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Research Article

Risk for Opioid Abuse is Diminished by Inhibiting Aldehyde Dehydrogenase-2 (ALDH-2) in Rats

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Abstract

Significant opiate addiction is known to follow prescribed opiate use for pain. There is a serious unmet need for non-addicting medications to prevent subsequent opiate addiction after a short period of opioid treatment for temporary pain. Recent evidence indicates that selective inhibition of aldehyde dehydrogenase-2 (ALDH-2) reduces drug-seeking and trained self-administration of alcohol, cocaine and nicotine, apparently by preventing a concomitant surge of dopamine in the ventral tegmental area (VTA) and nucleus accumbens (NAc). Activation of the same dopaminergic pathway is also implicated in opioid-induced reinforcement. Therefore, we asked whether the selective ALDH-2 inhibitor, ANS-6637, would attenuate opioid self-administration in drug-naïve rats for opioid self-administration. Rats received oral doses of ANS-6637 (9, 18, 36 or 72 mg/kg) or an equal volume of control vehicle 2 h before exposure to remifentanil and a light cue to accentuate self-administration over 5 consecutive days. Self-administration and the numbers of lever presses on both active and inactive levers were recorded. ANS-6637 significantly reduces remifentanil self-administration over 5 sessions of treatment in rats without prior exposure to remifentanil. We also confirm that the highest dose of ANS-6637 (72 mg/kg) used in this study did not prevent remifentanil-induced analgesia using a classic hot plate test. Thus, ANS-6637 significantly reduces of initial exposure to remifentanil self-administration without affecting desired analgesia. These preliminary observations suggest that ANS-6637 appears to have potential value as a non-addictive therapeutic agent to prevent abuse of commonly used opiates in initiating pain management.

Keywords: addiction, treatment, animal model, dopamine, analgesia, hot plate test

1. Introduction

Opioid addiction is a major health problem with devastating effects on individuals and society. According to the CDC (https://www.cdc.gov/drugoverdose/data/statedeaths.html), more than 350,000 people died from an overdose of prescription and illicit opioids from 1999 to 2016. The opioid induced death toll is increasing; 72,000 opioid overdose deaths in the US were reported in 2017. Recent evidence indicates that a significant number of patients develop opioid addiction for the first time following the legitimate use of prescription opiates for pain. Current treatments have not prevented this pathway to addiction. Despite availability of opiate receptor antagonists to reverse acute CNS depression and methadone to substitute for opiates, there is an urgent unmet need during pain management for non-addictive agents to prevent the acquisition of opioid use disorder and deaths due to overdose. Possible targets for therapeutic intervention include dopaminergic and serotonergic systems implicated in responses to opioids [1-6]. Importantly, a significant dopaminergic surge in the VTA and NAc appears to play a primary role in addictive drug-seeking behavior. Activated tyrosine hydroxylase is the rate-limiting step in dopamine synthesis. It has been reported that ANS-6637, a highly selective reversible inhibitor of ALDH-2, prevents cocaine-seeking behavior in rats by rate-limiting step in dopamine synthesis in the VTA and dopamine release into the NAc [7].

Remifentanil is one of the most powerful and rapidly onset/offset acting opioids used for analgesia during anesthesia which does not depend on bioconversion by liver enzymes. In this study we first exposed drug naïve rats to oral ANS-6637 simultaneously with self-administered remifentanil. We hypothesized that the oral administration of ANS-6637 will diminish self-administration of remifentanil without blocking its analgesic effect. To test this hypothesis, we used the standard IV self-administration method and the classic hot plate test of nociception. We find that this novel highly selective reversible ALDH-2 inhibitor significantly reduces initiating remifentanil self-administration without interfering with it analgesic effect.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats (Charles River Labs, Raleigh, NC, USA) were singly (for the self-administration study) or group (for the pain study) housed in approved standard laboratory conditions in a Duke University vivarium facility next to the testing room to minimize stress induced by transporting the rats. The day-night cycle was reversed (7:00 am to 7:00 pm dark) so that the rats were in their active phase during the behavioral testing. All behavioral tests were carried out during the dark phase of the dark-light cycle between 09:00 am and 4:00 pm. Rats for the self-administration experiment had *ad lib* access to water and fed a standard rat chow once daily throughout the study to keep them at approximately 85% *ad lib* weight with food amounts adjusted from 8-16 g per day as they grow to provide a lean healthy growth curve. Rats for the pain study had ad lib access to both food and water at all times except during the 30 min hot plate test. All procedures for testing the animals were approved by the Duke University Animal Care and Use Committee and conformed to the Animal Care Guide by NIH.

2.2 Preparation of drugs

Solutions of remifentanil hydrochloride (National Institute on Drug Abuse) were prepared in pyrogen-free glassware in sterilized isotonic saline and passed through a 0.2 μm filter (Millipore Corp, Billerica, MA, USA). Solutions of ANS-6637 (Amygdala Neuroscience, Inc.) were prepared in pyrogen-free glassware in distilled H_2O and the pH of the solutions was adjusted to 7.0 using NaOH. ANS-6637 solution was given by oral gavage in a volume of 4 ml/kg BW two hours before the initiation of remifentanil self-administration. All solutions were kept refrigerated in the dark between experiments and brought to room temperature before administration.

2.3 Experimental design and procedure

First, the effect of ANS-6637 on remifentanil self-administration was assessed providing a dose and time effect function. Second, the effect of the highest dose of ANS-6637 (72 mg/kg) on remifentanil-induced analgesia was assessed in another group of male rats.

2.4 ANS-6637 and intravenous remifentanil self-administration and nociception

We tested the dose-response of ANS-6637 to reduce intravenous self-administration of the opioid remifentanil. For 5 consecutive days rats were given an oral dose of 9, 18, 36 or 72 mg/kg of ANS-6637 or the vehicle 2 h before initiating opioid self-administration. The same experiment was repeated after 2 days of no treatment for another 5 consecutive days. To be clinically effective in attenuating abuse liability of opioids used for pain relief, a compound must reduce opioid self-administration without blocking its analgesic effect. Therefore, we also asked whether or not oral administration of ANS-6637 interfered with opioid-induced analgesia by using the hot pale test.

2.5 Intravenous remifentanil self-administration

Chronically indwelling intravenous jugular catheters were implanted IV under ketamine (60 mg/kg) and dexmedetomidine hydrochloride (15 mg/kg) anesthesia and were flushed daily with a 0.3 ml solution containing 100U/ml heparinized saline. After each self-administration session, the remifentanil remaining in each port was drawn out and a sterile lock consisting of heparinized saline 500 U/ml with 0.4 mg gentamicin was infused. A barbiturate injection test through the catheter was used to verify the patency of catheters. Only the data from the patent rats were used for analysis [8-11].

For behavioral training, rats were placed in dual lever operant test chambers (Med Associates, Georgia, VT, USA). Each chamber is equipped with a tone generator, house light, cue light above each lever, and a metal tether to cover the drug delivery line. A computer programmed with MED-PC software controls experimental events and data collection. Each catheter was connected to a microliter syringe pump, and tethers made of polyethylene tubing with huber needles for access to ports and catheters. During each self-administration session, the rats wear infusion harnesses to connect them to the tethers. Initially, the rats were trained daily for 30 min, to press the levers for food pellet reinforcers. Approximately half the animals were rewarded for responding on the right lever and the other half for responding on the left lever. Only the cue light over the correct lever was illuminated while the light over the incorrect lever was off. Pressing on the correct lever was rewarded by immediate delivery of one 45-mg food pellet and activation of the feedback tone for 0.5 sec. There was no timeout during these training sessions.

After the pellet sessions, animals had catheters surgically implanted under ketamine anesthesia to provide access for remifentanil self-administration by IV infusion. A plastic SoloPort was attached intraoperatively to a polyurethane catheter and inserted into a subcutaneous interscapular pocket and sutured to underlying fascia. 2-4 days after the surgery, the rats began self-administration sessions with remifentanil (0.3 mg/kg/infusion, IV) as the reinforcer.

A lever press on the active side resulted in the activation of the feedback tone for 0.5 sec, the immediate delivery of one 50-μl infusion of remifentanil in less than 1 sec. Each infusion was immediately followed by a 20 sec. timeout in which the house light goes on and cue lights go out and responses are recorded but not reinforced. The benchmark infusion dose of remifentanil was set at 0.3 mg/kg/infusion, IV FR was set at FR1. Each remifentanil infusion sessions was 1-hour [10-12]. Animals were tested only once/day between 9 am and 4 pm.

2.6 ANS-6637 and remifentanil analgesia

The purpose of this study was to determine whether ANS-6637 interfered with remifentanil-induced analgesia in rats. A standard hot plate test was used. To become adapted to the test environment, rats were exposed to the hot plate instrument for at least 15 min/day for two days without turning on the heat and without any treatment. Then, using a 2 by 2 design, each one of the following combinations was administered to rats and their paw withdrawal latency (PWL) was measured on the hotplate (at 55 °C) at 5, 10 and 15 min after the administration of remifentanil. ANS-6637 or the control solution was given orally two hours before subcutaneous administration of remifentanil. The volume of oral administration of ANS-6637 and the control solution was 4 ml/kg and the volume of subcutaneous administration of remifentanil was 2 ml/kg. All rats were tested with all combinations following a random order design with at least one day interval between administrations. The following combinations were used:

dH_2O + Saline

Remifentanil (0.4 mg/kg) + dH_2O

ANS-6637 (72 mg/kg) + Saline

ANS-6637 (72 mg/kg) + Remifentanil (0.4 mg/kg)

2.7 Statistical analysis

The data were evaluated with analysis of variance (ANOVA). Analysis was done for between and within-subjects factors. The between subjects factor was ANS-6637 treatment and the within subject factor was repeated self-administration test sessions. Alpha of $p<0.05$ (two-tailed) was used as the threshold for statistical significance. The following numbers of rats used for each treatment: Control=15, 9 mg/kg=6, 18 mg/kg=10, 36 mg/kg=15 and 72 mg/kg=13. The N of at least 10/dose has been found in previous studies to provide sufficient power to detect biologically significant effects.

3. Results

3.1 ANS-6637 and remifentanil self-administration

Our results show that during five daily sessions 36 mg/kg ($F(1,54)=5.92$, $p<0.025$) and 72 mg/kg ($F(1,54)=4.25$, $p<0.05$) of ANS-6637 significantly reduced remifentanil self-administration in rats (Figure 1). After two days of abstinence the rats were re-tested for another five days. During these sessions, ANS-6637 inhibition did not reach significance. The session-by-session detailed data are shown in Figure 2.

3.2 ANS-6637 and remifentanil analgesia

At five minutes post injection the majority of the animals injected with remifentanil were too sedated to test the hotplate response. Remifentanil (0.4 mg/kg) s.c. caused a significant ($F(1,7)=33.10$, $p<0.001$) antinociceptive effect 10 min post injection (Figure 3). Remifentanil is a rapid onset/offset opioid drug and the rat totally recovered at 15 min post injection with no residual effect. The oral administration of 72 mg/kg ANS-6637 given 2 hours before testing did not attenuate remifentanil-induced analgesia at 10 min post injection (Figure 3).

4. Discussion

The major finding in this study is that oral administration of ANS-6637, a selective and reversible inhibitor of ALDH-2, limits initiation of self-administration of remifentanil in drug-naïve rats without affecting remifentanil-induced analgesia. A dose response for ANS-6637 inhibition of remifentanil self-administration is shown in 5 sessions of exposure to both drugs. The efficacy of ANS-6637 to attenuate self-administration of
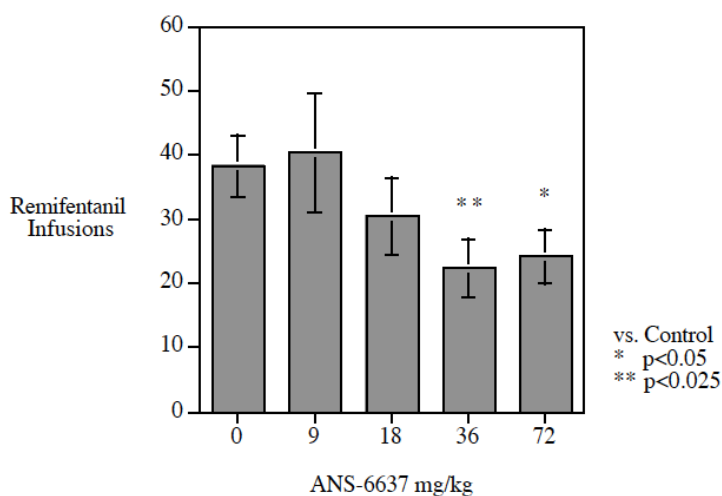

Figure 1: Acute ANS-6637 dose response study on initiation of remifentanil self-administration in drug-naïve rats. Data represent means ± sem for 5 sessions at all doses. N=saline: 15; 9 mg/kg: 6; 18 mg/kg 10; 36 mg/kg; 15 and 72 mg/kg, N=13 rats. During five daily sessions 36 mg/kg (p<0.025) and 72 mg/kg (p<0.05) of ANS-6637 significantly reduced remifentanil self-administration in rats.

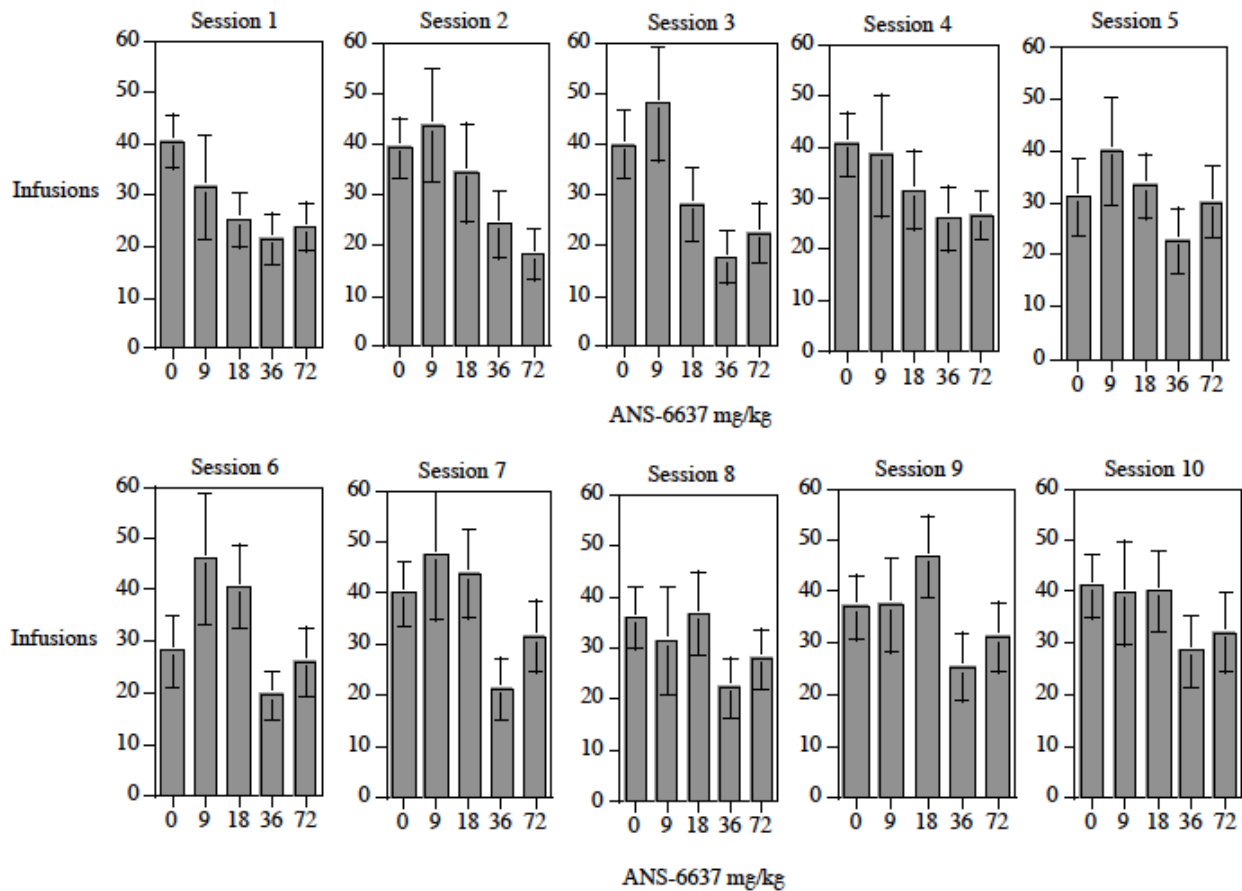## ANS-6637 Effects on Remifentanil Self-Administration

Figure 2: The effect of ANS-6637 on remifentanil self-administration session-by-session. Data represent means ± sem. N=saline: 15; 9 mg/kg: 6; 18 mg/kg 10; 36 mg/kg; 15 and 72 mg/kg, N=13 rats.

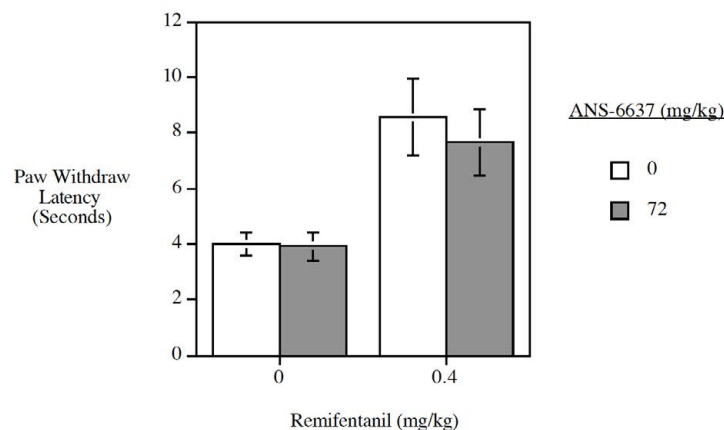## ANS-6637 and Remifentanil-Induced Analgesia

Figure 3: Remifentanil-induced analgesia is unaffected by 72 mg/kg oral ANS-6637. Data represent means ± sem, N=12 rats. Remifentanil (0.4 mg/kg, s.c) caused a significant $F_{(1,7)}=33.10$, $p<0.001$ and nociceptive effect 10 min post injection.

remifentanil is immediate. This suggests the possibility that ANS-6637 might prevent very early development of opioid use disorder in patients initiating opiate treatment for pain. These initial findings complement similar results reported with other non-addictive reversible selective inhibitors of ALDH-2 in more routine paradigms of trained drug-seeking rats. In alcohol-preferring Fawn-Hooded rats ALDH-2 inhibitors significantly suppress alcohol drinking, deprivation-induced increases in alcohol intake and reinstatement in the absence of alcohol [13], IV nicotine self-administration [11] and cue-induced reinstatement of cocaine seeking in rats [7]. Efficacy in blocking cue-induced reinstatement of cocaine seeking behavior was directly correlated with a

dramatic reduction in dopamine surges in the VTA and NAc despite the absence of cocaine [7]. Comparable results were also demonstrated in experimental food craving, thought to involve a common dopamine pathway in brain. In a model of rodent obesity, the ALDH-2 inhibitor CVT-2016 reduced carbohydrate and fatty acid seeking correlated with a reduction in dopamine surges [14, 15]. Taken together, findings in this preliminary report and published studies suggest that non-addictive selective reversible ALDH-2 inhibitors hold promise for effectively preventing and treating early acquisition of opioid addiction during initiation of pain management. This would also be relevant for patients who drink alcohol excessively while co-using opioids for pain relief. These patients are at even greater risk for opioid overdose and death. Fortunately, ANS-6637 appears to be simultaneously effective in attenuating both conditions.

The important role of the VTA and the NAc in mediating the rewarding effects of addictive drugs including opioids is well documented [16,17]. The mesocorticolimbic dopaminergic system has been implicated in reinforcing properties of several addictive substances [5,17-20]. Similar to alcohol, cocaine, methamphetamine and nicotine, opioids such as remifentanil and morphine have also been shown to increase forebrain dopamine release. The mechanism of action of opioids involves inhibiting GABAergic neurons in the VTA causing disinhibition of VTA DA neurons leading to increased release of dopamine into the NAc [21]. Thus, inhibition of drug-induced dopamine release in the NAc by selective ALDH-2 inhibitors such as ANS-6637 reduces the reinforcing effects of addictive drugs [7,11].

The mechanism of action of ALDH-2 in the brain has been described in detail [7]. ALDH-2 inhibition causes the formation of an endogenous tyrosine hydroxylase inhibitor THP which selectively prevents addiction-related pathophysiologic surges in dopamine without affecting normal levels. However, involvements of other neurotransmitters such as cholinergic, glutamatergic, GABAergic, serotonergic and endogenous opioid systems in drug seeking behaviors has also been documented by many investigators [22-29].

Remifentanil is a very powerful IV medication with a very fast onset and offset of action with a half-life of only about 0.3 min in blood. Remifentanil is not used in an outpatient setting. In addition, remifentanil is not used as an addictive agent by addicts [25-29].

5. Conclusion

Earlier reports confirmed efficacy of ALDH-2 inhibition on self-administration of addictive agents in fully trained drug-seeking animals. In this study we examined initiation of remifentanil use in untrained drug-naïve animals rather than treatment of trained drug-seeking addictive behavior. This paradigm may account for the variable

dose-response properties we find in this preliminary report. Our study was designed to shed light on the possibility of using ANS-6637 in patients just beginning to use opiates for pain relief. Although not as striking as results with rodents trained for self-administration, our findings in drug-naïve rats demonstrate that oral administration of ANS-6637 significantly reduces early acquisition of remifentanil self-administration without affecting its analgesic properties. It is not clear why ANS-6637 attenuation of remifentanil self-administration during the second 5-day exposures did not reach significance. It is possible that two days of abstinence between treatments increased efficacy of remifentanil. Despite this limitation, the findings in this study and in trained rodent models of addiction suggest that selective ALDH-2 inhibitors may have therapeutic potential for prevention and treatment of opioid addiction in patients just beginning a course of opiate therapy for pain. The food restriction used in the self-administration study caused the subjects to be leaner than those without food restriction in the nociception study. There may have been some alteration in pharmacokinetics of the drugs in animals with greater or lesser amount of adipose tissue. We see no obvious reason why this should have altered the pharmacodynamic effects of the drugs or their interactions.

In conclusion, inhibition of aldehyde dehydrogenase-2 (ALDH-2) reduces opioid self-administration without affecting opioid-induced nociception. Non-addictive selective reversible ALDH-2 inhibitors hold promise for effectively preventing and treating opioid addiction during initiation of pain management.

6. Acknowledgement and Disclosure

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