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Tetrodotoxin reduces cue-induced drug craving and anxiety in abstinent heroin addicts

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ABSTRACT

Background: Tetrodotoxin (TTX) is a neurotoxin found in puffer fish and other marine animals. New clinical studies suggest that low-dose TTX can safely relieve severe, treatment-resistant cancer pain. The therapeutic potential of TTX in addiction is supported by studies in laboratory animals. The purpose of this double-blind, placebo-controlled study was to assess the effect of a single intramuscular dose of TTX on cue-induced craving and anxiety in abstinent heroin addicts.
Methods: Forty-five abstinent heroin addicts were randomly assigned to three treatment groups: placebo, 5 μg TTX, or 10 μg TTX. Participants were exposed to a neutral video or a heroin-related video. Craving, anxiety, blood pressure, and heart rate were measured pre- and post-exposure.
Results: Heroin-related cues increased both craving and anxiety and had no effect on blood pressure and heart rate. A single dose of TTX dose-dependently attenuated the increases in craving and anxiety while having no effect on blood pressure or heart rate.
Conclusion: The results suggest that low-dose TTX is acutely effective in reducing cue-induced increases in heroin craving and associated anxiety.

1. Introduction

Successful treatment of heroin dependence is deterred by the high rate of relapse. Even after long periods of abstinence, heroin-dependent individuals are vulnerable to impulsive drug use when in the presence of stimuli related to previous episodes of use (Carter and Tiffany, 1999), and relapse frequently occurs (O'Brien, 1997). Thus, prevention and attenuation of relapse is a high priority for clinicians. Numerous preclinical and clinical studies have shown that relapse to drug seeking can be induced after extended periods of abstinence by exposure to drug-associated environmental cues (Carter and Tiffany, 1999) or exposure to stressors (Kosten et al., 1986; Sinha et al., 1999; Shaham et al., 2000; Lu et al., 2003). These findings correspond with the clinical observation that drug-dependent individuals appear to use drug or relapse more frequently in environments associated with prior drug use.

Sensitivity to cues in humans has frequently been studied in terms of physiological and psychological responses in the cue-reactivity paradigm (Carter and Tiffany, 1999; Sinha et al., 1999). Drug-associated cues tested in this paradigm can include the sight of drug paraphernalia (Yu et al., 2007), imagery of craving (Weinstein et al., 1997), drug-related pictures (Waters et al., 2003), or drug-related words, sentences, and videos (Ooteman et al., 2006; Ren et al., 2009, in press; Shi et al., 2008). Such cues tend to induce changes in physiological measures such as heart rate, blood pressure, and withdrawal signs (Carter and Tiffany, 1999), and in psychological measures such as craving and mood (Fox et al., 2005). In daily life, these responses may contribute to relapse to drug abuse. Thus, identifying treatments that diminish such responses is greatly important (Sinha et al., 1999).

One seemingly unlikely candidate for the prevention of relapse is tetrodotoxin (TTX), a neurotoxin found in puffer fish and other marine animals (Narahashi et al., 1994). TTX inhibits the generation of electrical impulses in neurons by blocking voltage-dependent sodium channels (Narahashi, 1972). Until recently, TTX has been used mainly as a tool in physiological and pharmacological studies. However, new clinical studies suggest that TTX in low doses can safely relieve severe, treatment-resistant cancer pain (Hagen et al., 2007, 2008).

The therapeutic potential of TTX in addiction is supported by studies in laboratory animals. Most of these studies have involved microinjections of TTX into specific brain regions. For example, TTX injected bilaterally into the basolateral amygdala and prefrontal cortex in rats selectively decreased conditioned responding for cocaine after extinction, whereas TTX inactivation of the nucleus accumbens significantly blocked the primary rewarding effects of cocaine after extinction (Grimm and See, 2000; Shaham et al., 2000; McLaughlin and See, 2003). In another such study, TTX injected into the

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Table 1
Schedule for laboratory session.

Time	Event / activity
– 65: 00 min	Participant arrival; measures of heart rate, blood pressure, craving, anxiety.
– 60: 00 min	Tetrodotoxin/placebo administration.
Neutral-cue video	
0: 00 min	Baseline period, online measurement of heart rate, blood pressure, craving, anxiety.
0: 05 min	Neutral-cue video shown.
0: 10 min	Online measurement of heart rate, blood pressure, craving, anxiety.
0: 15 min	10 min relaxation period.
Heroin-cue video	
0: 25 min	Baseline period, online measurement of heart rate, blood pressure, craving, anxiety.
0: 30 min	Heroin-cue video shown.
0: 35 min	Online measurement of heart rate, blood pressure, craving, anxiety.

basolateral amygdala in rats abolished the ability of heroin-paired stimuli and heroin priming to reinstate responding for heroin (Fuchs and See, 2002). However, a finding of more direct clinical relevance is that *systemic* administration of TTX significantly inhibited morphine withdrawal symptoms in rats and mice (Chen et al., 2001). In this study, it is demonstrated that intramuscular TTX pretreatment significantly reduced the naloxone-precipitated withdrawal symptoms including jump in morphine-dependent mice and weight loss both in morphine-dependent rats and mice, an effect similar to clonidine pretreatment. Moreover, this study also indicated that the same dose of TTX in attenuation of withdrawal symptoms did not change the heart rate, blood pressure, and breathe rate in anesthetic rats (Chen et al., 2001). Although little is known about the mechanisms by which this occurred, it suggests that TTX may be useful in the treatment of opiate dependence.

We conducted a double-blind, placebo-controlled study to assess the effect of single doses of intramuscular TTX on cue-induced craving and anxiety in abstinent heroin addicts. Given the pharmacological action of TTX on the cardiovascular system, we also evaluated the cardiovascular effects of the doses administered.

2. Materials and methods

2.1. Participants

Participants were recruited from an inpatient treatment center of the Yichang Addiction Rehabilitation Center, Yichang, China. Inclusion criteria included the following: (1) men or nonpregnant/nursing women 18–45 years old, (2) heroin dependence as assessed by the Structured Clinical Interview for DSM-IV (SCID), but opiate-free for at least 1 month, and (3) no prior or current use of cocaine or other illicit drugs. Exclusion criteria included the following: (1) current or past cardiovascular disease, (2) history of allergy (food, medicine), (3) current or past psychiatric illness, (4) neurological signs and/or history of neurological disease, (5) current medical illness, and (6) participation in other clinical trials of medications within the past 3 months. All participants gave written informed consent. The study was approved by the Human Investigation Committee of Peking University Health Center.

2.2. Study design

Fifty participants provided informed consent and underwent baseline assessments. Five were excluded due to abnormal laboratory tests ($n=3$) or heart problems ($n=2$). The remaining 45 participants were randomly assigned to three groups: 5 μg TTX, 10 μg TTX, and placebo. The placebo used was 0.21 μg solution of citric acid, which was the vehicle for TTX. The TTX and placebo were administered by intramuscular injection, because previous studies have shown that intramuscular TTX is well tolerated at doses from 7.5 mg b.i.d. to 30 mg b.i.d. (Hagen et al., 2007; 2008).

Each participant underwent a screening interview for demographic and heroin-abuse characteristics and administration of the Hamilton Anxiety Scale (HAMA) and Beck Depression Inventory (BDI). On the morning of the experimental day, participants were intramuscularly administered TTX or placebo 1 h before cue exposure. Heart rate and blood pressure were monitored online using a 9062D monitor (Baozhong Biotechnology Company, Beijing, China). Side effects were recorded as they occurred and were treated as necessary. Cue exposure occurred over two sessions, with a neutral videotape first and a heroin-related videotape second. This fixed order was chosen to prevent a "carry-over" effect from drug-related cues to neutral cues (Weinstein et al., 1997). Baseline psychological measures (including craving and anxiety ratings) and physiological measures (including blood pressure and heart rate) were obtained 5 min before each session and also obtained immediately after cue presentation. A 10 min interval occurred between the two sessions. Participants were allowed to leave when their physiological measures had returned to baseline levels. The schedule of assessments is shown in Table 1.

2.3. Psychological and physiologic measurements

The HAMA and BDI were used to assess anxiety and depression. Craving and anxiety before and after cue exposure were assessed with a 10-point visual analog scale (VAS; (Sinha et al., 1999), in which participants marked from 1 ("not at all") to 10 ("extremely high") their response to the question, "How much do you feel an urge to use heroin?" Two kinds of videotapes (neutral and heroin-related), each 5 min in length, were used as cues. The neutral videotape involved scenes that were non-emotional in content, such as birds, flowers, or trees. The heroin-related videotape included heroin-use scenes (Shi et al., 2007; Yu et al., 2007; Ren et al., 2009, in press).

2.4. Data analysis

Demographic and clinical characteristics of the three groups were assessed using one-way analysis of variance (ANOVA) and *post hoc t*-test if the omnibus F was significant. One-way ANOVA was used to analyze baseline scores on craving and anxiety measures. Repeated-measures ANOVAs with the within-subjects factors Stimulus Type (heroin cue and neutral cue) and Exposure Time (pre- and post-cue exposure) and the between-subjects factor Group (placebo, 5 and 10 μg TTX) were used to compare scores on craving and anxiety measures. Change scores were analyzed by two-way ANOVA with the between-group factor Treatment (placebo, 5 and 10 μg TTX) and the within-group factor Stimulus Type (heroin cue and neutral cue). *Post hoc* Fisher LSD tests were used when appropriate. P values less than or

Table 2
Demographic and clinical characteristics of participants.

	Placebo	TTX (5 μg)	TTX (10 μg)	p
	$n=15$	$n=15$	$n=15$	
Age (years)	31.53 ± 5.95	31.80 ± 5.17	31.20 ± 5.65	0.96
Male/female (n)	10/5	10/5	10/5	
Education (years)	10.73 ± 2.76	10.40 ± 1.84	10.33 ± 1.50	0.86
Duration of heroin use (years)	5.97 ± 3.18	5.53 ± 2.97	5.90 ± 1.61	0.89
Average dose abused (g/day)	0.55 ± 0.32	0.50 ± 0.23	0.57 ± 0.26	0.79
Duration of abstinence (months)	5.08 ± 0.76	5.51 ± 1.78	5.42 ± 1.46	0.68
HAMA score	5.80 ± 0.76	5.87 ± 0.80	6.07 ± 0.71	0.96
BDI score	11.27 ± 1.08	11.13 ± 1.10	11.67 ± 1.22	0.94

Values are expressed as mean ± SD. No significant differences were found on any demographic measures.