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Glutamate transporter (GLT-1) activation enhances nicotine antinociception and attenuates nicotine analgesic tolerance

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Abstract

Analgesic tolerance is partially mediated by enhanced glutamatergic transmission in the CNS. Beta lactam antibiotics, through glutamate transporter subtype 1 (GLT-1) activation, reduce extracellular glutamate levels and attenuate tolerance to morphine analgesia in rats. Like opioids, nicotine has potent analgesic properties that are subject to tolerance. The purpose of this study was to evaluate the effects of ceftriaxone (CTX), a beta lactam antibiotic and GLT-1 activator on nicotine antinociception and its tolerance. Rats were pre-treated for five days with CTX (200 mg/kg, ip) prior to evaluating their analgesic response to nicotine (1.0 or 2.5 mg/kg sc) for seven consecutive days using the tail flick assay. CTX-treated rats displayed an enhanced antinociceptive response to nicotine and unlike saline injected controls, did not develop tolerance to nicotine's analgesic effects. These results suggest that GLT-1 transporter activation enhances and preserves nicotine antinociception and identify beta lactam antibiotics as potential complementary therapeutic agents for the treatment of chronic pain.

Keywords

nicotine; analgesia; glutamate; GLT-1; tolerance; beta lactam

Introduction

The analgesic effects of nicotine are long established [1] and have been extensively characterized (reviewed in [2]). Nicotine antinociception is subject to tolerance, which has been documented by both acute [3, 4] and chronic [5, 6] administration paradigms, yet the mechanism of nicotine's analgesic tolerance is not clear. Several neurotransmitter and neuropeptide systems have been demonstrated to play a role in cholinergic analgesia (see [7] for review). Among these, the glutamatergic system has received considerable attention as a mediator of nociceptive sensation and transmission. Both ionotropic and metabotropic glutamate receptors are expressed in brain areas responsible for pain processing and a majority of evidence suggests that glutamate antagonists, especially mGlu1 antagonists,

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might be effective broad-spectrum analgesics (reviewed in [8]). It also suggests that attenuating glutamatergic transmission might enhance the effectiveness of known analgesics.

Clearance of extracellular glutamate by the glutamate transporter (GLT-1) is the primary mechanism for terminating glutamatergic neurotransmission. The widely used and well tolerated beta-lactam class of antibiotics have recently been shown to activate and increase the expression of GLT-1 [9] and produce a dose-dependent reduction in brain glutamate levels that persist for 20 days following discontinuation of drug exposure [10]. Recent pain studies have shown that the beta lactam antibiotic ceftriaxone (CTX) attenuates neuropathic pain [11], reduces morphine [12] and cannabinoid [13] analgesic tolerance, and reduce mechanical allodynia and hyperalgesia [14]. The current study evaluated the effect of CTX on nicotine analgesia. Results demonstrate that CTX both enhances acute nicotine analgesia and prevents nicotine analgesic tolerance.

Methods

Animals and drug administration

Forty, male Sprague-Dawley rats, (Charles River Laboratories) weighing 180–210g were housed in pairs on a twelve-hour light/dark schedule and had ad libitum access to food and water. Animals were handled daily for one week prior to the start of the study. All procedures were consistent with the NIH Guide for Care and Use of Laboratory Animals (NIH Publication #80–23) and all experimental protocols were approved by the Connecticut College Institutional Animal Care and Use Committee. CTX, (American Regent, Inc.) was dissolved in sterile saline at a concentration of 200 mg/ml. Nicotine hydrogen tartrate (Sigma-Aldrich) was dissolved in sterile saline at concentrations of 2.5 and 1.0 mg/ml calculated from the weight of the salt. Animals received daily intraperitoneal injections of CTX (200 mg/kg) or saline (1.0 mg/ml) for five days prior to the start of analgesia assessment and through the duration of testing. All animals also received subcutaneous injections of nicotine (1.0 or 2.5 mg/kg) in the nape of the neck immediately prior to the start of analgesia testing. Rats were divided into five groups as follows: CTX/Nic 2.5 (N=8); Sal/Nic 2.5 (N=8); CTX/Nic 1.0 (N=8); Sal/Nic 1.0 (N=8); Sal/Sal (N=8).

Procedure

Nicotine analgesia was assessed on seven consecutive days following 5 days of CTX or saline pre-treatment. On the days of testing, animals were lightly restrained in thin cotton towels secured with rubber bands with their tails exposed. A Life Science Instruments, Model 33 Tail Flick Analgesia Meter was used to measure tail flick latency in response to a radiant heat source focused on a ~25 mm² area of the distal tail. Maximum tail flick latency was set to 10 seconds. Prior to nicotine administration, baseline latencies for each animal were calculated from three tail flick measurements spaced 5–7 minutes apart. Following nicotine or saline administration, five tail flick latencies were measured at 2, 4, 6, 8 and 10 minutes post injection. In order to prevent tissue damage and inaccurate tail flick latencies, a slightly more proximal (~0.5 cm) section of each animal's tail was used to record latencies on consecutive days.

Data Analysis

Response-latency values were expressed as a percentage of maximum possible effect (% MPE): % maximum possible effect (% MPE) = (drug response latency - baseline response latency)/(10 s - baseline response latency) × 100%. Two-way repeated measures analyses of variance (ANOVA) followed by Bonferroni's post-hoc comparisons identified differences between individual treatment groups. Values of $P < 0.05$ were considered significant.

Results

The maximum analgesic effect of nicotine occurred at 2 minutes post injection. The present data is consistent with previous nicotine tail-flick experiments demonstrating similar dose and time course effects [15]. Figure 1 displays the time course of the analgesic effect of nicotine or nicotine + CTX as a function of %MPE. Figure 2 displays the average $\pm$ SD of the 2-minute %MPE on days 1, 4 and 7. A two-way ANOVA conducted on the 2-minute %MPE on days 1, 4 and 7 revealed significant group [$F(4, 105) = 217.0, P < 0.0001$] and day [$F(2, 105) = 7.580, P < 0.001$] effects as well as a significant interaction [$F(8, 105) = 6.982, P < 0.0001$]. Post-hoc comparisons revealed that acute (day 1) nicotine analgesia was enhanced by CTX at a dose of 2.5 mg/kg, ($P < 0.01$ compared to Sal/Nic 2.5) but not 1.0 mg/kg ($P > 0.05$ compared to Sal/Nic 1.0). On days 4 and 7, CTX dose dependently enhanced nicotine analgesia compared to saline matched controls. By day 7 nicotine (2.5 mg/kg) alone still exerted an analgesic effect ($P < 0.01$ compared to Sal/Sal, not indicated on figure 2) however this effect was drastically reduced compared to the %MPE on day 1 (21.2% on day 7 vs. 60.7% on day 1). By contrast the %MPE exhibited by CTX/Nic 2.5 animals was unchanged from day 1 to day 7 (78.6% on day 1 vs. 87.5% on day 7) suggesting that CTX prevented tolerance to nicotine (2.5 mg/kg) analgesia. Nicotine (1.0 mg/kg) alone no longer exerted analgesia by day 4 ($P > 0.05$ compared to Sal/Sal). However, CTX/Nic 1.0 treated animals exhibited analgesia on days 1, 4 and 7 ($P < 0.001$ vs. Sal/Sal on each day), indicating that CTX prevented nicotine (1.0 mg/kg) analgesic tolerance. In a separate preliminary study, no analgesia was observed following CTX (200 mg/kg), pre-treatment for 5 days compared to saline matched controls (data not shown).

Discussion

CTX activates GLT-1 [9] and enhances clearance of extracellular glutamate [10]. Rats pre-treated for 5 consecutive days with CTX displayed an enhancement of acute nicotine antinociception. In addition, CTX co-administered with nicotine over the course of one week prevented antinociceptive tolerance. These results suggest that extracellular glutamate plays a key role in nicotine analgesia.

Nicotine dependence studies have suggested that chronic nicotine administration is accompanied by enhanced glutamatergic transmission and that NMDA and mGlu(5) antagonists may be effective for treating nicotine dependence (reviewed by [16]). Comparatively little is known about the role of the glutamate system in nicotine analgesia. Iwamoto [17] demonstrated that nicotine antinociception is mediated by the tegmental pedunculopontine and dorsal raphe nuclei. These two areas have been shown to receive dense glutamatergic projections and express glutamate receptors [18, 19, 20]. While it is unclear from the results of the present study precisely how glutamate is involved in the development of nicotine antinociceptive tolerance it can be postulated that repeated nicotine administration results in a gradual enhancement of glutamatergic transmission in brain areas responsible for pain processing. CTX's activation of GLT-1 enhances cellular uptake of glutamate preventing extracellular glutamate levels from rising during repeated nicotine administration and preserving nicotine's analgesic efficacy.

Pre-treatment with CTX dose-dependently reduces morphine analgesic tolerance using the hot-plate assay [12]. In that study, the effects of CTX were prevented by pharmacologic blockade of GLT-1. These findings, taken together with the results of the current study, suggest that increased extracellular glutamate is a common factor in the development of analgesic tolerance exhibited by drugs with different mechanisms of action. Although the effects of an acute injection of CTX on nicotine antinociception were not investigated in this study, behavioral, cellular, and neurochemical data from a number of laboratories indicate

that repeated administration of CTX is required for the expression of anti-glutamate effects [9, 21, 22]. Moreover, it has been demonstrated that a single injection of CTX does not alter behavioral responses to morphine or amphetamine [23, 24]. This suggests that instead of exerting an acute effect, repeated CTX administration causes an upregulation in GLT-1 activity and expression [9] that increases the efficiency of glutamate uptake that may be necessary for the prevention of analgesic tolerance.

The dose of CTX used in this study (200 mg/kg) produces a significant and sustained reduction in extracellular glutamate [10] however, this dose is equivalent to a clinical dose of 13mg/d, significantly greater than the typical dose of 2mg/d administered to patients as an antibiotic [9]. Multiple doses of CTX were not evaluated in the current study, however lower doses of CTX have been shown to be effective in enhancing morphine analgesia [12]. These studies may have important clinical implications for the management of chronic pain. Clavulanic acid is a beta lactamase inhibitor that is structurally similar to CTX but lacks anti-bacterial efficacy. Future studies will evaluate the pharmacovigilance and effectiveness of this and other drugs as GLT-1 activators. Chronic pain sufferers often experience tolerance to pain medication while also being exposed to dangerously addictive drugs. Research highlighting the mechanisms of analgesic tolerance may promote the development of adjuvant therapies for the treatment of pain.

Conclusion

Pre-treatment with the beta lactam antibiotic and glutamate transporter activator ceftriaxone dose dependently potentiates nicotine antinociception. Continued enhancement of extracellular glutamate clearance prevents nicotine analgesic tolerance.

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Figure 1.

Time course of the analgesic effect of nicotine or nicotine + CTX as a function of %MPE for days 1, 4 and 7 of analgesia testing. Animals were injected with nicotine (1.0 or 2.5 mg/kg) at time point zero. Maximal effect was observed at 2 minutes post-injection. Saline-treated controls are not included in the figure and SEM error bars are not included for clarity.

Figure 2.

Average $\pm$ SEM of the 2-minute %MPE analgesia on days 1, 4 and 7 for nicotine-treated animals and saline controls. Two-way repeated measures ANOVA post-hoc comparisons revealed that CTX (2.5 mg/kg) significantly enhanced nicotine antinociception and was not subject to tolerance. Tolerance to nicotine (1.0 mg/kg) alone was observed by day 4 of testing, whereas nicotine's (1.0 mg/kg) analgesic efficacy was preserved when co-administered with CTX. * and ** $P < 0.01$, $P < 0.001$ compared to Sal/Nic 2.5 respectively. + and ++ $P < 0.01$, $P < 0.001$ compared to Sal/Nic 1.0 respectively. # and ## $P < 0.05$, $P < 0.001$ compared to Sal/Sal respectively.