocaine-induced paranoia. *Mol Psychiatry* 2000;5:56–63. [PubMed: 10673769]
- Daniel DG, Swallows A, Wolff F. Capgras delusion and seizures in association with therapeutic dosages of disulfiram. *South Med J* 1987;80:1577–9. [PubMed: 3423903]
- Derlet RW, Albertson TE. Emergency department presentation of cocaine intoxication. *Ann Emerg Med* 1989;18:182–6. [PubMed: 2916784]
- Dhuna A, Pascual-Leone A, Langendorf F, Anderson DC. Epileptogenic properties of cocaine in humans. *Neurotoxicology* 1991;12:621–6. [PubMed: 1745445]
- Fritschy JM, Grzanna R. Distribution of locus coeruleus axons within the rat brainstem demonstrated by Phaseolus vulgaris leucoagglutinin anterograde tracing in combination with dopamine-beta-hydroxylase immunofluorescence. *J Comp Neurol* 1990;293:616–31. [PubMed: 2329197]
- George TP, Chawarski MC, Pakes J, Carroll KM, Kosten TR, Schottenfeld RS. Disulfiram versus placebo for cocaine dependence in buprenorphine-maintained subjects: a preliminary trial. *Biol Psychiatry* 2000;47:1080–6. [PubMed: 10862808]
- Goldstein M, Anagnoste B, Lauber E, McKeregham MR. Inhibition of Dopamine-Beta-Hydroxylase by Disulfiram. *Life Sci* 1964;3:763–7. [PubMed: 14203977]
- Haile CN, During MJ, Jatlow PI, Kosten TR, Kosten TA. Disulfiram facilitates the development and expression of locomotor sensitization to cocaine in rats. *Biol Psychiatry* 2003;54:915–21. [PubMed: 14573319]
- Hameedi FA, Rosen MI, McCance-Katz EF, McMahon TJ, Price LH, Jatlow PI, Woods SW, Kosten TR. Behavioral, physiological, and pharmacological interaction of cocaine and disulfiram in humans. *Biol Psychiatry* 1995;37:560–3. [PubMed: 7619981]

- Hertel P, Lindblom N, Nomikos GG, Svensson TH. Modulation of central serotonergic neurotransmission by risperidone: underlying mechanism(s) and significance of action. *Prog Neuropsychopharmacol Biol Psychiatry* 1998;22:815–34. [PubMed: 9723122]
- Johansson B. Carbonyl sulfide: a copper chelating metabolite of disulfiram. *Drug Metab Dispos* 1989;17:351–3. [PubMed: 2568921]
- Judge SJ, Gartside SE. Firing of 5-HT neurones in the dorsal and median raphe nucleus in vitro shows differential alpha1-adrenoceptor and 5-HT1A receptor modulation. *Neurochem Int* 2006;48:100–7. [PubMed: 16256247]
- Kaminski RM, Shippenberg TS, Witkin JM, Rocha BA. Genetic deletion of the norepinephrine transporter decreases vulnerability to seizures. *Neurosci Lett* 2005;382:51–5. [PubMed: 15911120]
- Karamanakos PN, Pappas P, Stephanou P, Marselos M. Differentiation of disulfiram effects on central catecholamines and hepatic ethanol metabolism. *Pharmacol Toxicol* 2001;88:106–10. [PubMed: 11169169]
- Koppel BS, Samkoff L, Daras M. Relation of cocaine use to seizures and epilepsy. *Epilepsia* 1996;37:875–8. [PubMed: 8814101]
- Liddon SC, Satran R. Disulfiram (Antabuse) psychosis. *Am J Psychiatry* 1967;123:1284–9. [PubMed: 5336626]
- Lindvall O, Barry DI, Kikvadze I, Brundin P, Bolwig TG, Bjorklund A. Intracerebral grafting of fetal noradrenergic locus coeruleus neurons: evidence for seizure suppression in the kindling model of epilepsy. *Prog Brain Res* 1988;78:79–86. [PubMed: 3073428]
- Marcinkiewicz M, Morcos R, Chretien M. CNS connections with the median raphe nucleus: retrograde tracing with WGA-apoHRP-Gold complex in the rat. *J Comp Neurol* 1989;289:11–35. [PubMed: 2478595]
- McCance-Katz EF, Kosten TR, Jatlow P. Chronic disulfiram treatment effects on intranasal cocaine administration: initial results. *Biol Psychiatry* 1998;43:540–3. [PubMed: 9547934]
- McCance-Katz EF, Kosten TR, Jatlow P. Disulfiram effects on acute cocaine administration. *Drug Alcohol Depend* 1998;52:27–39. [PubMed: 9788003]
- McConchie RD, Panitz DR, Sauber SR, Shapiro S. Disulfiram-induced de novo seizures in the absence of ethanol challenge. *J Stud Alcohol* 1983;44:739–43. [PubMed: 6632888]
- McIntyre DC. Amygdala kindling in rats: facilitation after local amygdala norepinephrine depletion with 6-hydroxydopamine. *Exp Neurol* 1980;69:395–407. [PubMed: 7409053]
- Musacchio JM, Goldstein M, Anagnoste B, Poch G, Kopin IJ. Inhibition of dopamine-beta-hydroxylase by disulfiram in vivo. *J Pharmacol Exp Ther* 1966;152:56–61. [PubMed: 5937402]
- Nousiainen U, Torronen R. Differentiation of microsomal and cytosolic carboxylesterases in the rat liver by in vivo and in vitro inhibition. *Gen Pharmacol* 1984;15:223–7. [PubMed: 6735136]
- O'Dell LE, George FR, Ritz MC. Antidepressant drugs appear to enhance cocaine-induced toxicity. *Exp Clin Psychopharmacol* 2000;8:133–41. [PubMed: 10743914]
- O'Dell LE, Kreifeldt MJ, George FR, Ritz MC. The role of serotonin(2) receptors in mediating cocaine-induced convulsions. *Pharmacol Biochem Behav* 2000;65:677–81. [PubMed: 10764922]
- O'Dell LE, Kreifeldt MJ, George FR, Ritz MC. Serotonin(2C) receptors appear to mediate genetic sensitivity to cocaine-induced convulsions. *Psychopharmacology (Berl)* 1999;146:313–9. [PubMed: 10541732]
- Petrakis IL, Carroll KM, Nich C, Gordon LT, McCance-Katz EF, Frankforter T, Rounsaville BJ. Disulfiram treatment for cocaine dependence in methadone-maintained opioid addicts. *Addiction* 2000;95:219–28. [PubMed: 10723850]
- Peyron C, Luppi PH, Fort P, Rampon C, Jouvet M. Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *J Comp Neurol* 1996;364:402–13. [PubMed: 8820873]
- Price TR, Seiberfarb PM. Letter: convulsions following disulfiram treatment. *Am J Psychiatry* 1976a;133:235. [PubMed: 1251937]
- Price TR, Seiberfarb PM. Disulfiram-induced convulsions without challenge by alcohol. *J Stud Alcohol* 1976b;37:980–2. [PubMed: 972549]
- Pudovkina OL, Cremers TI, Westerink BH. Regulation of the release of serotonin in the dorsal raphe nucleus by alpha1 and alpha2 adrenoceptors. *Synapse* 2003;50:77–82. [PubMed: 12872296]

- Ritz MC, George FR. Cocaine-induced convulsions: pharmacological antagonism at serotonergic, muscarinic and sigma receptors. *Psychopharmacology (Berl)* 1997;129:299–310. [PubMed: 9085399]
- Savolainen K, Hervonen H, Lehto VP, Mattila MJ. Neurotoxic effects of disulfiram on autonomic nervous system in rat. *Acta Pharmacol Toxicol (Copenh)* 1984;55:339–44. [PubMed: 6528806]
- Schechter MD, Meehan SM. Serotonergic mediation of cocaine seizures in mice. *Pharmacol Biochem Behav* 1995;51:313–6. [PubMed: 7667346]
- Stanley WC, Li B, Bonhaus DW, Johnson LG, Lee K, Porter S, Walker K, Martinez G, Eglen RM, Whiting RL, Hegde SS. Catecholamine modulatory effects of nopicastat (RS-25560-197), a novel, potent and selective inhibitor of dopamine-beta-hydroxylase. *Br J Pharmacol* 1997;121:1803–9. [PubMed: 9283721]
- Szot P, Weinshenker D, White SS, Robbins CA, Rust NC, Schwartzkroin PA, Palmiter RD. Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli. *J Neurosci* 1999;19:10985–92. [PubMed: 10594079]
- Vandermaelen CP, Aghajanian GK. Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Res* 1983;289:109–19. [PubMed: 6140982]
- Weinshenker D, Szot P, Miller NS, Palmiter RD. Alpha(1) and beta(2) adrenoreceptor agonists inhibit pentylenetetrazole-induced seizures in mice lacking norepinephrine. *J Pharmacol Exp Ther* 2001;298:1042–8. [PubMed: 11504801]
- Weinshilboum RM. Human biochemical genetics of plasma dopamine-beta-hydroxylase and erythrocyte catechol-o-methyltransferase. *Hum Genet Suppl* 1978:101–12. [PubMed: 285022]
- Zemaitis MA, Greene FE. Impairment of hepatic microsomal and plasma esterases of the rat by disulfiram and diethyldithiocarbamate. *Biochem Pharmacol* 1976;25:453–9. [PubMed: 181003]

Fig. 1.

The effects of disulfiram *Dbh* genotype on cocaine-induced seizures. *Dbh* +/+ and *Dbh* -/- mice were pretreated with vehicle or disulfiram (3 doses of 100 mg/kg, i.p., each dose spaced 2 hours apart). Two hours after the last pretreatment, mice were injected with cocaine (60 mg/kg, i.p.), and behavior was observed for 30 min. Shown is (A) the percent of all mice tested having at least one seizure, (B) the mean ± SEM seizures observed in 30 min in all mice tested, and (C) the mean ± SEM seizures only in mice that had at least one seizure. N=14–20 per genotype and treatment group. * $P < 0.05$, *** $P < 0.001$ compared to vehicle control for that genotype.

Fig. 2.

The effects of nepicastat and *Dbh* genotype on cocaine-induced seizures. *Dbh* +/+ and *Dbh* -/- mice were pretreated with vehicle or nepicastat (3 doses of 100 mg/kg, i.p., each dose spaced 2 hours apart). Two hours after the last pretreatment, mice were injected with cocaine (60 mg/kg, i.p.), and behavior was observed for 30 min. Shown is (A) the percent of all mice tested having at least one seizure, (B) the mean ± SEM seizures observed in 30 min in all mice tested, and (C) the mean ± SEM seizures only in mice that had at least one seizure. N=9–15 per genotype and treatment group. * P < 0.05 compared to vehicle control for that genotype.

Fig 3.

The effects of disulfiram and *Dbh* genotype on cocaine metabolism. *Dbh* +/+ and *Dbh* -/- mice were pretreated with vehicle or disulfiram (3 doses of 100 mg/kg, i.p., each dose spaced 2 hours apart). Two hours after the last pretreatment, mice were injected with cocaine (20 or 60 mg/kg, i.p.), and blood was collected 5 min later. Shown is the mean $\pm$ SEM peak serum cocaine levels as measured by HPLC. N = 6-8 per genotype and treatment group. * P < 0.05 compared to vehicle control for that genotype.