Coronaridine congeners attenuate fentanyl seeking during prolonged abstinence

OKLAHOMA STATE UNIVERSITY
CENTER FOR HEALTH SCIENCES

Jacob D. Layne[1], Mason J. Hochstetler[1], Brandon M. Curry[1], Benjamin M. Williams[2], Christina A. Small[2], Scott C. Steffensen[2], Hugo Arias[1], Craig T. Werner[1]

[1]Oklahoma State University Center for Health Sciences, Department of Pharmacology and Physiology, Tulsa, OK 74107

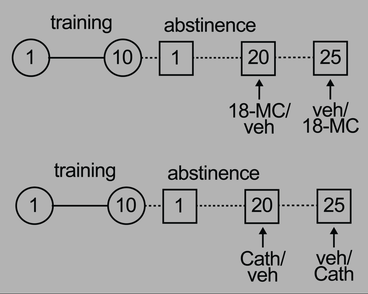
[2]Brigham Young University, Department of Psychology and Neuroscience, Provo, UT 84602

Background

The prevalence of opioid use disorder (OUD) has reached epidemic proportions with a record-breaking number of overdose deaths. Over 70% of the record-breaking number of overdose deaths are caused by synthetic opioids, including fentanyl. Fentanyl is commonly administered intravenously or by inhalation (smoking/vaping), which results in rapid drug bioavailability in the brain. There is a current need to identify a novel pharmacologic therapy to treat OUD, and there is increasing evidence to support the use of novel compounds referred to as coronaridine congeners to treat OUD and other psychiatric illnesses. In preclinical models, coronaridine congeners have been shown to decrease self-administration of drugs of abuse and induce antidepressant and anxiolytic effects. Here we used a preclinical fentanyl vapor self-administration model and fast-scan cyclic voltammetry (FSCV) to study the anti-addictive effects of two coronaridine congeners, 18-methoxycoronaridine (18-MC) and catharanthine (Cath).

Methods

C57BL/6J mice were trained to self-administer vaporized fentanyl (5 mg/mL) or vehicle in air-tight operant chambers. Mice self-administered vapor for 1 hour per day for 10 days (sessions were conducted for 5 consecutive days, followed by 2 days off). Chambers were equipped with two nosepokes, one active and one inactive. A successful response in the active nosepoke resulted in a vapor delivery that coincided with the presentation of a cue light, followed by a 1-minute timeout period. Mice learned to self-administer vapor with 3-second vapor deliveries for the first 3 days of training, which was then reduced to 1.5-second vapor deliveries the remaining 7 days. After training, mice were returned to their home cages for a forced abstinence period. Cue-induced drug seeking tests were conducted on abstinence days (AD) 20 and 25. During cue-induced seeking tests, successful responses in the active nosepoke resulted in presentation of the drug-associated cue, but no vapor was delivered (i.e. extinction conditions). Cue-induced drug seeking tests were conducted using a crossover design where half of subjects received coronaridine treatment (18-MC or Cath), while the other half received vehicle (ddH2O), on AD20. On AD25, subjects received the opposite treatment compared to AD20. Mice were injected (i.p.) with either vehicle or coronaridine treatment 1 hour before seeking tests. To examine the molecular mechanism of coronaridine congeners, FSCV was conducted on dopaminergic pre-synaptic terminals in the nucleus accumbens (NAc) neurons to measure dopamine (DA) release in the presence of 18-MC and Cath with or without nicotinic acetylcholine receptor antagonists.



Timeline of vapor self-administration and seeking tests with acute treatment of either 18-MC or Cath.

RESULTS

We found that both 18-MC and Cath significantly reduced fentanyl seeking during prolonged abstinence with no effect on mice that had previously self-administered vehicle. Furthermore, FSCV revealed that 18-MC and Cath significantly reduced DA release onto NAc neurons.

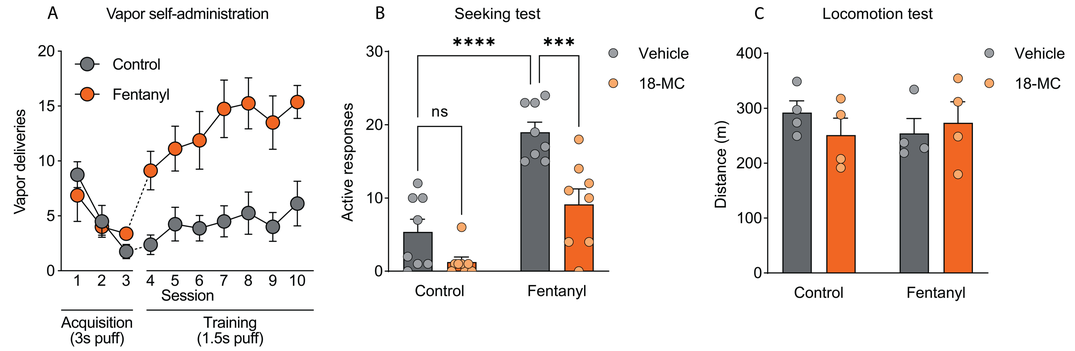


Figure 1. Acute treatment of 18-MC attenuates fentanyl seeking during prolonged abstinence. A) Vapor self-administration training behavior. **B)** Seeking tests 1-hour post-injection of 18-MC or vehicle, i.p., on AD20 and AD25 using a crossover experimental design. **C)** Locomotion test 1-hour post-injection of 18-MC or vehicle. ns = no significance; *** = p<0.001; **** = p<0.0001.

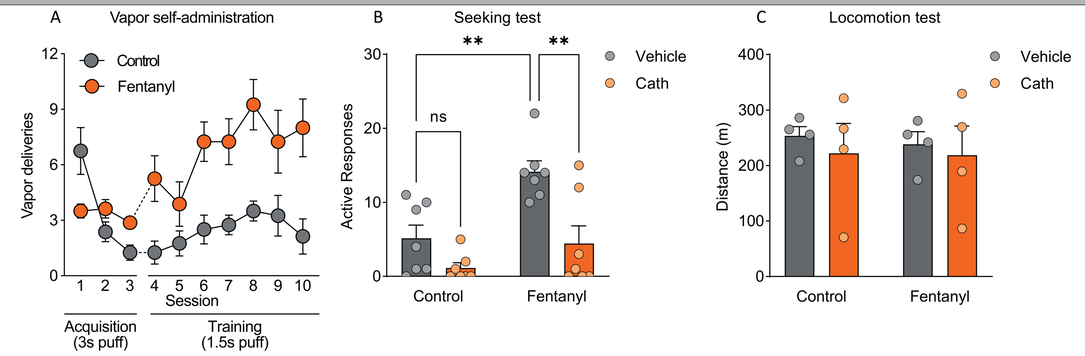


Figure 2. Acute treatment of Cath attenuates fentanyl seeking during prolonged abstinence. A) Vapor self-administration training behavior. **B)** Seeking tests 1-hour post-injection of Cath or vehicle, i.p., on AD20 and AD25 using a crossover experimental design. **C)** Locomotion test 1-hour post-injection of Cath or vehicle. ns = no significance; ** = p<0.01.

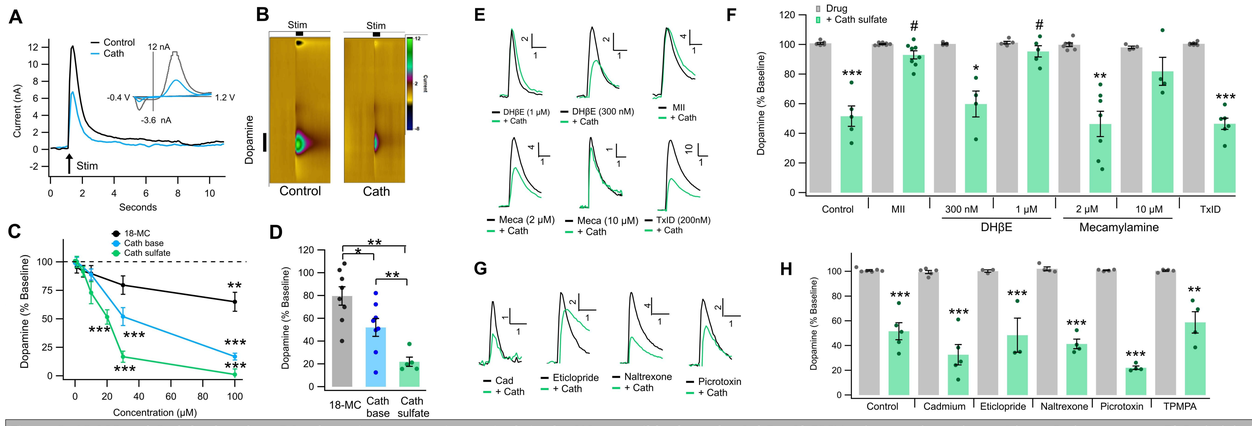


Figure 3. 18-MC and Cath both reduce DA release in presynaptic terminals in the NAc, possibly through nAchRs. A) FSCV voltage spike with or without Cath treatment. **B)** Radiolabeled images of FSCV spike. **C)** DA release in the presence of 18-MC, Cath base, or Cath sulfate. **D)** Quantified DA release at 40μM concentrations for 18-MC, Cath base, and Cath sulfate. **E)** Representative voltage spikes in the presence of selective and non-selective nicotinic acetylcholine receptor (nAchR) antagonists with or without Cath. **F)** The effect of Cath on DA release in the presence of selective and non-selective nAchR antagonists. **G)** Representative voltage spikes with or without Cath sulfate in the presence of the toxic metal cadmium, the selective D_2 and D_3 receptor antagonist eticlopride, the non-selective opioid antagonist naltrexone, the $GABA_A$ receptor antagonist picrotoxin, and the selective $GABA_{B-p}$ receptor antagonist TPMPA. **H)** The effect of Cath on DA release in the presence of antagonists. * = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001. NAc: nucleus accumbens; nAchR: nicotinic acetylcholine receptor; FSCV: fast scan cyclic voltammetry; DA: dopamine.

Conclusion

In this study, we report that both 18-MC and Cath decrease fentanyl seeking during prolonged abstinence. DA release is important for opioid-related behaviors, and we found that 18-MC and Cath reduce DA release in the NAc, a mechanism that may underlie the effect of coronaridine congeners on fentanyl seeking. Together, these results provide evidence that coronaridine congeners may be promising novel compounds for the pharmacotherapeutic treatment of OUD.

Future Directions

- Examine the effects of intra-NAc delivery of 18-MC and Cath on fentanyl seeking.
- Examine the effects of 18-MC and Cath treatment on fentanyl self-administration.
- Examine the state of nicotinic acetylcholine receptor expression in the NAc after treatment of 18-MC and Cath.
- Examine how 18-MC and Cath affect how neuronal ensembles process drug-related information during fentanyl seeking via miniscope calcium imaging.

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