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The β-lactam antibiotic ceftriaxone inhibits physical dependence and abstinence-induced withdrawal from cocaine, amphetamine, methamphetamine, and clorazepate in planarians

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Abstract

Ceftriaxone (a β-lactam antibiotic) has recently been identified as having the rare ability to increase the expression and functional activity of the glutamate transporter subtype 1 (GLT-1) in rat spinal cord cultures. GLT-1 has been implicated in diverse neurological disorders and in opioid dependence and withdrawal. It has been speculated that it might also be involved in the physical dependence and withdrawal of other abused drugs, but demonstration of this property can be difficult in mammalian models. Here, we demonstrate for the first time using a planarian model that ceftriaxone attenuates both the development of physical dependence and abstinence-induced withdrawal from cocaine, amphetamine, methamphetamine, and a benzodiazepine (clorazepate) in a concentration-related manner. These results suggest that physical dependence and withdrawal from several drugs involve a common – β-lactam-sensitive – mechanism in planarians. If these findings can be shown to extend to mammals, β-lactam antibiotics might represent a novel pharmacotherapy or adjunct approach for treating drug abuse or serve as a template for drug discovery efforts aimed at treating drug abuse, recovery from drug abuse, or ameliorating the withdrawal from chronic use of therapeutic medications.

Keywords: β-lactam antibiotic; (Meth)amphetamine, Benzodiazepine; Cocaine; Dependence; Withdrawal; Planaria

1. Introduction

A recent screen of more than 1000 clinically-approved drugs and nutritionals uncovered a surprising class of agents that are capable of significantly increasing glutamate reuptake in the mammalian central nervous system — β-lactam antibiotics (e.g., ceftriaxone, penicillin, etc.) (Rothstein et al., 2005). The β-lactam antibiotics significantly increased both the expression and functional activity of the glutamate transporter subtype 1 (GLT-1) in cultures of rat spinal cord (Beghi et al., 2005; Miller and Cleveland, 2005; Rothstein et al., 2005; Secko, 2005). The β-lactams also block glutamate-mediated pathophysiologic conditions in rats and mice. For example, repeated ceftriaxone administration protects against ischemic injury and motor neuron degeneration in vitro; delays the loss of neurons and muscle strength in a mouse model of amyotrophic lateral sclerosis; protects against the neurotoxicity of human immunodeficiency virus proteins; and displays neuroprotective properties in in vitro models of stroke (Rothstein et al., 2005; Rumbaugh et al., 2007; Lipski et al., 2007).

The GLT-1 transporter protein (excitatory amino acid transporter 2, EAAT2) is expressed in mammals, including humans, and is responsible for an estimated 90% of the glutamate uptake in mammalian brain (Rothstein, 1995–1996; Danbolt, 2001; Mitani and Tanaka, 2003). The GLT-1 transporter has been proposed to be a pharmacologic target for preventing physical and psychological dependence on opioids (Ozawa et al., 2001, 2004; Nakagawa and Satoh, 2004; Fujio et al., 2005).

However, the possible involvement of the GLT-1 transporter in dependence produced by other abused drugs remains understudied. One reason is that physical dependence to these other abused drugs is often difficult to quantify in mammals (McGregor et al., 2005; Segal et al., 2006; Segal and Kuczenski, 2006).

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To overcome some of the difficulties, we use a planarian model (Raffa and Valdez, 2001).

It is already known that an increase in glutamatergic transmission at the NMDA-subtype of glutamate receptors is involved in the development and expression of physical dependence in planarians (Cebrià et al., 2002; Rawls et al., 2007a) and that a functional role for glutamate signaling and a therapeutic role for NMDA receptor antagonists in physical dependence are consistent with evidence obtained in mammalian models (Koyuncuoglu et al., 1990; Tanganelli et al., 1991; Rasmussen et al., 1991; Tokuyama et al., 1996). However, NMDA receptor antagonists are ineffective in models of adolescent morphine dependence and cause a number of adverse side effects (e.g., lethality, catalepsy, and memory impairment) when administered with morphine (Zhu and Barr, 2001). Therefore it is of both mechanistic and potential clinical interest that recent evidence shows that an increase in glutamate reuptake following repeated ceftriaxone administration inhibits morphine-evoked hyperthermia in rats (Rawls et al., 2007b). This reveals a functional link between β-lactam antibiotics and opioid systems in conscious animals. What remains unclear however is whether the pharmacological activation of glutamate transporters by β-lactam antibiotics decreases the physical dependence to opioid or non-opioid abused drugs (Nakagawa and Satoh, 2004). Therefore, we used planarians to test if the β-lactam antibiotic ceftriaxone decreases the development or expression of physical dependence to representatives of abused classes of drugs: cocaine, methamphetamine, amphetamine, and benzodiazepine.

2. Materials and methods

2.1. Animals and chemicals

Planarians (*Dugesia dorotocephala*) were purchased from the Carolina Biological Supply Co. (Burlington, NC), acclimated to laboratory conditions, and used within 72 h. Each planarian was used once (N=5–8 per group). Ceftriaxone was purchased from Apotex Corp. (Miami, FL). Cocaine, methamphetamine, amphetamine, and clorazepate were gifts from NIDA to M. W. Adler (Temple University Medical School). All drugs were dissolved in water and fresh solutions were prepared daily.

2.2. Procedure

Following pretreatment in one of the four drugs or vehicle for 1 h (Raffa and Valdez, 2001), planarians were placed singly into a clear plastic Petri dish (14 cm diameter) containing room-temperature water (pH=7.0) and located over grid paper (gridlines spaced 0.5 cm apart). The spontaneous locomotor velocity (pLMV) of each planarian was measured by counting the number of gridlines each planarian crossed or recrossed per min over a 5-min observation period. Five groups were examined: (i) planarians pretreated in water, then tested in water — as control for exposure to vehicle; (ii) planarians pretreated in one of the four test drugs (cocaine, methamphetamine, amphetamine, or clorazepate) and then tested in water — to demonstrate abstinence-induced withdrawal; (iii) planarians co-treated with test drug plus ceftriaxone, then tested in water — the experimental group for detecting an effect of ceftriaxone on development of physical dependence; (iv) planarians pretreated with test drug, then tested in ceftriaxone — the experimental group for detecting an effect of ceftriaxone on abstinence-induced withdrawal; and (5) planarians tested in ceftriaxone — as control.

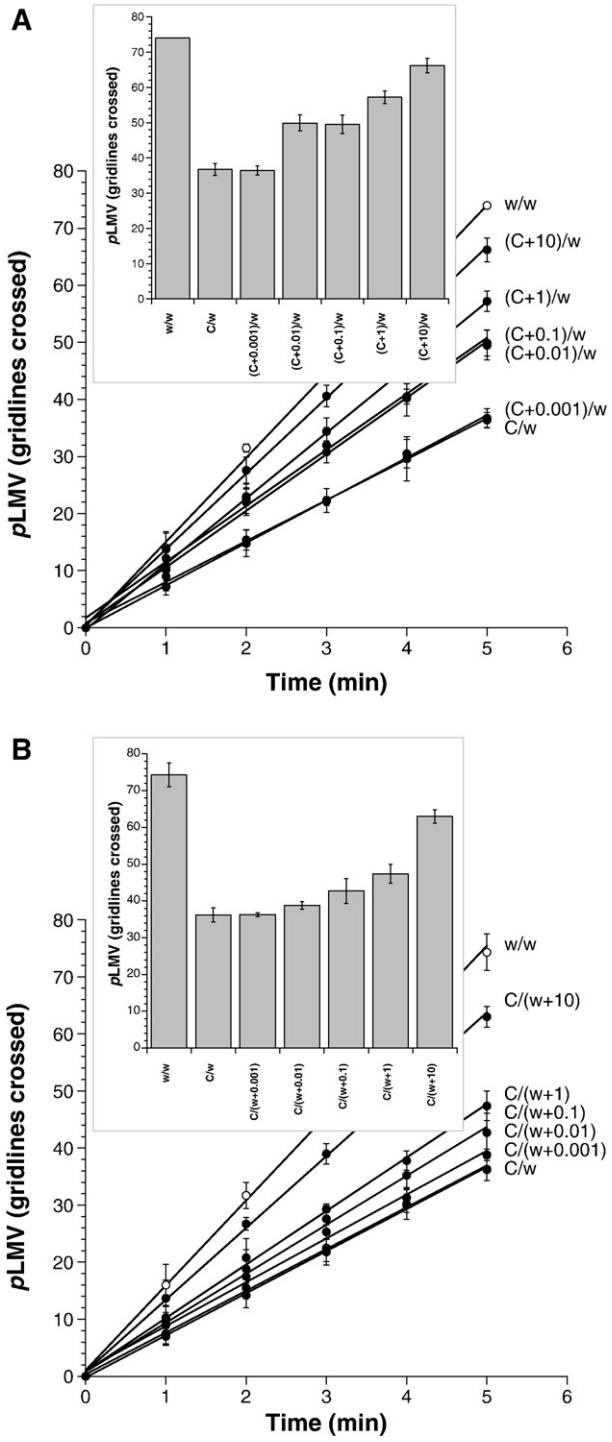

Fig. 1. Concentration-related attenuation of cocaine physical dependence development (A) and abstinence-induced withdrawal (B) by ceftriaxone at the concentration (μM) indicated. Inset=5-min totals. Abbreviations: w = water, C = cocaine.

2.3. Statistics

The group means were compared using one-way ANOVA followed, if $P<0.05$, by Tukey–Kramer multiple comparisons *post hoc* test (significance level $P<0.05$).

3. Results

3.1. Cocaine

Consistent with our previous findings (e.g., Raffa and Valdez, 2001; Raffa et al., 2001; Raffa et al., 2003), drug-naïve planarians displayed a nearly constant pLMV of about 14–16 gridlines per min when tested in water. This resulted in an essentially linear relationship between pLMV and observation time throughout the 5-min observation period. This near linear behavior persists for at least 10 min (Raffa et al., 2001). Also consistent with our previous findings (e.g., Raffa and Valdez, 2001; Raffa et al., 2001; Raffa et al., 2003), cocaine-naïve planarians displayed a nearly constant (linear) pLMV when tested in water, whereas cocaine-exposed planarians displayed abstinence-induced withdrawal when they were tested in water. The withdrawal was abstinence-induced, i.e., it was not induced when cocaine-exposed planarians were tested in the same concentration (10 μM) of cocaine.

Ceftriaxone had no effect of its own on pLMV, whether present during the pretreatment or the test phase. Co-incubation of planarians for 1 h with cocaine (10 μM) together with ceftriaxone attenuated the subsequent abstinence-induced cocaine withdrawal when the planarians were tested in water. The planarians still displayed a linear pLMV over the 5-min observation period. Pretreatment or testing in ceftriaxone alone had no effect on pLMV ($P>0.05$). The attenuation by ceftriaxone of cocaine withdrawal was concentration-related to the ceftriaxone concentration (0.001 to 10 μM) ($F=193.66$; $df=33$; $P<0.000$). The results are summarized in Fig. 1A, where the data are graphed as the means ± S.E.M. of the number of gridlines crossed each min over the 5-min observation period.

When planarians that were pretreated for 1-h in cocaine (10 μM) were tested in water containing ceftriaxone, which by itself had no effect, they still displayed linear pLMV over the 5-min observation period, but abstinence-induced cocaine withdrawal was attenuated in a concentration-related manner (0.001 to 10 μM ceftriaxone) ($F=214.33$; $df=33$; $P<0.0001$). The results are summarized in Fig. 1B, where the data are plotted as the means ± S.E.M. of the number of gridlines crossed each min over the 5-min observation period.

3.2. Amphetamine

Amphetamine-exposed planarians displayed abstinence-induced withdrawal when they were then tested in water; withdrawal was not induced when they were tested in the same concentration (100 μM) of amphetamine. Co-incubation of planarians for 1 h with ceftriaxone together with amphetamine (100 μM) attenuated the subsequent abstinence-induced amphetamine withdrawal. The attenuation by ceftriaxone was concentration-related to the ceftriaxone concentration (0.001 to 10 μM) ($F=191.32$; $df=54$; $P<0.0001$). The results are summarized in Fig. 2A. When planarians that were pretreated for 1-h in amphetamine (100 μM) were tested in water containing ceftriaxone, abstinence-induced withdrawal was attenuated in a concentration-related manner (0.001 to 10 μM ceftriaxone)

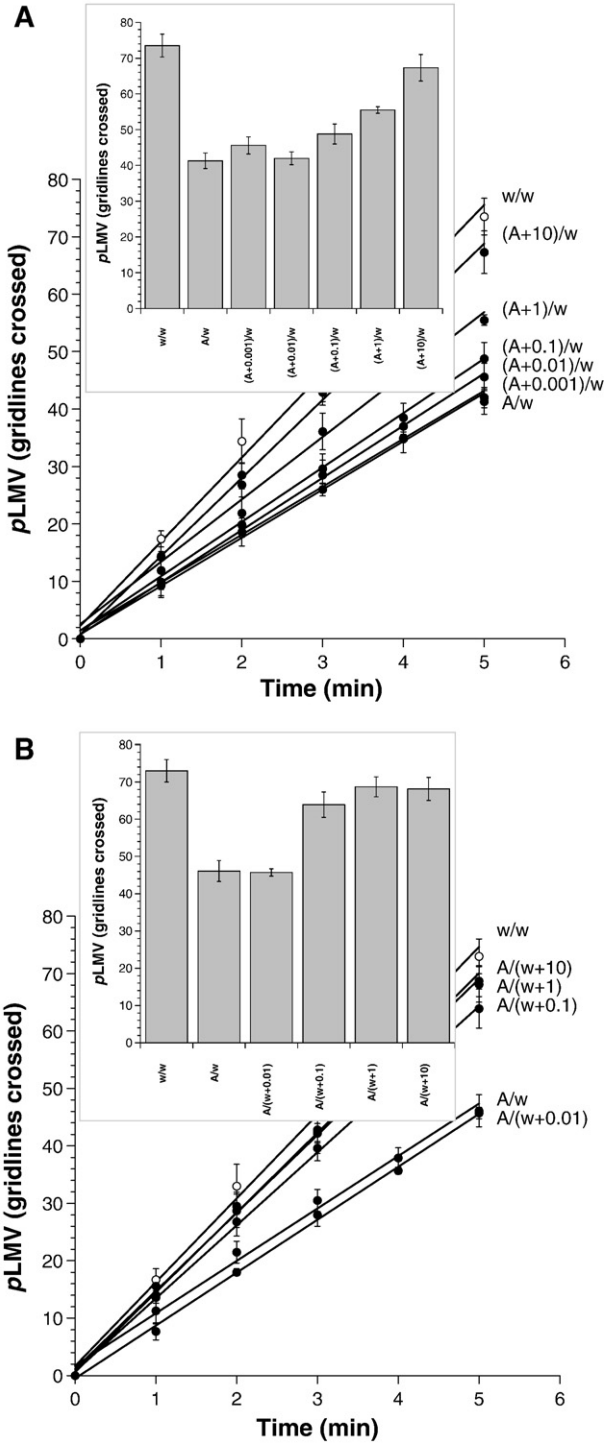

Fig. 2. Concentration-related attenuation of amphetamine physical dependence (A) and abstinence-induced withdrawal (B) by ceftriaxone at the concentration (μM) indicated. Inset = 5-min totals. Abbreviations: w = water, A = amphetamine.

(F=147.92; df=37; P<0.0001). The results are summarized in Fig. 2B.

3.3. Methamphetamine

The results with methamphetamine were similar to those obtained with amphetamine. Methamphetamine-exposed planarians displayed abstinence-induced withdrawal when they were tested in water, but not when they were tested in the same concentration (1 μM) of methamphetamine. Co-incubation of planarians for 1 h with ceftriaxone together with methamphetamine (1 μM) attenuated the subsequent abstinence-induced methamphetamine withdrawal when the planarians were tested in water. The attenuation by ceftriaxone was concentration-related to the ceftriaxone concentration (0.001 to 10 μM) (F=79.331; df=38; P<0.0001). The results are summarized

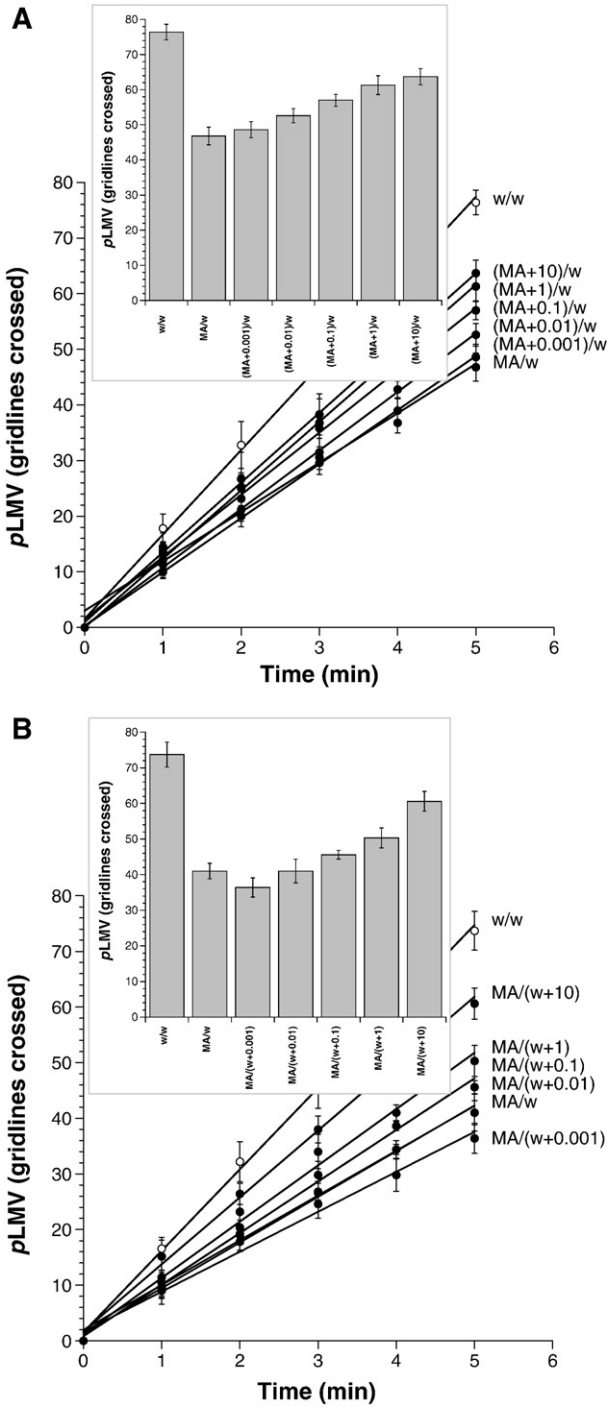

Fig. 3. Concentration-related attenuation of methamphetamine physical dependence development (A) and abstinence-induced withdrawal (B) by ceftriaxone at the concentration (μM) indicated. Inset=5-min totals. Abbreviations: w = water, MA = methamphetamine.

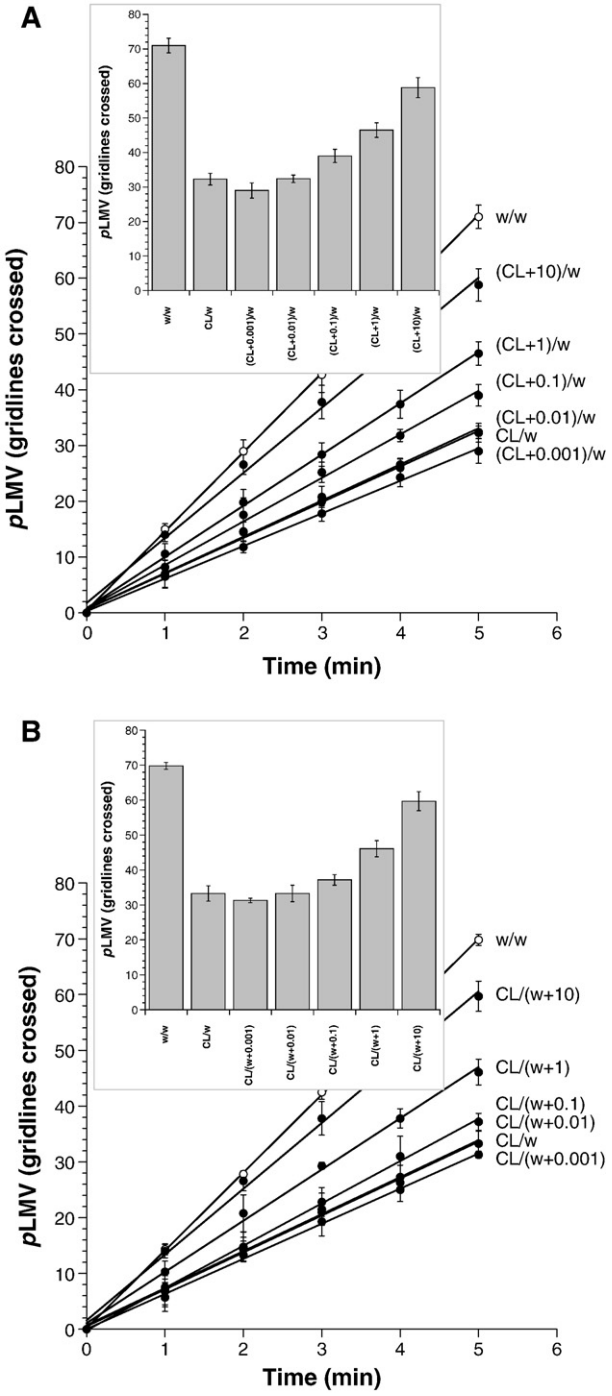

Fig. 4. Concentration-related attenuation of clorazepate physical dependence (A) and abstinence-induced withdrawal (B) by ceftriaxone at the concentration (μM) indicated. Inset=5-min totals. Abbreviations: w = water, CL = clorazepate.

in Fig. 3A. When planarians that were pretreated for 1-h in methamphetamine (1 μM) were tested in water containing ceftriaxone, abstinence-induced cocaine withdrawal was attenuated in a concentration-related manner (0.001 to 10 μM ceftriaxone) ($F=221.43$; $df=49$; $P<0.0001$). The results are summarized in Fig. 3B.

3.4. Clorazepate

The results obtained with the benzodiazepine clorazepate were similar to those obtained with amphetamine, methamphetamine, and cocaine. Clorazepate-exposed planarians displayed abstinence-induced withdrawal when they were tested in water, but not when they were tested in the same concentration (10 μM) of clorazepate. Co-incubation of planarians for 1 h with ceftriaxone together with clorazepate (10 μM) attenuated the subsequent abstinence-induced clorazepate withdrawal when the planarians were tested in water. The attenuation was concentration-related to the ceftriaxone concentration (0.001 to 10 μM) ($F=324.82$; $df=34$; $P<0.0001$). The results are summarized in Fig. 4A. When planarians that were pretreated for 1-h in clorazepate (10 μM) were tested in water containing ceftriaxone, withdrawal was attenuated in a concentration-related manner (0.001 to 10 μM ceftriaxone) ($F=206.39$; $df=27$; $P<0.0001$). The results are summarized in Fig. 4B.

4. Discussion

The GLT-1 transporter protein is important for normal excitatory synaptic transmission; its dysfunction is implicated in acute and chronic neurological disorders such as amyotrophic lateral sclerosis, neurotoxicity, stroke, Parkinson disease, adult motor neuron disease, and opioid dependence and withdrawal (Rothstein et al., 1995; Ye et al., 1999; Rao et al., 2001; Sepkuty et al., 2002). Although antagonism of the glutamate receptor has been studied as a pharmacologic target to decrease excess excitatory glutamatergic influence on neuronal function, there are no therapies directed at decreasing glutamatergic influence by activating the neuronal reuptake of glutamate. In a screen of over 1000 FDA-approved drugs and nutritionals to identify compounds capable of such activity, Rothstein et al. (2005) made the surprising discovery that several β-lactam antibiotics, including ceftriaxone, were the most active. Non-β-lactam antibiotics, such as kanamycin, fluconazole, minocycline, polymyxin, and doxycycline, were not. Furthermore, not all β-lactam antibiotics were active. For example, vancomycin had no effect. Rothstein et al. (2005) demonstrated that the active β-lactam antibiotics increase GLT-1 expression in the rat central nervous system, increase the functional and biochemical activity of GLT-1 in the rat brain, protect against ischemic injury and motor neuron degeneration in vitro, and delay the loss of neurons and muscle strength in a mouse model of amyotrophic lateral sclerosis. Given the favorable side-effect profile of β-lactam antibiotics at antibacterial doses, these findings suggest a role for these commonly prescribed drugs in the clinical management of glutamate-mediated conditions (Rothstein et al., 2005; Lipski et al., 2007; Rumbaugh et al., 2007). Prior to this time no practical pharmaceutical was known to increase GLT-1 expression and activity.

Previous reports of drug effects on planarians (e.g., Algeri et al., 1983; Venturini et al., 1989; Palladini et al., 1996) prompted us to establish a quantifiable metric of withdrawal in this species. Planarians are a type of flatworm that possess a simple centralized nervous system and mammalian-relevant neurotransmitters such as dopamine, 5-HT (serotonin), glutamate, endogenous opioids, and GABA (γ-aminobutyric acid) (Eriksson and Panula, 1994; Newmark and Sanchez Alvarado, 2002; Umeda et al., 2004; Rawls et al., 2006a). Planarians are capable of relatively complex behavioral adaptation, including learning and memory, and they display drug preference (Kimmel and Garrigan, 1973; Kusayama and Watanabe, 2000). We have previously demonstrated concentration-related abstinence-induced withdrawal from cocaine, cannabinoid, κ-opioid, amphetamine, and benzodiazepine ligands in planarians (Raffa and Valdez, 2001; Raffa et al., 2000, 2001, 2003; Umeda et al., 2004; Raffa and Desai, 2005; Raffa and Martley, 2005; Rawls et al., 2006b, 2007b). Withdrawal in planarians is manifested as a decrease in spontaneous locomotor velocity (pLMV) (Raffa et al., 2000; Raffa and Valdez, 2001) when drug-exposed planarians are placed into drug-free water, but not when they are placed into water containing the same concentration of drug. Factors such as osmolarity or pH are not the cause of the reduced pLMV (Umeda et al., 2004) and no withdrawal is observed from opioid antagonists, glutamate antagonists, nitric oxide synthase blockers, or vanilloids (Raffa et al., 2003, 2006; Rawls et al., 2006b, 2007b). Thus, a change in pLMV is a reproducible metric that quantifies abstinence-induced withdrawal in planarians.

In the present study, ceftriaxone attenuated both the development of physical dependence and the expression of physical dependence by an abstinence-induced withdrawal. The first effect (attenuation of development of physical dependence) was demonstrated by co-treatment of the planarians with ceftriaxone along with the test drug (cocaine, amphetamine, methamphetamine, or clorazepate) and then challenging for demonstration of development of physical dependence by transferring the planarians to water. Planarians that were not co-treated with ceftriaxone during the exposure period displayed abstinence-induced withdrawal from the drug. In contrast, planarians that were co-treated with ceftriaxone during the exposure period displayed a reduced abstinence-induced withdrawal during the test period (in water). The magnitude of the reduction in abstinence-induced withdrawal was related to the concentration of the ceftriaxone during the treatment period. The second ceftriaxone effect (attenuation of abstinence-induced withdrawal) was demonstrated by 1 h pretreatment of the planarians with test drug (cocaine, amphetamine, methamphetamine, or clorazepate) and then challenging for withdrawal by transferring the planarians to water that contained ceftriaxone. Abstinence-induced withdrawal was attenuated in a concentration-related manner by the presence of ceftriaxone during the test phase. That is, planarians that were tested in water alone displayed abstinence-induced withdrawal from the drug. In contrast, planarians that were tested in water containing ceftriaxone displayed a reduced abstinence-induced withdrawal.

The magnitude of the reduction was related to the concentration of the ceftriaxone.

The mechanism of ceftriaxone's attenuation of physical dependence and withdrawal is unknown. It is unrelated to its antibiotic action per se, but might be related to its ability to increase glutamate neuronal reuptake. On the basis of the current literature, the most compatible explanation for our results is that ceftriaxone reduced withdrawal and physical dependence by stimulating glutamate reuptake (Rothstein et al., 2005; Miller and Cleveland, 2005; Secko, 2005; Beghi et al., 2005; Rawls et al., 2007a). Molecular evidence indicates that ceftriaxone increases GLT-1 transcription in the mammalian central nervous system (Rothstein et al., 2005). More recently, our laboratory reported pharmacological evidence that ceftriaxone blocks morphine hyperthermia in rats by increasing the cellular reuptake of glutamate (Rawls et al., 2007a). It might also be due to some other, heretofore unrecognized, property. In either case, the ability of ceftriaxone to attenuate the dependence and withdrawal from the diverse drugs tested in the current study suggests that some common mechanism is involved, that is, shared by each of the drugs, not a mechanism tied to only one. If the present results are confirmed in mammalian models of physical dependence, then the β-lactam antibiotics, with their excellent safety profile, may be useful in the clinical management of dependence to a broad spectrum of abused drugs.

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