

β -Lactam antibiotic produces a sustained reduction in extracellular glutamate in the nucleus accumbens of rats

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Abstract We investigated the short- and long-term effects of ceftriaxone on glutamate transporter subtype 1 (GLT-1) transporter activity and extracellular glutamate in the rat nucleus accumbens. Repeated ceftriaxone administration (50, 100 or 200 mg/kg, i.p.) produced a dose-dependent reduction in glutamate levels that persisted for 20 days following discontinuation of drug exposure. The ceftriaxone effect was prevented by the GLT-1 transporter inhibitor dihydrokainate (1 μ M, intra-accumbal). These results suggest that β -lactam antibiotics produce an enduring reduction in glutamatergic transmission in the brain reward center.

Keywords Ceftriaxone · Glutamate ·
Nucleus accumbens · β -Lactam antibiotic ·
Microdialysis · GLT-1

Introduction

β -Lactam antibiotics enhance cellular glutamate uptake through activation of glutamate transporter subtype 1

(GLT-1), a predominantly astrocytic protein expressed in rats and humans (excitatory amino acid transporter-2, EAAT2) that is responsible for the majority of cellular glutamate uptake in the mammalian brain (Rothstein et al. 2005; Rothstein 1996). The physiological significance of GLT-1 transporters to the maintenance of normal glutamate transmission is evident from prior work demonstrating that knockdown of striatal GLT-1 protein expression to approximately 40% of control levels decreases glutamate uptake to 50% of control in rats and that glutamate transport activity is reduced by >90% in cortical synaptosomes prepared from mice lacking GLT-1 transporter expression (Rothstein et al. 1996; Mitani and Tanaka 2003). Because GLT-1 transporter dysfunction leads to abnormal increases in extracellular glutamate and glutamate transmission, consequences that contribute to the development and expression of a number of glutamate-related pathologies, GLT-1 transporter activation is widely recognized by neuroscientists as a promising therapeutic approach to manage a broad range of CNS diseases that currently lack safe and effective therapies. Unfortunately, investigation of the approach in preclinical animal models has been hampered by a lack of agents that activate the transporter. This viewpoint changed in 2005, when a screen of over 1,000 clinically approved drugs and nutritionals identified β -lactam antibiotics as the only class of agents capable of increasing GLT-1 transporter activity (Rothstein et al. 2005). Functional studies have since shown that the representative β -lactam antibiotic ceftriaxone displays efficacy in rodent models of amyotrophic lateral sclerosis, multiple sclerosis, stroke, neurotoxicity, Huntington's disease, depression, addiction, dependence and tolerance (Rothstein et al. 2005; Miller et al. 2008; Lee et al. 2008; Lipski et al. 2007; Rawls et al. 2010a, b; Sari et al. 2009; Knackstedt et al. 2010). Although it is a consistent finding

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that the anti-glutamate properties of ceftriaxone in rodents are dependent on GLT-1 transporter activation, it remains unclear whether the neurochemical changes accompanying repeated β -lactam antibiotic exposure are transient or persistent. To directly investigate this question, we used the technique of in vivo microdialysis to quantify extracellular glutamate levels in the rat nucleus accumbens, 1, 5 and 20 days following discontinuation of repeated ceftriaxone exposure. The nucleus accumbens was selected because it is a principal component of the brain reward circuitry that possesses well-defined glutamate systems, which are targeted by abused drugs (Kalivas 2009).

Methods

Experiment 1

Short- and long-term effects of ceftriaxone on accumbal glutamate levels were investigated. Animal use procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (200–250 g) were injected once daily for 5 days with ceftriaxone (50, 100 or 200 mg/kg) or saline. Microdialysis experiments were conducted 1, 5 and 20 days following discontinuation of ceftriaxone exposure. On the day of microdialysis experiments, CMA/12 microdialysis probes were implanted unilaterally into the nucleus accumbens of rats implanted with guide cannulae 5 days earlier (AP +2.0 mm, ML +1.6 mm, DV –7.8 mm). Artificial CSF NaCl [(147 mM), CaCl₂ (1.2 mM), KCl (2.7 mM), MgCl₂ (0.85 mM)] was then perfused through the probe at a flow rate of 1 μ l/min. Following a 120-min washout/acclimation period (to allow for stabilization of extracellular glutamate following probe implantation) during which samples were not collected for analysis, six consecutive samples were collected from each rat every 15 min for 90 min. Separate groups of animals were used for each day of microdialysis. Following histological verification of probe placement, glutamate was derivatized and then quantified by fluorescence detection (Werkheiser et al. 2006).

Experiment 2

The mechanism contributing to the effects of β -lactam antibiotics on accumbal glutamate levels was investigated by co-administering ceftriaxone (200 mg/kg) and dihydrokainate (DHK). Experiments were repeated as described above except that glutamate was sampled during a 90-min intra-accumbal perfusion of ACSF by itself or ACSF containing DHK (1 μ M).

Data analysis

The six glutamate samples (μ M) were averaged to obtain a mean glutamate value (μ M $\pm$ SEM). Data were analyzed using a two-way ANOVA (group, day) followed by pairwise multiple comparisons incorporating the Bonferroni correction. $P < 0.05$ was considered to be statistically significant.

Results

Repeated ceftriaxone treatments produced a dose-dependent and persistent reduction in accumbal glutamate levels (Fig. 1). The ceftriaxone effect was most apparent on post-treatment days 1 and 5, where doses of 100 and 200 mg/kg reduced glutamate levels to 40–50% compared to saline-treated rats ($P < 0.05$). On post-treatment day 20, glutamate levels in rats treated with 200 mg/kg of ceftriaxone were still decreased compared to saline-treated rats ($P < 0.05$). In rats pre-treated with ceftriaxone (200 mg/kg), subsequent DHK (1 μ M) perfusion prevented the normal reduction in extracellular glutamate levels on post-ceftriaxone treatment days 1 and 5 (Fig. 2) ($P < 0.05$). The ceftriaxone (200 mg/kg) effect was also reduced by DHK (1 μ M) on post-ceftriaxone

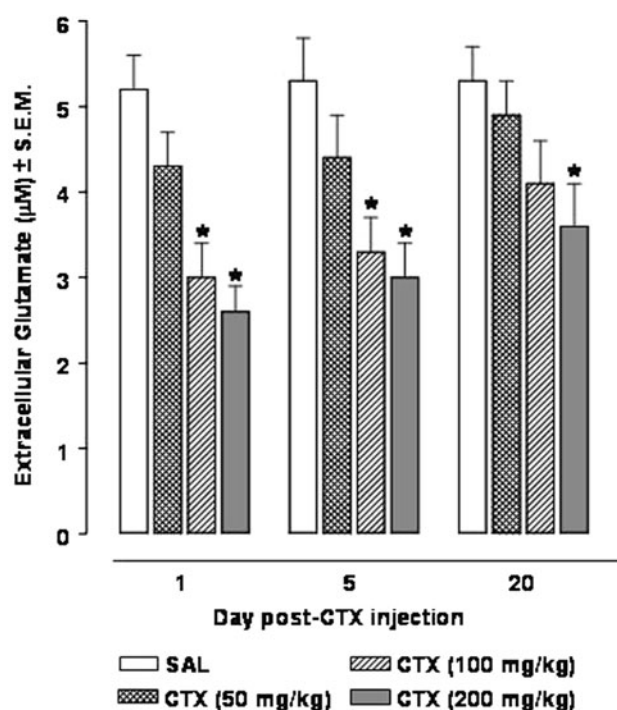


Fig. 1 Extracellular glutamate in the nucleus accumbens following repeated ceftriaxone (CTX) administration (50, 100, 200 mg/kg) or saline (SAL) ($N = 7$ –9 rats per group). Two-way ANOVA revealed a significant drug effect [$F(15,176) = 6.608$, $P = 0.0026$] and day effect [$F(2,56) = 18.97$, $P = 0.0002$]. * $P < 0.05$ compared to saline-treated rats

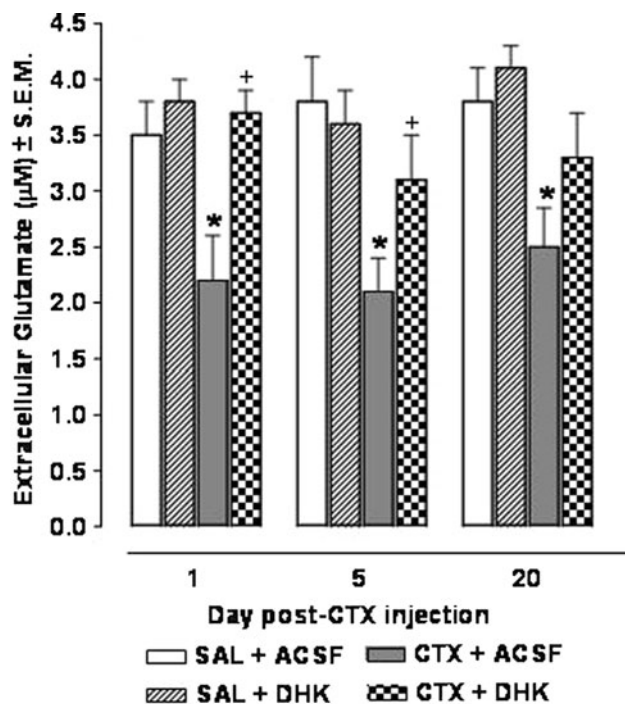


Fig. 2 Extracellular glutamate in the nucleus accumbens following local perfusion of ACSF by itself or ACSF containing DHK (1 μ M) in rats previously treated with SAL or CTX (200 mg/kg) ($N = 6$ rats per group). Two-way ANOVA revealed a significant drug effect [$F(3,20) = 13.87$, $P < 0.0001$]. * $P < 0.05$ compared to SAL + ACSF and + $P < 0.05$ compared to CTX + ACSF

treatment day 20, but the effect did not reach statistical significance ($P > 0.05$). In saline-treated rats, DHK (1 μ M) did not alter glutamate levels compared to the local perfusion of normal ACSF ($P > 0.05$).

Discussion

The present study provides evidence that β -lactam antibiotics produce a sustained reduction in extracellular glutamate. The ceftriaxone effect was dose related and dependent on increased GLT-1 transporter activity within the nucleus accumbens. It was not surprising that GLT-1 transporter activation by ceftriaxone simply reduced accumbal glutamate levels. Prior studies indicate that brain glutamate levels are elevated in mice lacking GLT-1 transporters and that ceftriaxone reduces glutamate levels 1 day after cessation of drug exposure (Mitani and Tanaka 2003; Miller et al. 2008). What was an unexpected discovery was the extended duration of action of ceftriaxone (i.e., extracellular glutamate levels were still suppressed 20 days following discontinuation of drug exposure). The sustained inhibition of glutamate levels by ceftriaxone is consistent with its ability to delay the loss of muscle strength and body weight in a mouse model of amyotrophic

lateral sclerosis for up to 6 weeks post-administration (Rothstein et al. 2005), but the functional consequences of an enduring reduction in extracellular glutamate produced is unclear. Agents (e.g., β -lactam compounds and neuro-immunophilin ligands) aimed at preserving or restoring glutamate homeostasis through widespread GLT-1 transporter activation may be therapeutic in certain pathological conditions, but harmful in other cases. Acute energy failure, in which GLT-1 transporters reverse their direction of glutamate transport and pump glutamate into the extracellular compartment, may be one such case because an enhancement in GLT-1 activity, over and above transporter reversal, would be expected to worsen the ischemic insults by further accelerating the rise in extracellular glutamate (Phillis et al. 2000).

The dose of ceftriaxone that produced the most persistent reduction in extracellular glutamate was 200 mg/kg, the standard dose used by our laboratory and other laboratories to investigate the anti-glutamate properties of ceftriaxone (Rothstein et al. 2005; Miller et al. 2008; Rawls et al. 2010a, b; Knackstedt et al. 2010). This dose is equivalent to approximately 13 g/day for a typical adult patient, whereas the maximal dose of ceftriaxone normally administered to humans as an antibiotic is 2 g/day. Assuming a linear, allometric relationship in ceftriaxone dose for a rat-to-human scale-up, plasma levels of ceftriaxone are clearly greater under our conditions than those levels achieved by a therapeutic dose in humans. It should be noted, however, that pharmacokinetic results suggest that i.p. administration of 200 mg/kg of ceftriaxone yields CNS concentrations of the antibiotic comparable to the CNS levels required to increase GLT-1 expression (3.5 μ M), and attained with therapy for meningitis (0.3–6 μ M) (Chandrasekar et al. 1984; Nau et al. 1993; Granero et al. 1995; Rothstein et al. 2005). In addition, to our knowledge, repeated administration of 200 mg/kg of ceftriaxone to rats or mice has not been reported to produce adverse effects. Nonetheless, there is a growing concern that the high doses of ceftriaxone required for anti-glutamate activity will also produce adverse effects that will limit the utility of the antibiotic as a CNS-active therapeutic in actual patients. Future studies will be directed toward designing, synthesizing and testing synthetic β -lactam compounds with the ultimate goal of identifying a patient-friendly alternative (e.g., enhanced brain penetrability, minimal anti-bacterial activity, oral administration, etc.) to direct ceftriaxone therapy.

The exact mechanism underlying the short- and long-term inhibition of glutamate levels by ceftriaxone is unknown. Increased GLT-1 activity played an important role, but the inability of DHK to completely abolish the ceftriaxone effect 20 days following discontinuation of antibiotic exposure suggests the involvement of additional

mechanisms, such as an alteration in signaling pathways downstream of GLT-1 activation, or compensatory responses aimed at countering the reduction in glutamate levels, such as increased cystine–glutamate exchange (Baker et al. 2002). It is also unclear whether ceftriaxone increased GLT-1 activity through upregulation or independent modulation of the transporter. GLT-1 transporter activation by ceftriaxone can occur in the absence or presence of protein upregulation, and GLT-1 transporter function is regulated at both the transcriptional and post-transcriptional levels (Miller et al. 2008; Chu et al. 2007; Lipski et al. 2007; Knackstedt et al. 2010). Important objectives of future studies are to: delineate the duration of the effect of ceftriaxone on glutamate function by providing an even longer time course than the 20-day post-treatment interval used here; determine if the anti-glutamate effects of ceftriaxone in conscious animals are specific to the nucleus accumbens or extend to other brain regions associated with the process of addiction, such as the prefrontal cortex, ventral tegmental area and dorsal striatum; determine if the neurochemical changes produced by ceftriaxone translate into effects in animal models of cocaine-induced behavioral sensitization, self-administration and conditioned place preference. In summary, the persistent reduction in accumbal glutamate levels produced by ceftriaxone indicates that repeated β -lactam antibiotic exposure may produce long-term changes in glutamate signaling in a primary component of the brain reward circuit.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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