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

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Abstract

There is no effective treatment for cocaine addiction despite extensive knowledge of the neurobiology of drug addiction[1–4]. 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 need[5]. Recent anecdotal clinical reports suggest that disulfiram may attenuate cocaine use[6]. 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 brain[7]. We and others have shown that highly selective ALDH-2 inhibitors potently reduce alcohol seeking in the presence or absence of acetaldehyde[8,9]. 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 accumbens[8], 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. Here 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 addiction[10,11]. 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 behavior, we pretreated them with ALDH2i (intraperitoneally (i.p.)) 30 min before rechallenging with i.p. cocaine or auditory (tone) and visual (light) cues. ALDH2i dose dependently inhibits cocaine priming- or cue-induced reinstatement (Fig. 1b,c). Furthermore, ALDH2i also reduces methamphetamine-induced reinstatement in rats (Fig. 1d).

Dopamine is synthesized in VTA neurons and axonally transported for release in the nucleus accumbens[12,13]. Addictive drugs activate VTA neurons, leading to increased dopamine release in the nucleus accumbens[14,15]. 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 spiperone 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 dopaminergic neurons in the VTA and involved in downstream dopamine metabolism[16]. ALDH-2 converts DOPAL to 3,4-dihydroxyphenyl-acetic acid (DOPAC)[17]. Inhibition of ALDH-2 increases DOPAL concentration[18], which condenses with dopamine to form THP[19]. We searched for evidence that selective inhibition of ALDH-2 induces THP formation during cocaine activation of dopamine production in PC12 cells. We found that ALDH2i increases THP formation in a dose-dependent manner in cocaine-treated cells (Fig. 2d). Of note, ALDH2i had no effect on basal THP abundance in the absence of cocaine (Fig. 2d). If ALDH2i-dependent formation of THP has a role in suppressing dopamine synthesis, then adding THP to cells should also inhibit dopamine synthesis. Indeed, we found that THP inhibits cocaine-stimulated dopamine production in cocaine-treated PC12 cells in a dose-dependent manner (Fig. 2e) and reduces basal dopamine production[20] (Supplementary Fig. 1).

Tyrosine hydroxylase is the first and rate-limiting step in dopamine production. TH converts L-tyrosine to L-dihydroxyphenylalanine (DOPA), a substrate DOPA decarboxylase to yield dopamine[17]. Inhibition of tyrosine hydroxylase, DOPA decarboxylase or both would be expected to lower dopamine synthesis. Therefore, we asked whether THP inhibits enzymes required for dopamine synthesis. We found that THP inhibited basal tyrosine hydroxylase activity with a half-maximal inhibitory concentration of 3.8 μM (Fig. 2f); dopamine decarboxylase was not affected (Supplementary Table 1).

Phosphorylation of tyrosine hydroxylase dramatically increases tyrosine hydroxylase activity[21]. We determined whether THP inhibits the phosphorylated (activated) form of tyrosine hydroxylase more effectively than unphosphorylated enzyme. THP inhibited phosphorylated tyrosine hydroxylase enzyme activity with a half-maximal inhibitory concentration of 50 nM. Activated tyrosine hydroxylase is 75 times more sensitive to THP inhibition than unphosphorylated tyrosine hydroxylase (Fig. 2f). 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 Cα and εPKC 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[32]. D2 inhibition blocks reinforcing effects of addictive drugs[33,34]. But, there might be a limited margin of selectivity in blocking psychostimulant-induced effects compared with normal behaviors[35,36]. Notably, ALDH2i only interferes with cocaine-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[8]. 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 ALDH-2 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/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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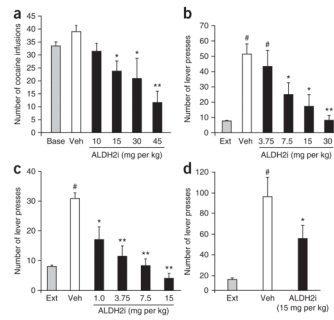
Figure 1.
ALDH2i reduces intravenous cocaine self-administration, cocaine-primed or cue-induced reinstatement and methamphetamine-induced reinstatement in Sprague Dawley rats. (**a**) The number of cocaine infusions recorded during the 2-h cocaine self-administration session (n = 7–12, $*P < 0.05$, $**P < 0.01$ compared with vehicle (Veh)). (**b,c**) The number of lever presses recorded during the 2-h cocaine-primed (**b**) or cue-induced (**c**) reinstatement session (n = 6–9 and n = 6–11 for **b** and **c**, respectively; $#P < 0.01$ compared with extinction (Ext); $**P < 0.01$ compared with Veh). (**d**) The number of lever presses recorded during the 2-h methamphetamine-induced reinstatement session (n = 7, $#P < 0.01$ compared with Ext; $*P < 0.05$ compared with Veh).
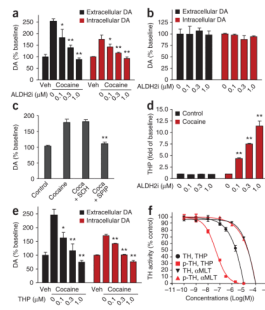
Figure 2.
ALDH2i decreases cocaine-induced dopamine (DA) production and increases THP abundance in PC12 cells. (**a**–**e**) Cells were incubated with or without cocaine (Coca, 1 μM) in the presence or absence of ALDH2i, the D1 antagonist SCH 23390 (SCH, 10 μM), the D2 antagonist spiperone (SPIP, 10 μM) or THP for 24 h. Intracellular and extracellular dopamine (**a**,**b**,**e**), total dopamine (**c**) or THP amounts (**d**) were determined. *$P < 0.05$, **$P < 0.01$ compared with cocaine control. (**f**) Dose-response analysis of THP and α-methyl-L-tyrosine (αMLT) on the inhibition of total tyrosine hydroxylase (TH) or phosphorylated tyrosine hydroxylase (p-TH).
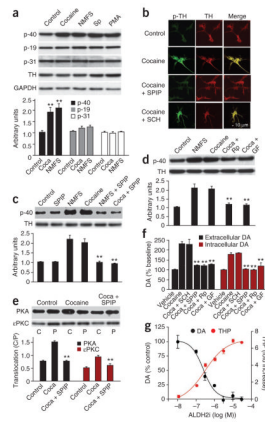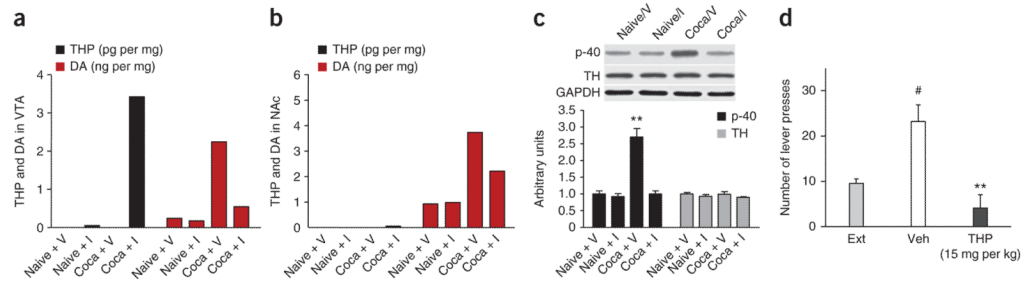
Figure 3.
Cocaine activates PKA and PKC to phosphorylate tyrosine hydroxylase and increase dopamine production in VTA neurons. (**a**–**e**) Cells were incubated for 24 h with or without cocaine (1 μM) or nomifensine (NMFS, 10 μM) in the presence or absence of the PKA activator Sp-cAMPS (Sp, 1 mM, 10 min), the PKC activator phorbol 12-myristate 13-acetate (PMA, 100 nM, 10 min), the D1 antagonist SCH23390 (10 μM), the D2 antagonist spiperone (10 μM), the PKA inhibitor Rp-cAMPS (Rp, 20 μM) or the PKC inhibitor GF 109203X (GF, 1 μM). TH phosphorylation at Ser40 p-40; (**a**–**d**), at Ser19 (p-19) and Ser31 (p-31; **a**) or the translocation of PKA and εPKC (**e**) was detected by western blotting (**a**,**c**–**e**) or by immunostaining (**b**). Green indicates phosphorylated tyrosine hydroxylase at Ser40 (p-TH), red, total tyrosine hydroxylase and yellow, the merged images. C/P, the ratio of PKA or ePKC in the cytosolic fraction (C) versus the particulate fraction (P). (**f**,**g**) Intracellular and extracellular dopamine (**f**) or total dopamine and THP (**g**). **$*P < 0.01$ compared with sham or cocaine control.

Figure 4.
ALDH2i increases THP production to inhibit tyrosine hydroxylase activity and decrease dopamine production in VTA in cocaine-addicted rats. (**a,b**) Dopamine and THP amounts measured in the VTA (**a**) and nucleus accumbens (**b**) pooled from the cocaine-seeking rats treated with ALDH2i (I, 15 mg per kg body weight, $n = 8$) or vehicle (V, $n = 8$) in Figure 1c or from naive rats treated with ALDH2i (15 mg per kg body weight, $n = 8$) or vehicle ($n = 8$). The experiment was repeated with similar results. (**c**) Tyrosine hydroxylase phosphorylation at Ser40 in the VTA, as detected by western blotting. $**P < 0.01$ compared with naive vehicle control. (**d**) The number of lever presses during the 2-h cue-induced cocaine reinstatement session ($n = 8$, $\#P < 0.01$ compared with extinction; $**P < 0.01$ compared with Veh).