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Effects of ACEA-1328, a NMDA receptor/glycine site antagonist, on U50,488H-induced antinociception and tolerance

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Abstract

Previously, we have shown that inhibition of the glycine site associated with the *N*-methyl-D-aspartate (NMDA) receptor is another viable approach to blocking morphine tolerance. In the present study, we sought to investigate the involvement of the NMDA receptor/glycine site in κ-opioid receptor-mediated antinociception and tolerance in CD-1 mice. In antinociception studies, mice were injected with 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328), a systemically bioavailable NMDA receptor/glycine site antagonist, or the vehicle (Bis-Tris, 0.2 M) and then immediately with *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U50,488H), a κ-opioid receptor agonist. Thirty minutes later, mice were tested for changes in nociceptive responses in the tail flick assay. ACEA-1328, per se, prolonged tail flick latencies with an ED_{50} of approximately 50 mg/kg. Concurrent administration of ACEA-1328, at doses that did not produce antinociception, with U50,488H increased the potency of U50,488H in a dose-dependent manner. In tolerance studies, mice were treated, either once a day for 9 days or twice daily for 4 days, with the vehicle or ACEA-1328. Immediately after the initial injection, mice then received an injection of saline or U50,488H. On the test day, mice were injected with U50,488H alone and tested for antinociception 30 min later. Chronic treatment with U50,488H by either method produced tolerance. Unlike the acute effect of the drug, chronic treatment with ACEA-1328 decreased the antinociceptive potency of U50,488H. Taken together, the data suggest that acute and chronic administration of ACEA-1328 differentially affected the antinociceptive effect of U50,488H. Furthermore, the decreased in the potency of U50,488H induced by chronic treatment with ACEA-1328 also confounded the interpretation of the tolerance data.

Keywords: Antinociception; Tolerance; NMDA receptor/glycine site; ACEA-1328; U50,488H; Tail flick test

1. Introduction

A series of compounds has been shown to modulate tolerance to and dependence on opioid analgesics (for review, see Bhargava, 1994). Of particular relevance to the present study is the use of *N*-methyl-D-aspartate (NMDA) receptor antagonists. Many studies have reliably demonstrated that activation of the NMDA receptor plays a critical role in the development of morphine tolerance. Inhibition of morphine tolerance, for example, can be achieved by blocking the NMDA receptor via antagonism of the NMDA receptor channel (Marek et al., 1991; Trujillo and Akil., 1991; Bhargava and Matwyshyn, 1993; Lutfy et al., 1993), the glutamate recognition site (Kolesnikov et al., 1993; Tiseo and Inturrisi, 1993), or the glycine co-agonist site (Lutfy et al., 1995, 1996).

In spite of the fact that κ-opioid receptor ligands have been shown to interact with the NMDA receptor (Caudle and Isaac, 1987, 1988; Bakshi and Faden, 1990; Isaac et al., 1990; Singh et al., 1990; Lambert et al., 1991; Skilling et al., 1992; Dumont and Lemaire, 1994; Chen et al., 1995a,b), previous studies have failed to consistently demonstrate the involvement of the NMDA receptor in κ-opioid receptor-mediated tolerance. For instance, while there is evidence to demonstrate that NMDA receptor antagonists block tolerance to *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U50,488H; Kolesnikov et al., 1993; Bhargava and Thorat, 1994), there are still some data showing the lack of an effect of NMDA receptor antagonists on tolerance to U50,488H (Elliott et al., 1994).

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Moreover, the effect of an antagonist at the glycine site associated with the NMDA receptor has not been determined on κ-opioid receptor-mediated tolerance.

As eluded above, we have previously shown that chronic treatment with 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328), a systemically bioavailable NMDA receptor/glycine site antagonist, attenuates morphine tolerance (Lutfy et al., 1995). Furthermore, concomitant administration of ACEA-1328 and morphine increases the antinociceptive potency of the opioid analgesic (Lutfy et al., 1998), indicating that ACEA-1328 and other NMDA receptor/glycine site might be somewhat unique, because other classes of NMDA receptor antagonists display no effect (Marek et al., 1991; Trujillo and Akil, 1991) or even block morphine-induced antinociception (Lipa and Kavaliers, 1990; Lutfy et al., 1993). In addition, ACEA-1328 and other NMDA receptor/glycine site antagonists have been shown to be devoid of PCP-like motor stimulatory and discriminative stimulus side effects (Balster et al., 1995; Lutfy et al., 1996), Recently, ACEA-1328 has also been shown to increase the antinociceptive potency of U50,488H (Lutfy et al., 1998), raising the possibility that blockade of the NMDA receptor via inhibition of the glycine co-agonist site may show differential effects on tolerance to U50,488H than other classes of NMDA receptor antagonists. The present study was therefore undertaken to evaluate the effect of acute and chronic administration of ACEA-1328 on tolerance to U50,488H in CD-1 mice using the tail flick test.

2. Materials and methods

2.1. Subjects

Male CD-1 mice (22–24 g) obtained from Charles River Laboratories (Hollister, CA) were used in all experiments. Mice were housed four to a cage with free access to food and water in a 12-h light/12-h dark cycle and used only once. All experiments were conducted during the light cycle in a blind manner in which the observers were unaware of the experimental treatment and drug doses.

2.2. Tail flick assay

A modification of the method of D'Amour and Smith (1949) was used. Briefly, a beam of light was focused on the dorsal surface of the tail of a mouse approximately 1 in. from the tip of the tail. The light intensity was adjusted so that the averaged basal nociceptive response (baseline) was 3–4 s. Mice that did not flick by ≥ 5 s prior to any treatment were discarded. A cut-off time of 10 s was employed in order to prevent tissue damage. A mouse that did not remove its tail from the noxious stimulus by 10 s after drug administration was defined as a responder to the opioid analgesic.

2.3. Drugs

U50,488H was purchased from Research Biochemicals (Natick, MA) and in part generously supplied by the National Institute on Drug Abuse (Rockville, MD). ACEA-1328 was prepared in the laboratory of Dr. S.X. Cai at CoCensys (Irvine, CA). U50,488H was dissolved in saline (0.9% NaCl) whereas ACEA-1328 was dissolved in Bis-Tris (0.2 M).

2.4. Effects of ACEA-1328 on U50,488H-induced antinociception

To examine the effect of ACEA-1328 on basal nociceptive responses in the tail flick assay (see below), a baseline tail flick latency was measured for each mouse. Mice were then weighed, injected with ACEA-1328 (5–100 mg/kg, i.p.; $n = 7$–10 mice/dose), and tested for antinociception 30 min later. To study the acute effect of ACEA-1328 on U50,488H-induced antinociception, separate groups of mice were tested for baseline tail flick latencies, weighed, and injected with either Bis-Tris (0.2 M) or ACEA-1328 (1, 5, and 10 mg/kg, i.p.). Mice were then, within a minute, injected with U50,488H (0.625–20.0 mg/kg, s.c.; $n = 5$–15 mice/dose), and tested for antinociception 30 min later. ACEA-1328 was given 1 min prior to U50,488H because the time-course of the two drugs is about the same.

2.5. Effects of ACEA-1328 on κ-opioid receptor-mediated tolerance

We previously used two different protocols to produce a reliable degree of tolerance to morphine (Lutfy et al., 1993, 1995). Furthermore, it was found that, using these paradigms, ACEA-1328 blocked morphine tolerance without affecting baseline tail flick latencies or the antinociceptive potency of morphine (Lutfy et al., 1995). Therefore, we used similar paradigms to induce tolerance to the effect of U50,488H and study the effect of ACEA-1328 on the baseline tail flick latency, the antinociceptive potency of U50,488H, and tolerance to the κ-opioid receptor agonist. We treated the mice either once a day or twice daily. In the former protocol, mice were injected once daily with Bis-Tris (0.2 M) or ACEA-1328 (20 mg/kg, i.p.). Within a minute following the initial treatments, mice were injected daily with either saline or U50,488H (10 mg/kg, s.c.). On day 10, mice were tested for a baseline tail flick latency prior to any treatment. Mice were then weighed, injected with only U50,488H (7.5–30.0 mg/kg, s.c.; $n = 8$–11 mice/dose), and tested for antinociception 30 min later.

In the twice-daily injection protocol, mice were treated once in the morning and once in the afternoon with either Bis-Tris (0.2 M) or escalating doses of ACEA-1328 (10, 15; 15, 20; 20, 30; 30, and 40 mg/kg, i.p., on days 1, 2, 3, and 4, respectively). Approximately 1 min following the

Table 1
Potentiation of U50,488H-induced antinociception by ACEA-1328 in CD-1 mice[a]

Treatment	ED_{50} (mg/kg)	Potency ratio
U50,488H		
Plus Bis-Tris (0.2 M)	10.23 (6.94–15.08)	1.00
Plus ACEA-1328		
(1 mg/kg)	10.02 (6.45–15.57)	1.02 (0.57–1.84)
(5 mg/kg)	5.54 (3.52–8.72)	1.85[b] (1.02–3.36)
(10 mg/kg)	2.48 (1.37–4.46)	4.13[c] (2.04–8.37)

[a]Mice were injected with either vehicle (Bis-Tris) or ACEA-1328 (1, 5, and 10 mg/kg, i.p.). Within a minute, mice were also injected with U50,488H (0.625–20.0 mg/kg, s.c.; $n = 5–15$ mice/dose) and tested for antinociception 30 min later. The 95% CLs are shown in parenthesis.

[b]Significantly different from the vehicle- and ACEA-1328 (1 mg/kg)-treated groups ($P < 0.05$).

[c]Significantly different from all other groups ($P < 0.05$).

first injection, mice were injected daily with either saline or increasing doses of U50,488H (5, 7.5; 7.5, 10; 10, 15; 15, 20 mg/kg, s.c., respectively, on days 1, 2, 3, and 4). On day 5, a baseline tail flick latency was measured for each mouse. Mice were then weighed, injected only with U50,488H (2.5–30.0 mg/kg, s.c.; $n = 6–14$ mice/dose), and tested for antinociception 30 min later. Unlike the acute studies, in these experiments, mice did not receive ACEA-1328 on the test day.

2.6. Data analysis

The baseline tail flick latencies were analyzed using a one-way analysis of variance (ANOVA) followed by the Newman–Keuls post-hoc test to reveal significant differences among various means. The ED_{50} and the potency ratio of the drug were estimated by the method of Litchfield and Wilcoxon (1949) as described in the Manual of Pharmacologic Calculations with Computer Programs (Tallarida and Murray, 1987). This method also estimates the upper and lower 95% confidence limits (CLs) of the ED_{50} and potency ratio. The program considers a decrease or an increase in the potency of a drug to be statistically significant, regardless of the overlapping of the 95% CLs of the ED_{50}, if the 95% CLs of the potency ratio are respectively smaller or greater than one.

3. Results

3.1. Effects of ACEA-1328 on U50,488H-induced antinociception

Systemic administration of ACEA-1328 increased the tail flick latency in a dose-dependent manner. The estimated ED_{50} (95% CLs) of ACEA-1328 was 48.2 (22.7–102.4) mg/kg. Co-administration of ACEA-1328 with U50,488H, at doses that showed minimal effects, if any, on the tail flick latency, shifted the dose–response curve of the opioid analgesic to the left which can be observed as a decrease in the ED_{50} of the drug (Table 1). There was a dose-related increase in the relative potency of the U50,488H in mice treated with ACEA-1328 as compared to the vehicle-treated control group (Table 1). ACEA-1328 did not alter the potency of U50,488H at 1 mg/kg, however, at higher doses (5 and 10 mg/kg) there was a significant dose-related increase in the potency of the κ-opioid receptor agonist (Table 1).

3.2. Effects of ACEA-1328 on κ-opioid receptor-mediated tolerance

Chronic treatment with U50,488H by either method produced an approximately two- to three-fold decrease in the potency of the drug (Table 2). Administration of ACEA-1328 by both methods showed no significant effect on basal nociceptive responses in the tail flick test ($F(3,43) = 0.12$ and $F(3,56) = 0.99$ for the once a day and twice daily methods, respectively; $P > 0.05$). However, the same treatment decreased the antinociceptive potency of the κ-opioid receptor agonist (Table 2). There was an approximately two-fold decrease in the relative potency of the drug in mice chronically treated with ACEA-1328 as compared to the vehicle-pretreated mice (Table 2; compare the ED_{50} of U50,488H in vehicle–saline

Table 2
Effects of ACEA-1328 on κ-opioid receptor-mediated tolerance in CD-1 mice[a]

Treatment	ED_{50} (mg/kg)	Potency ratio
U50,488H alone		
Once daily method		
Vehicle–saline	9.65 (5.94–15.69)	1.00
Vehicle–U50,488H	18.60 (12.00–28.81)	0.52[b] (0.27–0.99)
ACEA-1328–saline	18.01 (10.46–30.99)	0.54[b] (0.26–0.99)
ACEA-1328–U50,488H	15.50 (10.38–23.13)	0.62 (0.33–1.17)
Twice daily method		
Vehicle–saline	4.16 (1.94–8.92)	1.00
Vehicle–U50,488H	10.83 (6.92–16.97)	0.38[b] (0.16–0.93)
ACEA-1328–saline	10.36 (6.57–16.34)	0.40[b] (0.17–0.98)
ACEA-1328–U50,488H	8.49 (5.43–13.26)	0.49 (0.20–1.19)

[a]In the once-daily method, mice were injected for 9 days with either vehicle (Bis-Tris) or ACEA-1328 (20 mg/kg). Within a minute, mice were daily injected with saline or the opioid analgesic (10 mg/kg). The dose–response study for U50,488H alone was conducted in all four groups on day 10. In the twice-daily protocol, mice were injected once in the morning and once in the afternoon with either vehicle or escalating doses of ACEA-1328 for 4 days. Within a minute, mice received an injection of either saline or escalating doses of U50,488. On day 5, a dose–response relationship of U50,488H alone was generated in all groups. To conduct dose–response studies on the test day, mice were tested for a baseline tail flick latency and injected with U50,488H alone. Mice were then tested for antinociception 30 min later. The 95% CLs are shown in parenthesis.

[b]Significantly different from the respective vehicle–saline pretreated (control) group ($P < 0.05$).

and ACEA-1328–saline pretreated groups). There seemed to be a trend toward inhibition of κ-opioid receptor-mediated tolerance because chronic treatment with ACEA-1328, which by itself caused a decrease in the antinociceptive potency of the κ-opioid receptor agonist, did not further reduce the relative potency of the drug when co-administered with U50,488H. It even becomes apparent that ACEA-1328 partially restored the antinociceptive potency of the drug (Table 2). However, this restoration was not statistically significant ($P > 0.05$). Although it appeared that tolerance did not develop in mice chronically treated with ACEA-1328 (Table 2), the decrease in the antinociceptive potency of U50,488H by chronic administration of ACEA-1328 confounded the interpretation of the results.

4. Discussion

The results of the present study demonstrate that acute administration of ACEA-1328, a competitive and systemically bioavailable NMDA receptor/glycine site antagonist, increased the antinociceptive potency of U50,488H. On the other hand, chronic treatment with ACEA-1328 decreased the antinociceptive potency of the opioid analgesic. Furthermore, the decrease in the potency of U50,488H induced by chronic treatment with ACEA-1328 confounded the interpretation of tolerance data.

The antinociceptive effect of opioid analgesics can be modulated by NMDA receptor agonists and antagonists (Lipa and Kavaliers, 1990; Chapman and Dickenson, 1992; Kest et al., 1992; Lutfy et al., 1993; Advokat et al., 1994; Hunter et al., 1994; Saucier and Kavaliers, 1994). In the present studies, we found that acute administration of ACEA-1328 increased the antinociceptive effect of U50,488H. This is the first study to show that a systemically bioavailable NMDA receptor/glycine site antagonist increases the antinociceptive potency of U50,488H in the tail flick test in CD-1 mice. The increase in the antinociceptive potency of the opioid analgesic by ACEA-1328 is quite interesting, because other classes of NMDA receptor antagonist either show no effect (Kolesnikov et al., 1993; Bhargava and Thorat, 1994) or even block (Kest et al., 1992) the potency of U50,488H. As opposed to other NMDA receptor antagonists (Lipa and Kavaliers, 1990; Lutfy et al., 1993), ACEA-1328 has also been shown to increase the potency of morphine (Lutfy et al., 1998). Although the mechanism of such interaction is not clear at the present time, it could be due to augmentation of the apparent antinociceptive effect of ACEA-1328 by the opioid analgesics (see above and also Lutfy et al., 1998).

Chronic administration of ACEA-1328 was found to significantly decrease the antinociceptive potency of U50,488H. The decrease in the antinociceptive effect of U50,488H induced by chronic treatment with ACEA-1328 may simply indicate that tolerance developed to the effect of U50,488H. Although the mechanism of such tolerance is not clear at the present time, one possibility could be that ACEA-1328 acted as an agonist at the κ-opioid receptor and produced some changes, either at the NMDA and/or κ-opioid receptor or at their receptor–effector systems, that influenced the antinociceptive potency of U50,488H. Our initial experiments using naloxone, a non-selective opioid receptor antagonist, did not however support this notion (Lutfy et al., 1998). Further studies may therefore be needed to determine the effect of ACEA-1328 in the presence of a selective κ-opioid receptor antagonist to clarify this issue. An alternative explanation for the decrease in the potency of U50,488H after chronic treatment with ACEA-1328 could have been due to the development of tolerance to the effect of ACEA-1328. It is, however, less likely that tolerance to the effect of ACEA-1328 would be accountable for such an effect because acute administration of ACEA-1328 increases the potency of both morphine and U50,488H (Lutfy et al., 1998) but chronic treatment with ACEA-1328 only affects the potency of U50,488H.

Chronic treatment with U50,488H, either once a day for 9 days or twice daily for 4 days, produced a significant decrease in the potency of U50,488H (Table 2, compare the ED_{50} of the drug in vehicle–saline and vehicle–U50,488H groups), indicating that tolerance developed to the antinociceptive effect of U50,488H. During the time course of tolerance studies, we noticed that the potency of U50,488H was two-fold greater in the twice-daily injection protocol as compared to the once a day injection method (Table 2) or the ones used for acute studies (Table 1). Indeed, we observed a similar pattern for the effect of morphine (Lutfy et al., in press). In the present investigation, we found that concurrent treatment with ACEA-1328 did not produce any further increase in the ED_{50} of U50,488H (Table 2; compare the ED_{50} of the drug in ACEA-1328–saline and ACEA-1328–U50,488H pretreated groups), indicating that tolerance did not develop to the antinociceptive effect of the drug. It is, however, difficult to suggest that ACEA-1328 blocked κ-opioid receptor-mediated tolerance, because the decrease in the potency of U50,488H induced by chronic ACEA-1328 treatment confounded the interpretation of our data (Table 2; compare the ED_{50} of U50,488H in vehicle–saline and ACEA–saline groups). Despite the fact that ACEA-1328 reliably blocked morphine tolerance (Lutfy et al., 1995, 1996, 1999), the effect of this drug on κ-opioid receptor-mediated tolerance remains elusive. It is, therefore, necessary to further characterize the effect of ACEA-1328 and other NMDA receptor/glycine site antagonists on tolerance to U50,488H to specifically determine the role of the glycine site associated with the NMDA receptor in the mechanism of tolerance to U50,488H.

In summary, acute and chronic administration of ACEA-1328 with U50,488H differentially modulated the antinociceptive potency of the opioid analgesic. The effect of ACEA-1328 on κ-opioid receptor-mediated tolerance

was confounded by the decrease in the potency of U50,488H induced by chronic ACEA-1328 administration. Since ACEA-1328 acutely increased whereas chronically decreased the antinociceptive effects of U50,488H, the concomitant use of the NMDA receptor/glycine site antagonist with the κ-opioid receptor agonist would primarily be useful for acute management of pain.

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