Inhibition of aldehyde dehydrogenase-2 suppresses cocaine seeking by generating THP, a cocaine use–dependent inhibitor of dopamine synthesis

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There is no effective treatment for cocaine addiction despite extensive knowledge of the neurobiology of drug addiction1–4. Here we show that a selective aldehyde dehydrogenase-2 (ALDH-2) inhibitor, ALDH2i, suppresses cocaine self-administration in rats and prevents cocaine- or cue-induced reinstatement in a rat model of cocaine relapse-like behavior. We also identify a molecular mechanism by which ALDH-2 inhibition reduces cocaine-seeking behavior: increases in tetrahydropapaveroline (THP) formation due to inhibition of ALDH-2 decrease cocaine-stimulated dopamine production and release in vitro and in vivo. Cocaine increases extracellular dopamine concentration, which activates dopamine D2 autoreceptors to stimulate cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC) in primary ventral tegmental area (VTA) neurons. PKA and PKC phosphorylate and activate tyrosine hydroxylase, further increasing dopamine synthesis in a positive-feedback loop. Monoamine oxidase converts dopamine to 3,4-dihydroxyphenylacetaldehyde (DOPAL), a substrate for ALDH-2. Inhibition of ALDH-2 enables DOPAL to condense with dopamine to form THP in VTA neurons. THP selectively inhibits phosphorylated (activated) tyrosine hydroxylase to reduce dopamine production via negative-feedback signaling. Reducing cocaine- and craving-associated increases in dopamine release seems to account for the effectiveness of ALDH2i in suppressing cocaine-seeking behavior. Selective inhibition of ALDH-2 may have therapeutic potential for treating human cocaine addiction and preventing relapse.

The lack of effective medication for cocaine addiction and relapse is a major unmet medical need5. Recent anecdotal clinical reports suggest that disulfiram may attenuate cocaine use6. Disulfiram, an irreversible nonspecific inhibitor of ALDH-1 and ALDH-2, increases acetaldehyde accumulation to discourage alcohol drinking, owing to the adverse effects of acetaldehyde. Reduced cocaine use after disulfiram treatment has been attributed to disulfiram inhibition of dopamine β hydroxylase (DBH) in the brain7. We and others have shown that highly selective ALDH-2 inhibitors potently reduce alcohol seeking in the presence or absence of acetaldehyde8,9. These findings seem to be explained by changes in dopamine metabolism. Thus, the selective ALDH-2 inhibitor ALDH2i (CVT-10216) prevents alcohol-induced increases in dopamine in the nucleus accumbens4, which is not explained by inhibition of DBH. Indeed, ALDH2i does not inhibit DBH (Supplementary Table 1). Taken together, these observations suggest that a selective inhibitor of ALDH-2 might suppress cocaine seeking by reducing drug-associated increases in dopamine synthesis.

We test this possibility in vivo and in vitro. In a rat model of self-administration, ALDH2i inhibits intravenous cocaine infusions in a dose-dependent manner (Fig. 1a). Relapse is a serious limitation of effective medical treatment of cocaine addiction10,11. We therefore asked whether selective ALDH-2 inhibition can also prevent cocaine- or cue-induced cocaine relapse–like behavior in a reinstatement model. After rats deprived of cocaine extinguished cocaine seeking by generating THP, a cocaine use–dependent inhibitor of dopamine synthesis. Thus, the selective ALDH-2 inhibitor ALDH2i (CVT-10216) prevents alcohol-induced increases in dopamine in the nucleus accumbens4, which is not explained by inhibition of DBH. Indeed, ALDH2i does not inhibit DBH (Supplementary Table 1). Taken together, these observations suggest that a selective inhibitor of ALDH-2 might suppress cocaine seeking by reducing drug-associated increases in dopamine synthesis.

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Dopamine is synthesized in VTA neurons and axonally transported for release in the nucleus accumbens12,13. Addictive drugs activate VTA neurons, leading to increased dopamine release in the nucleus accumbens14,15. We thus determined whether ALDH2i inhibits cocaine-induced dopamine production in PC12 cells, a neural cell line derived from a rat adrenal medullary pheochromocytoma. We find that cocaine elevates extracellular and intracellular dopamine levels (Fig. 2a). ALDH2i prevents cocaine-induced dopamine increases in a dose-dependent manner (Fig. 2a). Notably, ALDH2i had no effect on basal dopamine (Fig. 2b). Moreover, blockade of dopamine D2 receptors by the D2 antagonist siperone prevented cocaine-induced increases in dopamine; the D1 antagonist SCH 23390 had no effect (Fig. 2c).

How does selective ALDH-2 inhibition block cocaine-induced increases in dopamine levels? ALDH-2 is highly expressed in...
Tyrosine hydroxylase is the first and rate-limiting step in dopamine synthesis. We searched for evidence that selective inhibition of ALDH-2 decreases cocaine-induced dopamine production in cocaine-treated PC12 cells. We found that ALDH2i decreases cocaine-induced dopamine production in cocaine-treated PC12 cells. We also found that ALDH2i had no effect on basal THP abundance in the absence of cocaine. THP inhibits dopamine metabolism, including DBH, monoamine oxidase A (MAO-A), MAO-B and ALDH-2. ALDH2i by itself had no effect on these enzymes other than ALDH-2 (Supplementary Table 1).

Tyrosine hydroxylase is activated by phosphorylation at Ser19, Ser31 and Ser40 (ref. 21). We asked whether cocaine activates dopamine synthesis by increasing tyrosine hydroxylase phosphorylation in primary VTA neurons. Western blotting showed that cocaine increases phosphorylation of tyrosine hydroxylase mainly at Ser40, which condenses with dopamine to form 3,4-dihydroxyphenyl-acetic acid (DOPAC), a substrate for ALDH-2. Unlike THP, the classic tyrosine hydroxylase inhibitor, α-methyl-l-tyrosine, was equally effective against tyrosine hydroxylase and phosphorylated tyrosine hydroxylase (Fig. 2f). THP did not inhibit the activities of other enzymes involved in dopamine metabolism, including DBH, monoamine oxidase A (MAO-A), MAO-B and ALDH-2. ALDH2i by itself had no effect on these enzymes other than ALDH-2 (Supplementary Table 1).

Tyrosine hydroxylase is activated by phosphorylation at Ser19, Ser31 and Ser40 (ref. 21). We asked whether cocaine activates dopamine synthesis by increasing tyrosine hydroxylase phosphorylation in primary VTA neurons. Western blotting showed that cocaine increases phosphorylation of tyrosine hydroxylase mainly at Ser40, with little or no effect at Ser19 and Ser31 (Fig. 3a). As a positive control we tested nomifensine, another dopamine reuptake inhibitor, and found that it produced similar changes in tyrosine hydroxylase phosphorylation (Fig. 3a). Immunostaining of VTA neurons confirmed that cocaine increases tyrosine hydroxylase phosphorylation at Ser40 (Fig. 3b).
VTA neurons express dopamine autoreceptors\(^22-24\). We asked whether dopamine receptors in VTA neurons are involved in cocaine-induced phosphorylation of tyrosine hydroxylase and dopamine production. Pretreatment of VTA neurons with the D2 antagonist spiperone completely blocked cocaine-induced tyrosine hydroxylase phosphorylation (Fig. 3b,c). In contrast, the D1 antagonist SCH23390 had no effect (Fig. 3b). These results suggest that D2 autoreceptors mediate phosphorylation of tyrosine hydroxylase in VTA primary neurons.

Tyrosine hydroxylase is a substrate for PKA and PKC\(^21\). As expected, activation of PKA by Sp-adenosine 3',5'-cyclic monophosphorothioate (Sp-cAMPS) or activation of PKC by phorbol 12-myristate 13-acetate mimics cocaine-induced phosphorylation of tyrosine hydroxylase in primary VTA neurons (Fig. 3a). By contrast, selective inhibition of PKA by Rp-cAMPS or PKC by GF109203X prevented cocaine-induced tyrosine hydroxylase phosphorylation at Ser40 (Fig. 3d). Inhibition of mitogen-activated protein kinase by U-0126 or Ca\(^{2+}\)-calmodulin-dependent protein kinase by KN-93 had no effect (Supplementary Fig. 2). Activation of D2 stimulates PKA and PKC\(^25\). Western blotting showed that cocaine induces translocation (activation) of PKA Cox and ePKC from the particulate fraction to the cytosol (Fig. 3e). Notably, translocation is blocked by the specific D2 antagonist spiperone (Fig. 3e), suggesting that cocaine-induced stimulation of D2 autoreceptors activates PKA and PKC signaling. Indeed, the PKA inhibitor Rp-cAMPS, the PKC inhibitor GF109203X and the D2 antagonist spiperone each blocked cocaine-induced increases in dopamine production in primary VTA neurons (Fig. 3f). Furthermore, we confirmed ALDH2i concomitantly increased THP and reduced dopamine concentrations in cocaine-treated VTA neurons (Fig. 3g).

To confirm and extend our findings on the central role of THP in the mechanism of action of ALDH2i during cocaine addiction, we measured THP and dopamine abundance in vivo in the VTA and nucleus accumbens after rats extinguished from cocaine-seeking underwent auditory (tone) and visual (light) cue-induced reinstatement (Fig. 1c). Cue-induced rats showed large increases in dopamine abundance in the VTA (Fig. 4a) and nucleus accumbens (Fig. 4b), consistent with previous reports\(^26\). THP was virtually undetectable in nucleus accumbens (Fig. 4b). In contrast, cocaine-extinguished rats pretreated with ALDH2i (15 mg per kg body weight i.p.) before exposure to cues showed marked increases in THP in the VTA (Fig. 4a) and decreases in dopamine abundance in the VTA and nucleus accumbens (Fig. 4a,b). This correlated with considerable in vivo decreases in tyrosine hydroxylase phosphorylation by ALDH2i in VTA (Fig. 4c) and suppression of cocaine-seeking behavior (Fig. 1c). THP was virtually absent in the VTA or nucleus accumbens in naive rats that had never been given cocaine (Fig. 4a,b). Of note, ALDH2i does not affect basal dopamine levels in both brain regions (Fig. 4a,b). To support the hypothesis that THP has a role in ALDH2i suppression of cocaine-seeking behavior, we pretreated cocaine-extinguished rats with THP (15 mg per kg body weight i.p.) 30 min before exposure to cues. THP eliminated cue-induced reinstatement of lever-pressing for cocaine (Fig. 4d). These results may be compared to the diverse effects of THP on alcohol intake under various experimental conditions. THP augments voluntary alcohol consumption when given by intracerebroventricular injection but reduces alcohol intake when injected into striatal sites such as the VTA and substantia nigra complex\(^27\).

Our major findings suggest that selective inhibition of ALDH-2 by ALDH2i suppresses cocaine self-administration and prevents cocaine- or cue-induced reinstatement of cocaine-seeking behavior. ALDH-2 inhibition during activation of dopamine signaling diverts accumulating DOPAL to condense with dopamine to form THP. THP seems to inhibit cocaine- or cue-dependent increases in dopamine synthesis in the VTA via negative feedback inhibition of phosphorylated tyrosine hydroxylase. A putative molecular mechanism by which ALDH2i restores dopamine homeostasis is illustrated in Supplementary Figure 3.

There is extensive evidence that dopamine transmission from the VTA to the nucleus accumbens has a central role in cocaine addiction\(^28-30\). Activation of D2 autoreceptors in the VTA\(^31\) enhances dopamine...
Neuron pacemaker activity. D2 inhibition blocks reinforcing effects of addictive drugs. Notably, ALDH2i only interferes with dopamine-related increases in dopamine signaling and does not change basal levels of dopamine in the VTA and nucleus accumbens. This is consistent with our observation that ALDH2i does not affect inactive lever responses (Supplementary Table 2), locomotor activity, water intake and food consumption. Moreover, we find no evidence of an additive effect of ALDH2i on cocaine self-administration (Supplementary Fig. 4). Although additional cocaine-seeking models can be used to extend our results, we believe our findings taken together demonstrate a new mechanism of action for ALDH2 inhibition of dopamine production in the VTA and release in nucleus accumbens during cocaine seeking and in a rat model of cocaine relapse–like behavior. We propose that a safe, selective, reversible ALDH-2 inhibitor such as ALDH2i may have the potential to attenuate human cocaine addiction and prevent relapse.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

L.Y. and I.D. designed and supervised the project, analyzed the data and wrote the manuscript. P.F. designed, carried out and analyzed molecular and cell biology studies. M.A. designed, performed and analyzed behavioral studies. Z.I. performed the cell biology experiments. M.F.O. carried out cocaine dose-response experiments. J.Z. and team synthesized CVT-10216. K.L. supervised and H.-L.S. and N.C. performed mass spectrometric analysis of in vitro dopamine and THP. J.L. and H.-Y.K. developed a mass spectrometric analysis method for dopamine and THP and determined their in vivo abundance. J.S. contributed to design and review of PC12 data. B.B. contributed to design and review of in vivo data.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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We purified DBH, tyrosine hydroxylase and dopamine decarboxylase from PC12 cells. Details can be found in the Supplementary Methods.

**Enzyme preparations.** We purified DBH, tyrosine hydroxylase and dopamine decarboxylase from PC12 cells. Details can be found in the Supplementary Methods.

**Enzyme activity assays.** Enzyme activity assays for tyrosine hydroxylase, dopamine decarboxylase and DBH were performed as previously described. MAO activity assay was performed as described by the manufacturer (Invitrogen). Details can be found in the Supplementary Methods.

**Cocaine self-administration and reinstatement.** Male Sprague Dawley rats (Charles River) with implanted jugular vein catheters were trained to self-administer cocaine on a fixed-ratio 1 schedule of reinforcement as previously described. Each active lever press produced a 0.05-ml infusion of 0.35 mg per kg body weight per infusion of cocaine over 3 s with a cue light and tone signaled during the drug delivery. ALDH2i (10, 15, 30 and 45 mg per kg body weight) or vehicle was administered i.p. 30 min before the self-administration session in a counterbalanced order. For reinstatement, rats underwent extinction training procedures, whereby activation of the lever that had previously delivered cocaine was recorded but did not result in cocaine infusions. After extinction, rats received i.p. ALDH2i (3.75, 7.5, 15 or 30 mg per kg body weight) or vehicle (0.5% methylcellulose) 30 min before a single injection of cocaine (10 mg per kg body weight i.p.); or ALDH2i (1.375, 7.5 and 15 mg per kg body weight) or THP (15 mg per kg body weight) 30 min before an auditory (tone) and visual (cue light) cue immediately before the beginning of the 2-h session. The session was conducted identically to the extinction training sessions without cocaine delivery. Active and inactive lever presses were recorded, and the number of active lever presses was the measure of reinstatement. All experiments were approved by the Gilead Institutional Animal Care and Use Committee following criteria outlined in the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. Details can be found in the Supplementary Methods for methamphetamine-induced reinstatement.

**Statistical analyses.** Data from rat studies were analyzed by repeated measures analysis of variance followed by Fisher’s post hoc test to determine statistical significance. Data from cell studies were expressed as means ± s.e.m. of three independent experiments and evaluated with one-way analysis of variance and Dunnett’s test.
**Corrigendum:** Inhibition of aldehyde dehydrogenase-2 suppresses cocaine seeking by generating THP, a cocaine use–dependent inhibitor of dopamine synthesis

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In the version of this article initially published online, Zhan Jiang's name was incorrectly spelled as Zhang Jiang. The error has been corrected for the print, PDF and HTML versions of this article.