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RESEARCH PAPER

The beta-lactam antibiotic, ceftriaxone, attenuates morphine-evoked hyperthermia in rats

SM Rawls[1], R Tallarida[2], W Robinson[1] and M Amin[1]

[1]*Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA, USA and* [2]*Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA, USA*

Background and purpose: Beta-lactam antibiotics are the first practical pharmaceuticals capable of increasing the expression and activity of the glutamate transporter, GLT-1, in the CNS. However, the functional impact of beta-lactam antibiotics on specific drugs which produce their pharmacological effects by increasing glutamatergic transmission is unknown. One such drug is morphine, which causes hyperthermia in rats, mediated by an increase in glutamatergic transmission. Since drugs (e.g. antibiotics) that enhance glutamate uptake also decrease glutamatergic transmission, we tested the hypothesis that ceftriaxone, a beta-lactam antibiotic, would block the glutamate-dependent portion of morphine-evoked hyperthermia.
Experimental approach: A body temperature assay was used to determine if ceftriaxone decreased morphine-induced hyperthermia in rats by increasing glutamate uptake.
Key results: Body temperatures of rats treated with ceftriaxone (200 mg kg^{-1}, i.p. $\times$ 7 days) did not differ from rats receiving saline. Morphine (1, 4, 8 and 15 mg kg^{-1}, s.c.) caused significant hyperthermia. Pre-treatment with ceftriaxone, as described above, decreased the hyperthermic response to these doses of morphine. The effects of ceftriaxone were prevented by TBOA (0.2 μmol, i.c.v.), an inhibitor of glutamate transport.
Conclusions and implications: Ceftriaxone attenuated the hyperthermia caused by morphine, an effect prevented by inhibiting glutamate transport. Thus this effect of ceftriaxone was most likely mediated by increased glutamate uptake. These data revealed a functional interaction between ceftriaxone and morphine and indicated that a beta-lactam antibiotic decreased the efficacy of morphine in conscious rats.
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Keywords: antibiotics; morphine; glutamate; GLT-1; hyperthermia; ceftriaxone; TBOA

Abbreviations: EAAT2, excitatory amino-acid transporter 2; GLT-1, glutamate transporter subtype 1; riluzole, 2-amino 6-trifluoromethoxy-benzothiazole; TBOA, DL-threo-β-benzyloxyaspartic acid

Introduction

Common antibiotics may do more than just kill bacteria. A recent screening of over 1000 clinically approved drugs revealed that the β-lactam antibiotics were the only agents capable of increasing both the expression and activity of the glutamate transporter subtype 1 (GLT-1) (Beghi *et al*., 2005; Miller and Cleveland, 2005; Rothstein *et al*., 2005; Secko, 2005). The GLT-1 transporter protein is expressed in rats and humans (excitatory amino-acid transporter 2, EAAT2) and is responsible for 90% of glutamate uptake in the central nervous system (CNS) (Rothstein, 1996; Danbolt, 2001). Earlier work reveals that extracellular glutamate increases in animals lacking the GLT-1 transporter (Mitani and Tanaka, 2003). Animal models demonstrate that GLT-1 dysfunction contributes to a number of clinical disorders, including amyotrophic lateral sclerosis, neurotoxicity, stroke, Parkinson's disease, adult motor neuron disease, opioid dependence and opioid withdrawal (Tanaka *et al*., 1997; Ye *et al*., 1999; Rao *et al*., 2001; Nakagawa and Satoh, 2004; Ozawa *et al*., 2001, 2004; Fujio *et al*., 2005).

In spite of the importance of GLT-1 in physiological and pathophysiological conditions, no practical pharmaceuticals were known to modulate its expression and activity until recently. This situation changed when Rothstein *et al*. (2005) discovered that the β-lactam class of antibiotics caused the following effects: increased GLT-1 expression in the rat brain; increased functional and biochemical activity of GLT-1 in the rat brain; protection against ischaemic injury and motor neuron degeneration *in vitro*; and delayed loss of neurons and muscle strength in a mouse model of ALS. Because β-lactam antibiotics are the most widely used antibiotics in the world, cause no known side effects at antibacterial doses,

Correspondence: Dr SM Rawls, Assistant Professor of Pharmacology, Department of Pharmaceutical Sciences, Temple University School of Pharmacy, 3307 North Broad Street, Philadelphia, PA 19140, USA.
E-mail: scott.rawls@temple.edu
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and increase GLT-1 transcription, they may be useful in the clinical management of glutamate-mediated conditions (Goodman *et al*., 2001; Rothstein *et al*., 2005).

The hyperthermic effect of morphine in rats is one end point mediated by glutamate (Geller *et al*., 1983; Rawls *et al*., 2003). Low doses of morphine cause hyperthermia in rats by stimulating μ-opioid receptors, whereas higher doses of morphine produce hypothermia by activating κ-opioid receptors (Spencer *et al*., 1988, 1990; Adler and Geller, 1993; Chen *et al*., 1996, 2001). Because glutamate increases body temperature and heat production, an enhancement in glutamatergic transmission is thought to cause hyperthermia (Singh and Gupta, 1997; Yasumatsu *et al*., 1998). Several lines of evidence suggest that glutamate is a key factor in morphine-induced hyperthermia. For example, pharmacological antagonism of NMDA receptors blocks the hyperthermia caused by systemically administered morphine (Rawls *et al*., 2003). In addition, a hyperthermic dose ($10\,mg\,kg^{-1}$) of morphine causes a downregulation of NMDA receptor subunit mRNAs in the hypothalamus, the major thermoregulatory centre in the brain (Boulant, 1981; Le Greves *et al*., 1998). An *in vivo* microdialysis study showed that extracellular glutamate levels in the striatum, a structure that plays an indirect role in thermoregulation, are elevated following the systemic injection of morphine (Lin *et al*., 1992; Huang *et al*., 1997). Collectively, these results suggest that morphine-evoked hyperthermia is mediated in part by an increase in glutamatergic transmission at NMDA receptors located in regions of the brain that regulate body temperature. A number of other behavioural and neuro-adaptive effects of morphine, including neural plasticity, dependence, withdrawal and antinociception, are also mediated by an increase in glutamatergic transmission at NMDA and non-NMDA receptors (Rasmussen and Aghajanian, 1989; Akaoka and Aston-Jones, 1991; Rasmussen *et al*., 1991; Aghajanian *et al*., 1994; Herman *et al*., 1995; Inturrisi, 1997; Larcher *et al*., 1998; Trujillo, 2000; Allen and Dykstra, 2001).

In the present study, we used a body temperature assay to test the hypothesis that ceftriaxone would block morphine-evoked hyperthermia. Ceftriaxone was chosen because it is water-soluble and penetrates the brain more effectively than penicillin. To test our hypothesis, rats treated with ceftriaxone for 7 days were injected with one of four doses of morphine and body temperatures were determined. To determine if an enhancement of glutamate uptake was the mechanism of ceftriaxone, we used DL-threo-β-benzyloxy-aspartic acid (TBOA), a glutamate uptake inhibitor, in a second set of experiments. To confirm that glutamatergic transmission mediates morphine-evoked hyperthermia, we tested the effects of 2-amino 6-trifluoromethoxy-benzothiazole (riluzole), a glutamate release inhibitor, and morphine on body temperature (Rawls *et al*., 2003).

Methods

Animals

Animal procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Temple University Animal Care and Use Committee. Male Sprague–Dawley rats (Zivic-Miller, Pittsburgh, PA, USA) weighing 200–250 g (age, 60–80 days) were housed two per cage for a minimum of 5 days before experimental use. Rats were maintained on a 12-h light/dark cycle and fed rat chow and water *ad libitum*. Individual rats were used in one experiment and then killed humanely. A total of 134 rats were used in the entire study.

Cannula implantation

Rats were anaesthetized with an intraperitoneal (i.p.) injection of ketamine hydrochloride ($100–150\,mg\,kg^{-1}$) and acepromazine maleate ($0.2\,mg\,kg^{-1}$). A polyethylene cannula was implanted stereotaxically into the right lateral ventricle (Rawls *et al*., 2005). Dental acrylic cement was used to secure the cannula to the cranium. The route of TBOA administration was intracerebroventricular (i.c.v.) and it was given by inserting the needle tip of a 10-μl syringe into the polyethylene cannula. TBOA ($0.2\,\mu mol$) was administered in a volume of $5\,\mu l$. The dose of TBOA was determined from a previous *in vivo* study (Cechova and Zuo, 2006). Following i.c.v. experiments, injection sites were verified with an injection of 0.1% Evan's blue ($5\,\mu l$). If the ventricles of a rat were not labelled with dye, the injections were considered as misplaced and those rats were omitted from the data analysis.

Dosing schedule

Rats were randomly divided into two groups. One group of rats received a single injection of ceftriaxone ($200\,mg\,kg^{-1}$, i.p.) daily for 7 consecutive days. The second group received injections of saline for 7 days. The ceftriaxone and saline injections were made each day between 0700 and 0800 hours. On day 8, rats from both groups were injected with morphine between 1100 and 1200 hours Ceftriaxone was not administered to rats on day 8.

Body temperature experiments

Body temperature experiments were always started between 0800 and 0900 hours on day 8 to minimize the effects of circadian variation. Rats were placed individually into an environmental room maintained at a constant temperature of $21 \pm 0.3^{\circ}C$ and relative humidity of $52 \pm 2\%$. The animals were allowed to acclimatize for 60 min before taking the first temperature reading. Before drug administration, baseline temperatures were taken every 30 min for 90 min using a thermistor probe (YSI series 400, Yellow Springs Instrument Co., Yellow Springs, OH, USA; sensitivity of $0.10^{\circ}C$), which was lubricated and inserted approximately 7 cm into the colon. A digital thermometer (Model 49 TA, YSI) was used to record body temperature. Rats were unrestrained throughout the experiment, with only the tail being held gently between two fingers. Following the baseline interval, rats were injected with saline or morphine (1, 4, 8, or $15\,mg\,kg^{-1}$, subcutaneously (s.c.)). Body temperatures were recorded at 30, 60, 90, 120 and 150 min following injection. Doses of ceftriaxone and morphine were based on previous studies in conscious rats (Benamar *et al*., 2001; Rawls *et al*., 2003; Rothstein *et al*., 2005). To determine if ceftriaxone affected morphine-evoked hyperthermia by decreasing glutamatergic

transmission, a separate set of experiments tested the effects of morphine and TBOA on the body temperatures of rats pretreated for 7 days with ceftriaxone (200 mg kg^{-1}, i.p.) or saline. Rats treated with ceftriaxone (200 mg kg^{-1}, i.p.) or saline for 7 days were given TBOA (0.2 μmol/ rat, i.c.v.) or vehicle (5 μl, 20% dimethylsulphoxide (DMSO)/water) on day 8, followed by either morphine (4 mg kg^{-1}, s.c.) or saline 15 min later.

As a positive control, we investigated the effect of riluzole on the hyperthermic response to a single dose of morphine (4 mg kg^{-1}, s.c.). This experiment was performed to confirm that an increase in glutamatergic transmission is necessary for morphine to produce its full hyperthermic effect (Rawls *et al*., 2003). Following a 90-min baseline interval, drug-naïve rats were given riluzole (2.5 or 5 mg kg^{-1}, s.c.) or vehicle (0.1 N hydrochloric acid). Thirty minutes later, rats were administered saline or morphine (4 mg kg^{-1}, s.c.). Body temperatures were recorded 30, 60, 90, 120 and 150 min post-injection. The riluzole dose range was based on work showing that riluzole (2, 4 and 8 mg kg^{-1}, s.c.) attenuated the formation of conditioned place aversion induced by naloxone in rats undergoing a single morphine exposure (Jin *et al*., 2006).

Data analysis

Three consecutive temperature readings were measured and averaged to establish a baseline temperature before drug injection. Data were calculated as the mean ± s.e.m. of the change in body temperature. Before analysis, all data were transformed into 'normalized ranks' to address non-normality. Transformed data were analysed using either a Student's *t*-test or two-way (group, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pairwise multiple comparisons incorporating the Bonferroni correction. Area under the body temperature time curve (AUC) values were calculated from 15 to 150 min using the difference score from 0 min (trapezoidal rule) and differences between individual groups were determined by Tukey's *post hoc* analysis. In all cases, values of $P<0.05$ were considered to be statistically significant.

Drug preparation and administration

Morphine sulphate was obtained from the National Institute on Drug Abuse (Rockville, MD, USA). Ceftriaxone hydrochloride was purchased from Apotex Corporation (Weston, FL, USA). Riluzole (2-amino 6-trifluoromethoxy-benzothia-

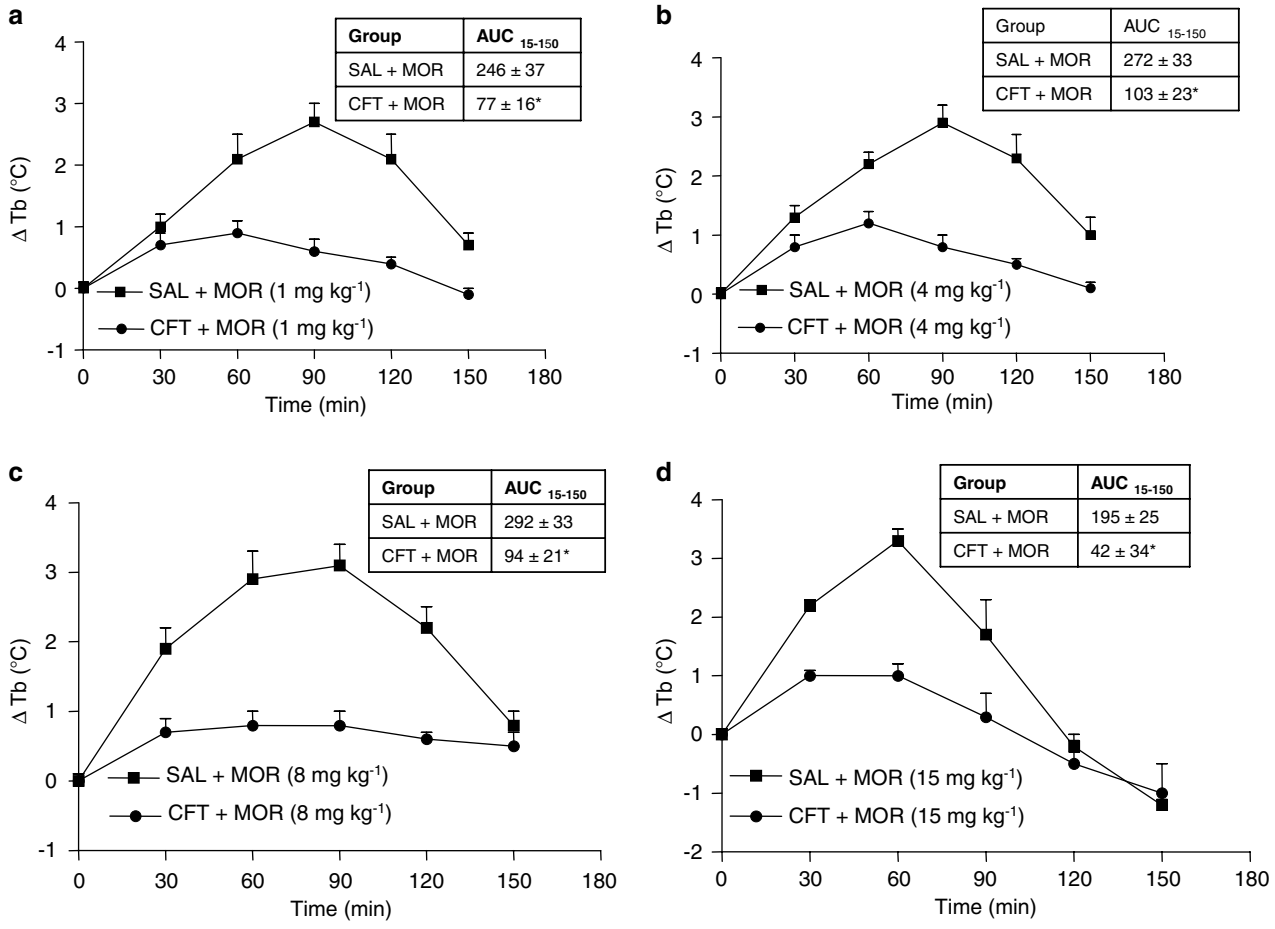

Figure 1 Repeated ceftriaxone attenuated the hyperthermia caused by morphine. (**a**–**d**) Time courses, separated according to dose of morphine: ceftriaxone (CFT) (200 mg kg^{-1}, intraperitoneally (i.p.)) or saline (SAL, i.p.) was injected for 7 consecutive days. On day 8, all rats received an injection of morphine (MOR) (1, 4, 8, or 15 mg kg^{-1}, subcutaneous (s.c.)) or SAL. Data are expressed as the mean ± s.e.m. of the change in body temperature (ΔT_b) from baseline (time 0). Inserted table, AUC_{15-150} profile: Area under the body temperature time curve (AUC) was calculated from 15 to 150 min using the difference score from 0 min (trapezoidal rule). *$P<0.05$, Student's *t*-test.

zole), a glutamate release inhibitor, and TBOA, a glutamate uptake inhibitor, were purchased from Tocris Bioscience (St Louis, MO, USA). Morphine and ceftriaxone were dissolved in pyrogen-free saline. TBOA was dissolved in a 20% dimethyl sulphoxide (DMSO)/ water solution. Riluzole was dissolved in 0.1 N hydrochloric acid. Morphine and riluzole were injected s.c. and ceftriaxone was injected i.p.. TBOA was administered centrally. Systemically injected drugs were given in a volume of 1 ml kg^{-1}, whereas TBOA was administered in a volume of 5 μl (intracerebroventricular, i.c.v.).

Results

Ceftriaxone attenuates morphine-evoked hyperthermia

The body temperatures of rats receiving repeated administrations of ceftriaxone (200 mg kg^{-1}, i. p.) for 7 days did not differ significantly from rats receiving saline for 7 days ($P>0.05$) (data not shown). Morphine (1, 4, 8 and 15 mg kg^{-1}, s.c.) produced hyperthermia in saline pretreated rats (Figure 1). For combined administration, the hyperthermic responses to graded doses of morphine (1, 4, 8 and 15 mg kg^{-1}, s.c.) were attenuated in rats that received a pretreatment of ceftriaxone (200 mg kg^{-1}, i.p.) for 7 days (Figures 1a–d) ($P<0.05$). Morphine did not produce any other overt behavioural effects over the duration of our experiments. The acute injection of ceftriaxone (200 mg kg^{-1}, i.p.) did not affect the hyperthermia caused by a single dose of morphine ($P>0.05$) (data not shown). This result indicates that repeated administration of the antibiotic is required for the attenuation of morphine-induced hyperthermia (Rothstein *et al.*, 2005).

To quantitate more effectively the time course data in Figures 1a–d, we compared the dose–response relation of the active agent (morphine) and the dose–response relation of that agent (four doses) in combination with the inactive agent (ceftriaxone). A simply additive interaction would lead to the same dose–response relation, whereas a significant shift in the combination curve means that an interaction has occurred (Tallarida, 2001). The regression lines for these two dose–response data sets, using the peak elevation in body temperature (60 or 90 min following morphine administration) are shown in Figure 2. The dose–response data of both morphine (1, 4, 8 and 15 mg kg^{-1}, s.c.) by itself and morphine (1, 4, 8 and 15 mg kg^{-1}, s.c.) with a fixed dose (200 mg kg^{-1}, i.p.) of ceftriaxone are linear ($r=0.98$ and 0.94, respectively) and do not differ significantly in slope ($P>0.05$). There is a pronounced downward shift in the combination's regression line, indicating antagonism of the morphine hyperthermia that resulted in a mean temperature decrease of 2.2 ± 0.06°C (ANOVA, $F=1240$; $P<0.001$). The downward shift in the regression line of the combination means that morphine is less efficacious when given with ceftriaxone.

Glutamate uptake inhibitor (TBOA) prevents the inhibition of morphine-evoked hyperthermia by ceftriaxone

The effects of TBOA (0.2 μmol/ rat, i.c.v.) on the inhibition of morphine-induced hyperthermia by ceftriaxone are shown in Figure 3. Two-way ANOVA revealed a significant drug

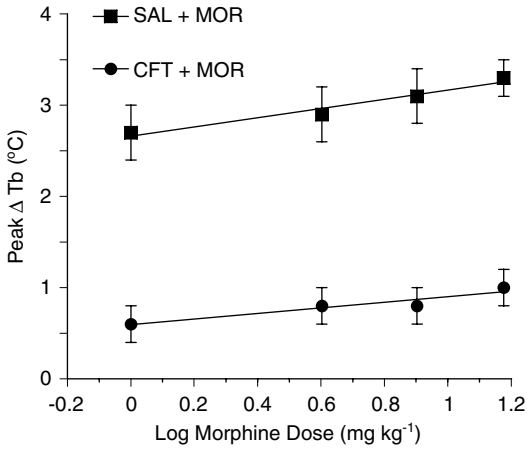

Figure 2 Combination of ceftriaxone and morphine produced subadditive hypothermia. Using the data from Figure 1, regression lines for morphine (MOR) (1, 4, 8 and 15 mg kg^{-1}, subcutaneous (s.c.)) by itself and in combination with an inactive dose of ceftriaxone (CFT) (200 mg kg^{-1}, intraperitoneal (i.p.) were constructed. The effect was a decrease in the peak body temperature. The dose–response data of both MOR (1, 4, 8, and 15 mg kg^{-1}, s.c.) by itself and MOR (1, 4, 8, and 15 mg kg^{-1}, s.c.) with a fixed dose (200 mg kg^{-1}, s.c.) of ceftriaxone are linear ($r=0.98$ and $r=0.94$, respectively) and do not differ significantly in slope ($P>0.05$). There is a pronounced downward shift in the regression line for the combination, indicating antagonism of the MOR hyperthermia that resulted in a mean temperature decrease of 2.2 ± 0.06°C (analysis of variance (ANOVA), $F=1240$; $P<0.001$). The significant downward shift in the regression line of the combination indicates an interaction between the two drugs and reveals that morphine is less efficacious in the presence of ceftriaxone.

interaction (F (6,36) = 46.09, $P<0.0001$), time interaction (F (4,124) = 21.09, $P<0.0001$) and drug × time interaction (F (10,124) = 15.34, $P<0.0001$). In rats pretreated with saline for 7 days, morphine (4 mg kg^{-1}, s.c.) produced its usual hyperthermia compared to the injection of an equivalent volume of saline, with significant increases in body temperature occurring 30, 60, 90 and 120 min post-administration ($P<0.05$). In rats pretreated with saline for 7 days, TBOA (0.2 μmol/ rat, i.c.v.) did not produce a change in body temperature that was significantly different compared to saline ($P>0.05$). Consistent with results presented in Figure 1, morphine (4 mg kg^{-1}, s.c.) produced significantly less hyperthermia in rats pretreated for 7 days with ceftriaxone (200 mg kg^{-1}, i.p.) than in rats that received saline for 7 days ($P<0.05$). This inhibition of morphine-induced hyperthermia by repeated injections of ceftriaxone (200 mg kg^{-1}, i.p.) was prevented when TBOA (0.2 μmol/ rat, i.c.v.) was administered 15 min before morphine (4 mg kg^{-1}, s.c.) ($P<0.05$). A one-way ANOVA on AUC means followed by a Tukey's *post hoc* analysis confirmed the effects of TBOA (Figure 3b).

Glutamate release inhibitor attenuates morphine-evoked hyperthermia

The effects of riluzole (2.5 and 5 mg kg^{-1}, i.p.) on the hyperthermia caused by a single dose of morphine (4 mg kg^{-1}, s.c.) are presented in Figure 4. Two-way ANOVA revealed a significant drug interaction (F (4,26) = 39.05,

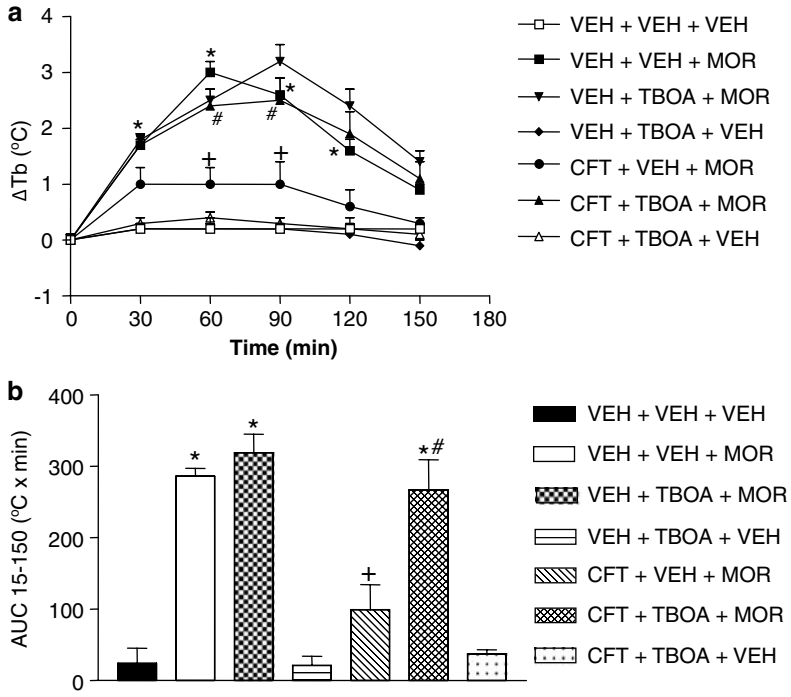

Figure 3 The glutamate uptake blocker, DL-threo-β-benzyloxyaspartic acid (TBOA), prevented the inhibition of morphine-evoked hyperthermia by ceftriaxone. **(a)** Time course: rats were divided into two groups and given either ceftriaxone (CFT) (200 mg kg^{-1}, i.p.) or saline for 7 consecutive days. On day 8, rats were injected with either TBOA (0.2 μmol/ rat, i.c.v.) or vehicle (VEH), followed by the administration of either morphine (MOR) (4 mg kg^{-1}, s.c.) or VEH 30 min later. Data are expressed as the mean $\pm$ s.e.m. of the change in body temperature (ΔT_b) from baseline (time 0). *$P<0.05$, compared to VEH + VEH + VEH; $^{+}P<0.05$, compared to VEH + VEH + MOR; and $^{\#}P<0.05$, compared to CFT + VEH + MOR using a two-way analysis of variance (ANOVA) followed by the Bonferroni correction at the different time points. **(b)** AUC_{15-150} profile: area under the body temperature time curve (AUC) was calculated from 15 to 150 min using the difference score from 0 min ($F_{6,36}=28.60$, $P<0.0001$). *$P<0.05$, compared to VEH + VEH + VEH; $^{+}P<0.05$, compared to VEH + VEH + MOR; and $^{\#}P<0.05$, compared to CFT + VEH + MOR using a one-way ANOVA followed by Tukey's *post hoc* analysis.

$P<0.0001$), time interaction (F (4,104) = 17.03, $P<0.0001$) and drug $\times$ time interaction (F (8,104) = 12.00, $P<0.0001$). Rats given a pretreatment of riluzole (5 mg kg^{-1}, i.p.) and injected with saline displayed body temperatures that were not significantly different than riluzole-naïve rats injected with saline ($P>0.05$). Morphine (4 mg kg^{-1}, s.c.) produced its usual hyperthermia compared to vehicle/ saline-treated rats, with significant increases in body temperature occurring at 30, 60, 90, 120 and 150 min following morphine administration ($P<0.05$). For combined administration, rats given a pretreatment of riluzole (5 mg kg^{-1}, i.p.) and then injected with morphine (4 mg kg^{-1}, s.c.) displayed significantly lower body temperatures than riluzole-naïve rats injected with morphine (4 mg kg^{-1}, s.c.) 60, 90 and 120 min post-injection ($P<0.05$). A lower dose (2.5 mg kg^{-1}, i.p.) did not affect morphine-evoked hyperthermia ($P>0.05$). A one-way ANOVA on AUC means followed by a Tukey's *post hoc* analysis confirmed our results with riluzole (Figure 4b).

Discussion

The present study investigated the effect of ceftriaxone, a representative β-lactam antibiotic, on the hyperthermia caused by morphine. We hypothesized that repeated ceftriaxone administration would block morphine-induced hyperthermia. This is, in fact, what was found. Ceftriaxone blocked a significant proportion of the hyperthermia caused by morphine and these effects were prevented by a broad spectrum glutamate transport inhibitor (TBOA). The effects of ceftriaxone on morphine-induced hyperthermia were similar to the effects of a glutamate release inhibitor and a NMDA antagonist (Rawls *et al*., 2003), which both significantly inhibit the hyperthermic response to morphine. These data suggest that morphine-evoked hyperthermia is controlled by the endogenous glutamate system and can be inhibited by drugs that increase glutamate uptake (β-lactam antibiotics), decrease glutamate release (riluzole) and block glutamatergic transmission at NMDA receptors (dextromethorphan) (Rawls *et al*., 2003).

The attenuation of morphine-induced hyperthermia by riluzole supports our finding that NMDA receptor blockade reduces the hyperthermic response to morphine and extends the finding to include a role for glutamate release in the hyperthermia caused by morphine (Rawls *et al*., 2003). The major mechanism of action of riluzole is the inhibition of glutamate release from presynaptic terminals in the CNS (Malgouris *et al*., 1989; Martin *et al*., 1993; Prakriya and Mennerick, 2000). Riluzole affects a number of ion channels that regulate glutamate release, including voltage-activated calcium channels (Huang *et al*., 1997), voltage-dependent sodium channels (Stefani *et al*., 1997) and potassium channels (Duprat *et al*., 2000). Riluzole also increases glutamate uptake in synaptosomal preparations and blocks

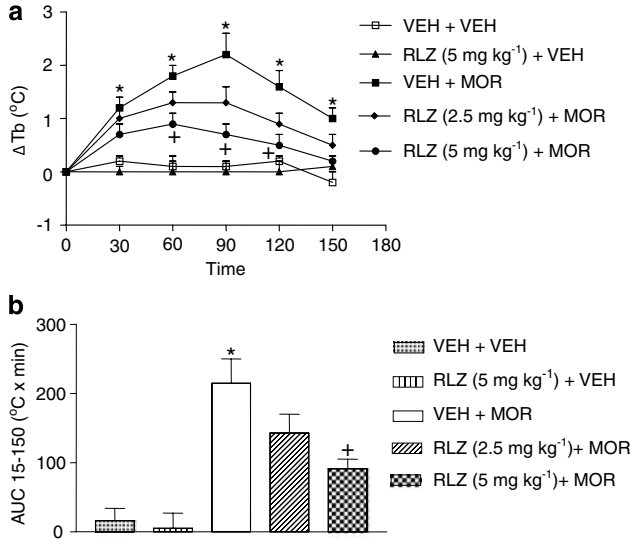

Figure 4 Riluzole (RLZ), an inhibitor of glutamate release, attenuated morphine-evoked hyperthermia. (**a**) Time course: rats were injected with riluzole (2.5 or 5 mg kg^{-1}, i.p.) or vehicle (VEH). Thirty minutes later, morphine (MOR) (4 mg kg^{-1}, s.c.) or VEH was injected. Data are expressed as the mean ± s.e.m. of the change in body temperature (ΔT_b) from baseline (time 0). *$P<0.05$, compared to VEH + VEH + VEH and $^{+}P<0.05$, compared to VEH + VEH + MOR using a two-way ANOVA followed by the Bonferroni correction at the different time points. (**b**) AUC_{15-150} profile: area under the body temperature time curve (AUC) was calculated from 15 to 150 min using the difference score from 0 min ($F_{5,26}=8.874$, $P=0.0001$). *$P<0.05$, compared to VEH + VEH + VEH and $+P<0.05$, compared to VEH + VEH + MOR using a one-way (ANOVA) analysis of variance followed by Tukey's *post hoc* analysis.

some of the post-synaptic effects of glutamate by blocking NMDA receptors (Doble, 1996; Frizzo *et al*., 2004). Regardless of the exact mechanism, the outcome is that riluzole decreases glutamatergic transmission.

The major finding of the present study is that ceftriaxone decreased a significant proportion of the hyperthermic response to morphine. The effect was observed after 7 days of repeated ceftriaxone administration, but not after a single, acute injection of ceftriaxone. These data demonstrate an interaction between morphine and ceftriaxone and reveal that ceftriaxone reduces the efficacy of morphine. One explanation is that increased glutamatergic transmission mediates a component of morphine-evoked hyperthermia that is suppressed by ceftriaxone. In this model, morphine administration to ceftriaxone-naïve rats increases glutamate release in CNS regions that regulate body temperature (Boulant, 1981). The elevation in extracellular glutamate leads to the activation of NMDA receptors and enhancement of the hyperthermic response to morphine (Rawls *et al*., 2003). In rats pretreated with ceftriaxone, both the expression and activity of GLT-1 transporters in the rat brain are elevated before morphine administration (Rawls *et al*., 2003; Beghi *et al*., 2005; Ji *et al*., 2005; Miller and Cleveland, 2005; Rothstein *et al*., 2005; Secko, 2005). The resultant increase in glutamate uptake accelerates glutamate clearance from the extracellular compartment, leading to a reduction in glutamatergic transmission at NMDA receptors. Thus, the usual increase in glutamatergic transmission caused by morphine does not occur in rats treated with ceftriaxone. This means that the β-lactam antibiotic blocks the component of morphine-evoked hyperthermia, which is dependent on enhanced glutamatergic transmission.

A glutamate uptake inhibitor, TBOA, prevented the inhibition of morphine-evoked hyperthermia by ceftriaxone (Shimamoto *et al*., 1998). TBOA is a potent, competitive, non-transportable blocker of excitatory amino acid transporters (EAATs) with high selectivity for EAATs versus ionotropic and metabotropic glutamate receptors (Shimamoto *et al*., 1998, 2000). Although the mechanism of ceftriaxone is not entirely clear, the lack of an interaction between ceftriaxone and morphine in the presence of TBOA supports a role for the glutamate transport system. The most probable explanation and the explanation best supported by the current literature, is that TBOA counteracted the increase in glutamate uptake caused by ceftriaxone. In this event, the repeated administration of ceftriaxone increased GLT-1 expression before TBOA administration, but the actual uptake of glutamate normally caused by ceftriaxone is prevented by the uptake block. The decline in extracellular glutamate and subsequent reduction in glutamatergic transmission expected to arise following ceftriaxone administration is prevented by TBOA (Rawls and McGinty, 1997; Xi *et al*., 2002). Therefore, when morphine is administered with ceftriaxone and TBOA, the component of morphine-induced hyperthermia that depends on an increase in glutamatergic transmission is apparent. This results in morphine producing its full hyperthermic response.

The effect of centrally administered TBOA suggests that ceftriaxone acted in the brain to antagonize morphine-evoked hyperthermia. One possible locus for the interaction between ceftriaxone and morphine is the hypothalamus, the major thermoregulatory centre in the brain and a primary site of opioid-induced effects on body temperature (Boulant, 1981). For example, μ-opioid agonists microdialysed into the preoptic anterior hypothalamus increase body temperature (Geller *et al*., 1986; Chen *et al*., 1996; Xin *et al*., 1997). Future studies will investigate a role for the hypothalamus and other brain regions in the ceftriaxone–morphine interaction.

The dose of ceftriaxone that attenuated the hyperthermic response to morphine is equivalent to a dose of 13 g day^{-1} for a typical adult patient. The maximal dose of ceftriaxone administered to humans as an antibiotic is 2 g day^{-1}. 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. Future studies will determine if lower doses of ceftriaxone attenuate the hyperthermic response to morphine. Because it is likely that ceftriaxone inhibited morphine-evoked hyperthermia by increasing the expression and activity of GLT-1 transporters in the brain, a more important consideration in the present study was the concentration of ceftriaxone achieved in the brain (Rothstein *et al*., 2005). Pharmacokinetic results suggest that the i.p. administration of 200 mg kg^{-1} of ceftriaxone yields CNS concentrations of the antibiotic comparable to those CNS levels required to increase GLT-1 expression (3.5 μM), and attained with therapy for meningitis (0.3–6 μM) (Chandrasekar *et al*., 1984; Nau *et al*., 1993;

Granero *et al*., 1995; Rebuelto *et al*., 2003; Rothstein *et al*., 2005). A dose of 200 mg kg^{-1} of ceftriaxone administered to rats and mice displays neuroprotective and antidepressant properties, both effects that are thought to be mediated by GLT-1 activation (Rothstein *et al*., 2005; Chu *et al*., 2007; Mineur *et al*., 2007).

Although a pharmacokinetic interaction may have contributed to the attenuation of morphine-evoked hyperthermia by ceftriaxone, two lines of evidence argue against this possibility. First, TBOA administered into the brain abolished the effects of ceftriaxone. Given that both ceftriaxone and TBOA directly affect glutamate uptake by acting at the GLT-1 transporter protein, the most probable explanation for our findings is that ceftriaxone altered the glutamate transport system, not the morphine concentration, to attenuate morphine-evoked hyperthermia (Shimamoto *et al*., 1998, 2000; Miller and Cleveland, 2005; Rothstein *et al*., 2005). Second, our data show that ceftriaxone blocks a significant proportion of the hyperthermia caused by each dose (1, 4, 8, or 15 mg kg^{-1}) of morphine. An earlier work from our laboratory demonstrates that this dose range of morphine produces significant hyperthermia in rats (Rawls *et al*., 2003). Given that the lowest dose (1 mg kg^{-1}) of morphine produced a significant hyperthermia when administered by itself, a hyperthermic response would still be predicted even if plasma, or brain, concentrations of morphine were lower than normal following the concurrent administration of ceftriaxone and a dose of morphine such as 8 mg kg^{-1}.

In summary, the data presented here show that a common antibiotic, ceftriaxone, blocked a significant proportion of the hyperthermia caused by morphine. These data reveal a functional interaction between ceftriaxone and morphine and indicate that morphine is less efficacious in the presence of a β-lactam antibiotic. A glutamate uptake blocker, TBOA, antagonized the effects of ceftriaxone, suggesting that an increase in glutamate transport mediated the effects of ceftriaxone. It is possible that the effects of ceftriaxone extend to other morphine-evoked effects, such as analgesia, constipation, tolerance and dependence, which depend on increased glutamatergic transmission. In this event, β-lactam antibiotics may prove to be a useful clinical alternative to treat some of the adverse effects that accompany morphine therapy.

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Conflict of interest

The authors state no conflict of interest.

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