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β-lactam antibiotic inhibits development of morphine physical dependence in rats

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Abstract

β-lactam antibiotics enhance cellular glutamate uptake. Because increased glutamatergic transmission is a primary mediator of opiate dependence, we tested the hypothesis that a β-lactam antibiotic (ceftriaxone) prevents development of morphine physical dependence in rats. Morphine (20 mg/kg) was injected twice daily for 10 days to induce physical dependence. Naloxone (10 mg/kg) administration 1, 48 and 96 h after the last morphine injection induced a withdrawal syndrome characterized by the appearance of wet-dog shakes, teeth chattering, eye blinking, jumping, and paw tremor. Ceftriaxone (150, 200 mg/kg) injected once daily during chronic morphine exposure inhibited each naloxone-precipitated withdrawal sign. Ceftriaxone efficacy persisted even after the 96 h naloxone (10 mg/kg) injection. These results suggest β-lactam antibiotics inhibit processes leading to development of morphine physical dependence.

Keywords

β-lactam antibiotic; ceftriaxone; morphine; glutamate transporter; dependence; withdrawal; GLT-1; EAAT2, rat

Introduction

Glutamate activity is regulated by two categories of glutamate receptors, N-methyl-D-aspartate (NMDA) and non-NMDA, and two distinct transporter systems, the high-affinity, sodium-dependent, excitatory amino acid transporters (EAATs) and the cystine-glutamate antiporter (Bridges and Esslinger, 2005). Increased glutamate activity produced by repeated morphine exposure mediates physical dependence, a phenomenon defined by the appearance of a withdrawal syndrome following discontinuation of morphine exposure or administration of an opioid antagonist (Noda and Nabeshima, 2004). NMDA receptor activation plays a critical role in morphine physical dependence as the development, expression, and maintenance of the phenomenon are inhibited by NMDA receptor antagonists (Trujillo and Akil, 1991; Popik and Skolnick, 1996). Metabotropic glutamate (mGlu2/3) receptor activity also contributes to both development and expression of morphine physical dependence, whereas AMPA receptor activity is a major mediator of the process in infant rats (Fundytus and Coderre, 1994; Zhu and Barr, 2004). A far less studied – and understood – approach

than glutamate receptor antagonism is EAAT activation. EAAT2, defined as glutamate-transporter subtype 1 (GLT-1) in rodents, is a particularly attractive target. GLT-1 is an astrocytic transporter which mediates 90% of cellular glutamate uptake in the brain, underscoring its unrivaled importance in the maintenance of extracellular glutamate levels and termination of glutamatergic transmission (Rothstein et al., 1994). The primary reason GLT-1 activation is understudied is the lack of available compounds which activate the transporter. This perspective changed when a screen of 1040 clinically approved agents identified β -lactam antibiotics as the only pharmaceuticals which increase glutamate uptake through GLT-1 activation. Therefore, the present study tested the hypothesis that ceftriaxone, a representative β -lactam antibiotic, blocks development of morphine physical dependence in rats.

Methods

Subjects

Animal use procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals*. Male Sprague-Dawley rats (250–275 g) were housed 2 per cage and maintained on a 12-hr light/dark cycle.

Procedure

Morphine sulphate and naloxone hydrochloride were injected subcutaneously (s.c.) and ceftriaxone was injected intraperitoneally (i.p.). Morphine (20 mg/kg) or saline was injected twice daily for 10 days (07.00 and 19.00 h). Rats from both groups were injected once daily with either ceftriaxone (200 mg/kg) or saline, beginning 2 days prior to the start of chronic morphine (or saline) dosing. One hour following a morning injection of morphine (20 mg/kg) or saline on day 11, rats were injected with naloxone (10 mg/kg). Rats were injected again with naloxone (10 mg/kg) 48 and 96 h following the last morphine (20 mg/kg) or saline injection. Immediately after naloxone administration, rats were placed individually into test chambers consisting of boxes (50 cm $\times$ 35 cm $\times$ 45 cm) and 5 withdrawal signs (wet-dog shakes, teeth chattering, eye blinking, jumping, and paw tremor) were counted for 60 min by trained observers who were blind to treatment. Lower ceftriaxone doses (50, 100, and 150 mg/kg) were also tested.

Analysis

Two-way ANOVA was used to identify main effects followed by a Bonferroni's *post-hoc* analysis to identify differences between treatment groups. Values of $P < 0.05$ were considered statistically significant.

Results

Two-way ANOVA revealed a significant drug effect for each naloxone-precipitated withdrawal sign: wet-dog shakes [$F(3, 22) = 14.99$, $P < 0.001$]; teeth chattering [$F(3, 22) = 75.22$, $P < 0.001$]; eye blinking [$F(3, 22) = 28.72$, $P < 0.001$]; jumping [$F(3, 22) = 30.56$, $P < 0.001$]; and paw tremor [$F(3, 22) = 29.33$, $P < 0.001$] (Fig. 1). The frequency of each withdrawal sign (1, 48, and 96 h) was significantly greater in rats co-exposed to saline and morphine (SAL + MOR) than in previously drug-naïve rats (SAL + SAL) or ceftriaxone-exposed rats (CTX + SAL) ($P < 0.05$). Rats with prior 200 mg/kg ceftriaxone experience (CTX + SAL) did not display significantly different withdrawal signs following naloxone injection than previously drug-naïve rats (SAL + SAL) (NS). However, the frequency of each withdrawal sign was significantly less in rats co-exposed to ceftriaxone (200 mg/kg) and morphine (CTX + MOR) compared to rats exposed to morphine (SAL + MOR) ($P < 0.05$). The ceftriaxone (200 mg/kg) effect was evident regardless of the time of naloxone

injection ($P < 0.05$). The ceftriaxone effect was also dose-related as pretreatment with 150 mg/kg of ceftriaxone reduced the frequency of each naloxone-precipitated withdrawal sign whereas lower ceftriaxone doses (50 and 100 mg/kg) were ineffective (Fig. 2).

Discussion

The present demonstration that ceftriaxone inhibits development of morphine physical dependence in rats identifies a functionally significant interaction between a β -lactam antibiotic and morphine in opiate-dependent animals. The ceftriaxone effect is likely related to its capacity to increase cellular glutamate uptake (Rothstein et al., 2005; Lipski et al., 2007; Rawls et al., 2007). Glutamate transporter dysfunction and opiate dependence have been linked by evidence that chronic morphine exposure reduces GLT-1 mRNA levels in the brain and spinal cord (Ozawa et al., 2001; Nakagawa and Satoh, 2004). Our data are further supported by evidence that morphine physical dependence is reduced by MS-153, an agent which displays anti-glutamatergic activity through multiple mechanisms, including glutamate uptake enhancement in CS-7 cells and voltage-gated calcium channel inhibition (Nakagawa et al., 2001) and by genetic insertion of GLT-1 mRNA into the locus coeruleus (Ozawa et al., 2004).

It is unclear how GLT-1 transporter activation by ceftriaxone countered naloxone-precipitated morphine withdrawal signs. The development of morphine physical dependence and expression of somatic signs of morphine withdrawal are multifactorial processes which are mediated by several neural substrates (e.g. locus coeruleus, hypothalamus) and highly dependent on the activation of central glutamate systems (Trujillo and Akil, 1991; Rasmussen et al., 1996; Koob et al., 1992). It is well documented that repeated ceftriaxone exposure enhances GLT-1 transporter activity, either through upregulation or independent modulation of the transporter protein (Chu et al., 2007; Lipski et al., 2007). The most plausible explanation for the present finding is that GLT-1 transporter activity is either enhanced, or maintained, in rats co-exposed to ceftriaxone and morphine. In this case, the enhancement in cellular glutamate uptake produced by GLT-1 transporter activation inhibits the normal increase in glutamate transmission related to morphine dependence, perhaps by preventing the increase in extracellular glutamate levels produced by morphine withdrawal (Koob et al., 1992). The site of action of ceftriaxone is unknown, but the locus coeruleus is a likely candidate. This midbrain nucleus is the most sensitive anatomical site for the expression of somatic signs of morphine withdrawal, and GLT-1 transporter overexpression in the locus coeruleus blocks naloxone-precipitated morphine withdrawal signs (Koob et al., 1992; Ozawa et al., 2004).

It should be noted that the ceftriaxone doses (150, 200 mg/kg) which disrupted morphine physical dependence in our study yield brain ceftriaxone concentrations comparable to those levels required to increase GLT-1 expression (3.5 μ M) and attained with meningitis therapy (0.3–6 μ M) (Rothstein et al., 2005). However, these doses given to rats are significantly greater than the maximal dose (2 g/d) of ceftriaxone administered to patients as an antibiotic (Chandrasekar et al., 1984). Future studies will determine if the ceftriaxone effect demonstrated in the present experiments is mimicked by agents which display greater clinical versatility, such as potent β -lactam antibiotics (e.g. meropenem) and non-antibiotic β -lactam compounds (e.g. β -lactamase inhibitors).

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References

- Bridges RJ, Esslinger CS. The excitatory amino acid transporters: pharmacological insights on substrate and inhibitor specificity of the EAAT subtypes. *Pharmacol Ther* 2005;107:271–285. [PubMed: 16112332]
- Chandrasekar PH, Rolston KV, Smith BR, LeFrock JL. Diffusion of ceftriaxone into the cerebrospinal fluid of adults. *J Antimicrob Chemother* 1984;14:427–430. [PubMed: 6094424]
- Chu K, Lee ST, Sinn DI, Ko SY, Kim EH, Kim JM, Kim SJ, Park DK, Jung KH, Song EC, Lee SK, Kim M, Roh JK. Pharmacological Induction of Ischemic Tolerance by Glutamate Transporter-1 (EAAT2) Upregulation. *Stroke* 2007;38:177–182. [PubMed: 17122424]
- Fundytus ME, Coderre TJ. Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. *Br J Pharmacol* 1994;113:1215–1220. [PubMed: 7889275]
- Koob GF, Maldonado R, Stinus L. Neural substrates of opiate withdrawal. *Trends Neurosci* 1992;15:186–191. [PubMed: 1377426]
- Lipski J, Wan CK, Bai JZ, Pi R, Li D, Donnelly D. Neuroprotective potential of ceftriaxone in *in vitro* models of stroke. *Neuroscience* 2007;146:617–629. [PubMed: 17363173]
- Nakagawa T, Satoh M. Involvement of glial glutamate transporters in morphine dependence. *Ann N Y Acad Sci* 2004;1025:383–388. Review. [PubMed: 15542740]
- Nakagawa T, Ozawa T, Shige K, Yamamoto R, Minami M, Satoh M. Inhibition of morphine tolerance and dependence by MS-153, a glutamate transporter activator. *Eur J Pharmacol* 2001;419:39–45. [PubMed: 11348628]
- Noda Y, Nabeshima T. Opiate physical dependence and N-methyl-D-aspartate receptors. *Eur J Pharmacol* 2004;500:121–128. [PubMed: 15464026]
- Ozawa T, Nakagawa T, Sekiya Y, Minami M, Satoh M. Effect of gene transfer of GLT-1, a glutamate transporter, into the locus coeruleus by recombinant adenoviruses on morphine physical dependence in rats. *Eur J Neurosci* 2004;19:221–226. [PubMed: 14750980]
- Popik P, Skolnick P. The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence. *Pharmacol Biochem Behav* 1996;53:791–797. [PubMed: 8801580]
- Rasmussen K, Kendrick WT, Kogan JH, Aghajanian GK. A selective AMPA antagonist, LY293558, suppresses morphine withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. *Neuropsychopharmacology* 1996;15:497–505. [PubMed: 8914123]
- Rawls SM, Tallarida RJ, Robinson W, Amin M. The β -lactam antibiotic, ceftriaxone, attenuates morphine-evoked hyperthermia in rats. *Br J Pharmacol* 2007;151:1095–1102. [PubMed: 17592517]
- Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncel RW. Localization of neuronal and glial glutamate transporters. *Neuron* 2004;13:713–725. [PubMed: 7917301]
- Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. β -lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* 2005;433:73–77. [PubMed: 15635412]
- Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science* 1991;251:85–87. [PubMed: 1824728]
- Zhu H, Barr GA. The role of AMPA and metabotropic glutamate receptors on morphine withdrawal in infant rats. *Int J Dev Neurosci* 2004;22:379–395. [PubMed: 15380837]

Fig. 1.

Ceftriaxone (CTX) (200 mg/kg) effect on naloxone (NLX)-precipitated morphine (MOR) withdrawal. NLX was injected 1, 48, and 96 h after the last MOR injection. Data are expressed as the mean number ($\pm$ S.E.M.) of a specific withdrawal sign observed in the 60 min following NLX injection. * $P < 0.05$ compared with SAL + SAL and $^+P < 0.05$ compared with SAL + MOR.

Fig. 2.

Dose-related effects of ceftriaxone (CTX) (0, 50, 100, 150, or 200 mg/kg) on naloxone (NLX)-precipitated morphine (MOR) withdrawal. All rats were injected with NLX 1 h after the last MOR injection. Data are expressed as the mean number (+ S.E.M.) of a specific withdrawal sign observed in the 60 min following NLX injection. * $P < 0.05$ or ** $P < 0.01$ compared with 0 mg/kg CTX (i.e., CTX-naïve, morphine-dependent rats injected with NLX).