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Short communication

Blockade of morphine tolerance by ACEA-1328, a novel NMDA receptor/glycine site antagonist

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Abstract

Recent studies indicate that competitive and non-competitive NMDA receptor antagonists can block the development of morphine tolerance. Since glycine is considered to be a co-agonist for activation of NMDA receptors we examined the effect of a novel bioavailable NMDA receptor/glycine site antagonist, 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328), on the development of morphine tolerance. Administration of ACEA-1328 (20 mg/kg) completely blocked tolerance to morphine-induced antinociception in the tail flick test in CD-1 mice, without affecting the basal nociceptive response or potentiating morphine-induced antinociceptive effects. These data suggest that inhibition of NMDA receptor activity via blockade of the glycine co-agonist site is potentially viable as a therapeutic approach for preventing development of morphine tolerance.

Keywords: Morphine tolerance; NMDA receptor/glycine site antagonist; Tail flick test; (CD-1 mouse)

1. Introduction

Chronic treatment with morphine produces tolerance (Way et al., 1969). Though the mechanism of morphine tolerance is incompletely understood, recent studies suggest that activation of NMDA receptors may play an important role. For example, competitive and non-competitive NMDA receptor antagonists both appear to block development of tolerance (Trujillo and Akil, 1991; Marek et al., 1991; Tiseo and Inturrisi, 1993; Bhargava and Matwyshyn, 1993; Kolesnikov et al., 1993; Lutfy et al., 1993). It is now generally accepted that glycine is required for activation of NMDA receptors (Johnson and Ascher, 1987). Thus, another pharmacological approach to blocking morphine tolerance would be to antagonize NMDA receptor/glycine sites. In the present study we used 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione (ACEA-1328), a novel centrally active glycine site antagonist, to test this hypothesis.

2. Materials and methods

The pharmacology of ACEA-1328 was characterized in vitro at NMDA and non-NMDA receptors expressed in *Xenopus* oocytes by rat cerebral cortex poly(A)$^+$ RNA (see Chomczynski and Sacchi, 1987; Woodward et al., 1992 for methods). Antagonist dissociation constant (K_b values) were estimated from parallel displacement of concentration-response curves for glycine (NMDA receptor assays) or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, non-NMDA receptor assays) (see Swartz et al., 1992 for details).

Male CD-1 mice, weighing 22–24 g upon arrival from Charles River Laboratories (Hollister, CA, USA), were used in tolerance studies. A modification of the method of Kolesnikov et al. (1993) was used to produce tolerance in CD-1 mice. This method was used because it has been demonstrated that tolerance can develop in CD-1 mice using smaller doses of morphine. Briefly, mice were injected daily for a period of 9 days with either vehicle (Bis-Tris, 0.2 M; $n = 19$ mice) or ACEA-1328 (1, 10 and 20 mg/kg i.p.; $n = 8$–14 mice/group).

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Approximately half of the mice ($n = 10$) in the vehicle-treated group were then, within a minute, injected with saline and the other half ($n = 9$ mice) with morphine (10 mg/kg s.c.). ACEA-1328 treated groups were all injected with morphine (10 mg/kg s.c.) except in the highest dose (20 mg/kg) group where approximately 1/3 of the mice were injected with saline ($n = 5$ mice). On day 10 (test day), mice were weighed and a baseline tail flick latency was measured for each mouse by the method of D'Amour and Smith (1941). Mice were then injected with a single dose of morphine (7.5 mg/kg s.c.) and tested again for tail flick latencies 30 min later. We also examined the effect of chronic treatment with ACEA-1328 alone and in combination with morphine on morphine-induced antinociception. In this study mice were chronically treated as described above, except that on the test day (day 10) mice were injected with morphine (5 mg/kg s.c.) and tested for analgesia 30 min later. Mice that did not flick by 10 s were defined as analgesic. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test.

3. Results

In vitro electrophysiological characterization of ACEA-1328 suggested that inhibition at NMDA receptor glycine sites and non-NMDA receptor glutamate sites was consistent with competitive antagonism (Swartz et al., 1992). K_b values for ACEA-1328 were 39 (34–43) nM at NMDA receptor glycine sites, and 3.1 (2.7–3.6) μM at non-NMDA receptors; giving a steady-state selectivity index approximately 80-fold in favor of inhibition at glycine sites (data given as means, numbers in parentheses are 95% confidence intervals, $n = 4$; details will be presented elsewhere).

Chronic treatment with ACEA-1328, alone or in combination with morphine had no significant effect on the basal nociceptive response in the tail flick test ($F(5,43) = 1.1$; $P > 0.05$). We also found that chronic treatment with ACEA-1328 alone, and in combination with morphine, did not alter the antinociceptive effect of morphine (Table 1). Chronic treatment with morphine for 9 days resulted in development of tolerance

Table 1
Effect of chronic treatment with ACEA-1328 either alone, or in combination with morphine in CD-1 mice

Treatment	Percent analgesia
Vehicle-saline	3/5 (60%)
Vehicle-morphine	1/5 (20%)
ACEA-1328-saline	2/5 (40%)
ACEA-1328-morphine	3/5 (60%)

For 9 days mice were injected with either Bis-Tris (0.2 M) or ACEA-1328 (20 mg/kg i.p.) and subsequently, within a minute, injected with either saline or morphine (10 mg/kg s.c.). On day 10 mice were tested for baseline tail flick latencies, injected with morphine (5 mg/kg s.c.), and then tested for analgesia 30 min later. Percent analgesia was calculated as the number of mice that showed complete analgesia (latency of 10 s) divided by the total number of animals in that group.

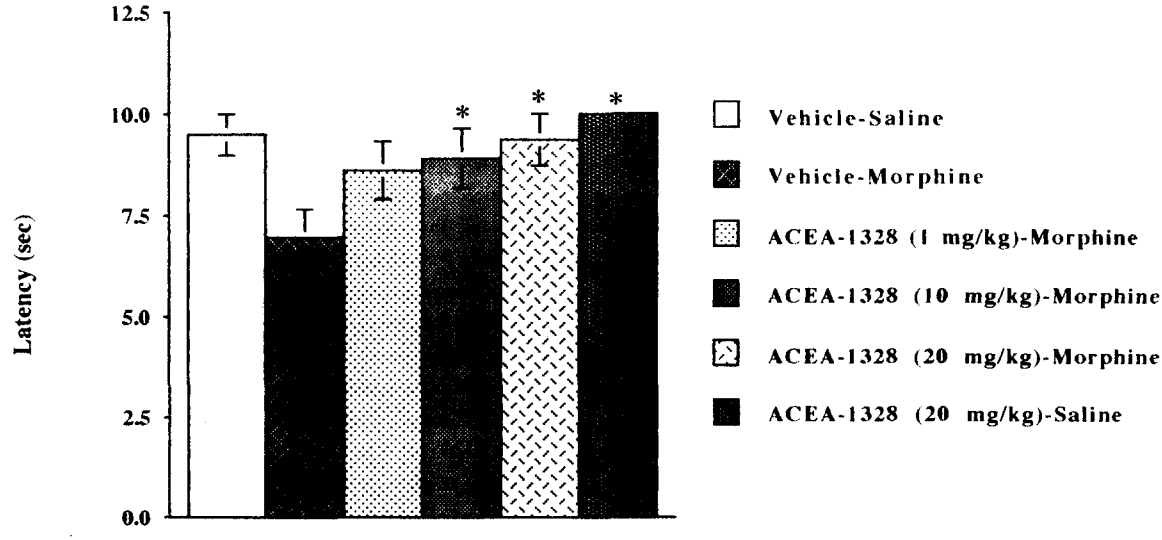

Fig. 1. Blockade of morphine tolerance by ACEA-1328 in the tail flick test in CD-1 mice. Mice were injected daily for a period of 9 days with either vehicle or ACEA-1328 (1, 10 or 20 mg/kg i.p.) alone or in combination with morphine as described in Materials and methods. On day 10, mice were weighed and a baseline tail flick latency was measured for each mouse. Mice were then injected with morphine (7.5 mg/kg s.c.) and 30 min later tested again for post-drug latencies. Data are presented as the mean ± S.E.M. * Significantly different from tolerant (vehicle followed by morphine) group ($P < 0.05$).

to the antinociceptive effect of the drug (Fig. 1). A one-way ANOVA followed by Newman-Keuls test revealed a statistically significant attenuation of morphine-induced antinociception in the tolerant (vehicle-treated followed by morphine) mice as compared to the control (vehicle-treated followed by saline) group ($F(5,43) = 2.68$; $P < 0.05$; Fig. 1). Mice co-administered with ACEA-1328 (1, 10 and 20 mg/kg i.p.) showed dose-dependent reductions in morphine tolerance. ACEA-1328 at 1 mg/kg attenuated morphine tolerance but this effect was not statistically significant ($P > 0.05$); however, at higher doses (10–20 mg/kg) this effect was significant ($P < 0.05$).

4. Discussion

The electrophysiological data suggests that ACEA-1328, a quinoxaline-2,3-dione with a novel pattern of benzene ring substitutions, shows selectivity for antagonism at NMDA receptor/glycine sites as compared to non-NMDA receptors. In the behavioral studies, chronic morphine administration produced tolerance and this was blocked by ACEA-1328. Blockade of morphine tolerance by ACEA-1328 is consistent with previous findings using other classes of NMDA receptor antagonists (Trujillo and Akil, 1991; Marek et al., 1991; Tiseo and Inturrisi, 1993; Bhargava and Matwyshyn, 1993; Kolesnikov et al., 1993; Lutfy et al., 1993), and supports the notion that activation of NMDA receptors is important in the development of tolerance. The mechanism underlying attenuation of morphine tolerance by ACEA-1328 remains unclear. However, the possibility that the effect is due to potentiation of morphine-induced antinociception by chronic ACEA-1328 seems unlikely, because chronic treatment with ACEA-1328 did not itself potentiate antinociceptive effects of morphine.

The present data indicate that antagonism at NMDA receptor glycine co-agonist sites is a viable pharmacological approach to attenuate the development of morphine tolerance. These results could be important because glycine site antagonists appear to have relatively favorable side-effect profiles compared to other classes of NMDA receptor antagonist, particularly with respect to phencyclidine (PCP)-like psychotomimetic behaviors (Singh et al., 1990; Koek and Colpaert, 1990), and might therefore have advantages as candidates for the clinical treatment of opioid tolerance.

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