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Short communication

Attenuation of morphine tolerance and dependence with the highly selective δ-opioid receptor antagonist TIPP[ψ]

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Abstract

We examined the effects of i.c.v. treatment with naltrindole, and the two highly selective peptide δ-opioid receptor antagonists H-Tyr-Tic-Phe-Phe-OH (TIPP) and H-Tyr-Ticψ[CH_2-NH]-Phe-Phe-OH (TIPP[ψ]), on the development of morphine tolerance and dependence. Each treatment significantly decreased naloxone-precipitated withdrawal, with TIPP[ψ] reducing the most symptoms. TIPP[ψ], but neither naltrindole nor TIPP, attenuated the development of analgesic tolerance in the tail-flick test. These results suggest that δ-opioid receptors are critically involved in the development of morphine tolerance and dependence.

Keywords: Analgesia; Opiate; δ-Opioid receptor antagonist; Opioid withdrawal; Abstinence

1. Introduction

Opioid drugs such as morphine are commonly used in the management of pain; however, their usefullness is compromised by the development of tolerance and dependence. Morphine is primarily a μ-opioid receptor agonist, but it also acts at both δ- and κ-opioid receptors (Takemori and Portoghese, 1987). Moreover, it has been proposed that activation of an allosterically coupled δ-opioid receptor modifies activity at the μ-opioid receptor (Rothman and Westfall, 1982; Tiseo and Yaksh, 1993). Due to this interaction between μ- and δ-opioid receptors, it has been hypothesized that activation of δ-opioid receptors may play a significant role in the development of opioid tolerance and dependence (Abdelhamid et al., 1991).

Blockade of δ-opioid receptors with the relatively selective non-peptide antagonists naltrindole, naltrindole-5′-isothiocyanate (5′-NTII) and naltriben has been shown to attenuate the development of morphine tolerance and dependence in mice (Abdelhamid et al., 1991; Miyamoto et al., 1993). However, naltrindole and naltriben also possess significant antagonist potencies at both μ- and κ-opioid receptors in the guinea pig ileum assay (Portoghese et al., 1991). The peptide H-Tyr-Tic-Phe-Phe-OH (TIPP) (Schiller et al., 1992) and its pseudopeptide analogue H-Tyr-Ticψ[CH_2-NH]Phe-Phe-OH (TIPP[ψ]) (Schiller et al., 1993) are potent and highly selective δ-opioid receptor antagonists which exhibit no μ- or κ-opioid receptor antagonistic properties in the guinea ilium assay at concentrations up to 10 μM. The purpose of the present investigation was to verify the specific involvement of δ-opioid receptors in the development of morphine tolerance and dependence by comparing the effects of naltrindole and the two highly selective δ-opioid receptor antagonists, TIPP and TIPP[ψ], in chronic morphine-treated rats.

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2. Materials and methods

2.1. Subjects and surgery

Subjects were male Long Evans rats (280–350 g). Rats were housed 2–4 per cage, with food and water available ad libitum.

On day 0, rats were anaesthetized with sodium pentobarbital (Somnotol, MTC Pharmaceuticals), and 23-gauge stainless steel cannulae, attached to model 2001 Alzet osmotic mini pumps filled with one of the δ antagonist solutions or saline, were implanted stereotaxically in the lateral ventrical (i.c.v.) (AP = −1.3 mm and L = −1.8 mm from bregma, and V = −3.8 mm from the skull surface. During surgery, the first of two model 2ML1 Alzet pumps containing 60 mg/ml morphine sulfate solution was implanted subcutaneously (s.c.) on the back; the second was implanted on day 1, while the rats were briefly anaesthetized with halothane. A second group of rats was given i.c.v.

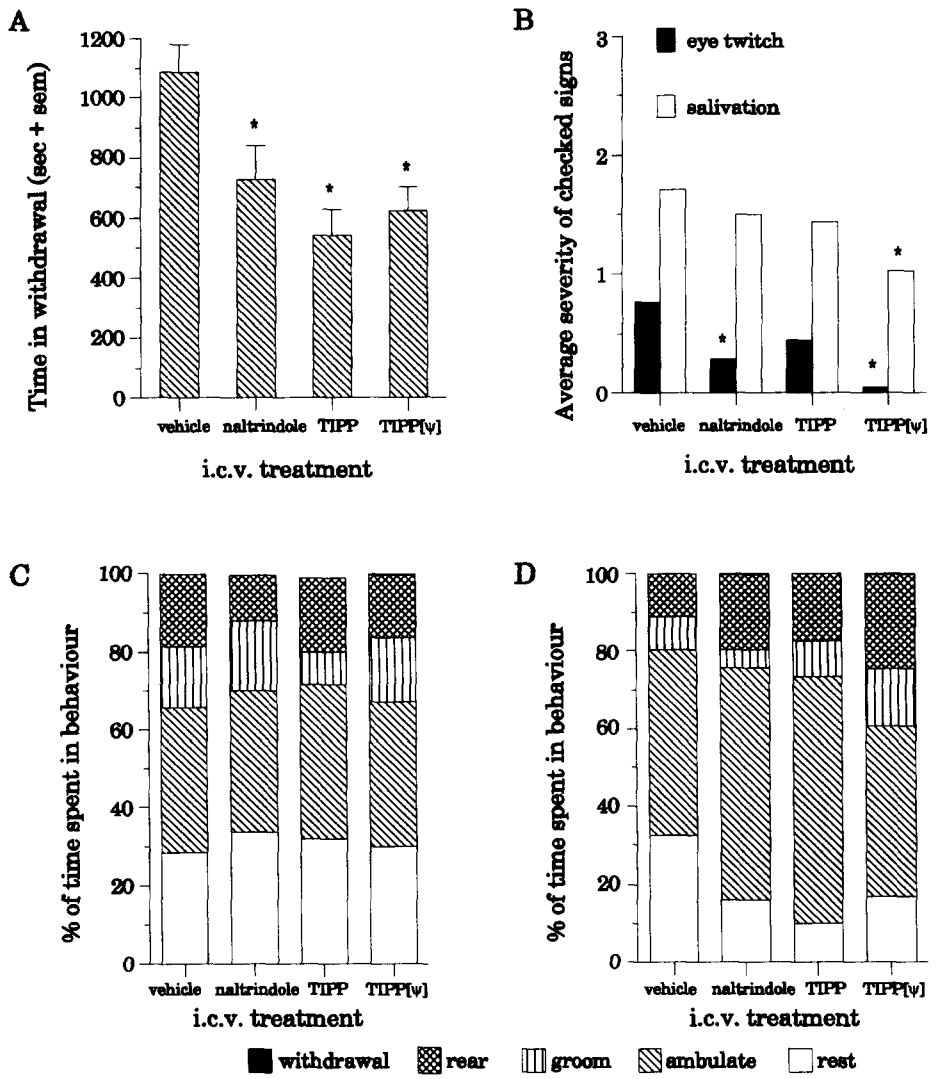

Fig. 1. A: Mean time spent teeth chattering and writhing during the 40 min withdrawal period for rats treated with 36.65 μmol/day morphine sulfate (s.c.) and either vehicle, naltrindole, TIPP or TIPP[ψ] (i.c.v.). ANOVA revealed a significant main effect of i.c.v. treatment for time spent in withdrawal (teeth chattering and writhing) ($F(3,35) = 6.63$, $P < 0.01$). * Significant difference from the vehicle control group ($P < 0.05$, LSD t-test). B: Average severity of checked signs (eye twitch and salivation, maximum = 3) for rats in each treatment group. Kruskal-Wallis ANOVA for non-parametric data indicated a significant effect of i.c.v. treatment for both eye twitch ($H(3,39) = 13.7$, $P < 0.01$) and salivation ($H(3,39) = 9.92$, $P < 0.05$). * Significant difference from the vehicle control group ($P < 0.05$, Mann-Whitney U-test). C: Percent of time spent in non-withdrawal (ambulating, rearing, grooming and resting) and withdrawal (teeth chattering and writhing combined) behaviours during the 10 min prior to precipitation of withdrawal for morphine-dependent rats in each i.c.v. treatment group. D: Percent of time spent in non-withdrawal and withdrawal behaviours during the 10 min prior to the precipitation of withdrawal for non-dependent rats. ANOVA of the data in C and D indicated that there were no differences in any of the behaviours between different i.c.v. treatment groups. ANOVA indicated a significant effect of morphine treatment on resting ($F(1,54) = 7.11$, $P < 0.01$), ambulating ($F(1,54) = 25.54$, $P < 0.01$) and grooming ($F(1,54) = 4.58$, $P < 0.05$). Morphine-dependent rats tended to rest and groom more, while ambulating less, than non-dependent rats.

treatment alone (non-dependent) to assess the effects of the δ-opioid receptor antagonists on general behaviour.

2.2. Drugs

TIPP and TIPP[ψ] were synthesized as described previously (Schiller et al., 1992, 1993). Naltrindole was obtained from Research Biochemicals (Natick, MA, USA). δ-Opioid receptor antagonists were infused i.c.v. at a rate of 1 μl/h in the following doses: naltrindole (10 nmol/day), TIPP (80 nmol/day) and TIPP[ψ] (40 nmol/day). These doses of antagonists were chosen to be equipotent based on K_e values obtained in the mouse vas deferens assay (Schiller et al., 1993). Morphine sulfate (Sabex, Montreal, PQ, Canada) was continuously delivered s.c. at a rate of 10 μl/h for a total dose of 36.65 μmol/day.

2.3. Tolerance measurement

To assess the development of analgesic tolerance, the ED_{50} of morphine in a radiant heat tail-flick test was determined both prior to chronic morphine treatment (pretreatment) and after 6 days of morphine treatment (chronic). A baseline tail-flick latency was measured for each rat prior to each morphine trial. For the pretreatment and chronic trials rats were injected s.c. with morphine sulfate (0, 0.5, 1 or 2 mg/kg for pretreatment tests; 0, 1, 2, 4 or 8 mg/kg for post-treatment tests) and tail-flick latencies were measured for 90 min post-injection at 10 min intervals. A percent maximum possible effect (%MPE) score for each tail-flick latency measurement following the injection of morphine was calculated using the following formula: %MPE = [(test latency − baseline latency)/(cutoff − baseline)] × 100. A cutoff latency of 10 s was used to prevent tissue injury. The area under the curve (AUC) for the %MPE scores over the 90 min test period was calculated for each rat. The AUC scores were then used to calculate ED_{50} values.

2.4. Withdrawal measurement

Precipitated abstinence symptoms were assessed on the seventh day of treatment after injection of naloxone (1 mg/kg s.c.). For 10 min before and 40 min after naloxone injection, withdrawal symptoms were assessed by measuring the amount of time spent teeth chattering and writhing. To ensure that chronic i.c.v. treatment with the δ-opioid antagonists had no effect on general behaviour, the time spent in non-withdrawal behaviours (ambulating, rearing, grooming and resting) was also assessed during the 10 min prior to the precipitation of naloxone in both morphine-dependent (given i.c.v. treatments and s.c. morphine) and non-dependent (given i.c.v. treatments alone) rats. The average severity of checked signs, eye twitch and salivation, was rated on a 4 point scale, where 0 = absent and 3 = severe, at the end of each 10 min period.

3. Results

Chronic treatment with 36.65 μmol/day morphine sulfate produced an intense and reliable withdrawal syndrome, as evidenced by the large amount of time spent teeth chattering and writhing, as well as the occurrence of jumps, wet dog shakes, eye twitch and salivation in i.c.v. saline-treated rats (Fig. 1). All three δ-opioid receptor antagonists significantly decreased the amount of time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period (Fig. 1A). Both naltrindole and TIPP[ψ] significantly decreased the severity of eye twitch, while TIPP[ψ] also significantly decreased the severity of salivation (Fig. 1B).

Fig. 1C illustrates the percent of time spent in non-withdrawal (ambulating, rearing, grooming and resting) as well as withdrawal (teeth chattering and writhing) behaviours during the 10 min prior to the precipitation of withdrawal (baseline period) for morphine-dependent rats. Fig. 1D shows the percent of time spent in non-withdrawal and withdrawal behaviours for non-dependent rats. As seen in Fig. 1C and D, there was a significant effect of morphine treatment, with morphine-dependent rats resting and grooming more and ambulating less than non-dependent rats. However, there were no significant differences between i.c.v. treatment groups, indicating that chronic i.c.v. treatment with δ-opioid receptor antagonists had negligible effects on general behaviour either in morphine-dependent (Fig. 1C) or non-dependent (Fig. 1D) rats.

TIPP[ψ], but neither naltrindole nor TIPP, attenuated the development of analgesic tolerance, with an ED_{50} following chronic morphine treatment (ED_{50} = 0.36 mg/kg) that was unchanged from the baseline ED_{50} (ED_{50} = 0.35 mg/kg) (Table 1).

Table 1
Morphine ED_{50} for rats prior to morphine treatment, and for rats in each i.c.v. treatment group following 6 days of morphine treatment

Treatment	ED_{50} (mg/kg) (95% C.I.)
Pretreatment	0.35 (0.33–0.53)
Vehicle, post-treatment	0.81 (0.30–1.66) [a]
Naltrindole, post-treatment	0.98 (0.06–3.16) [a]
TIPP, post-treatment	1.25 (0.22–3.47) [a]
TIPP[ψ], post-treatment	0.36 (0.05–0.81)

[a] Significantly greater than pretreatment ($P < 0.05$, Student's t-test).

4. Discussion

The present results suggest that activity at δ-opioid receptors is critical to the development of morphine tolerance and dependence. All three δ-opioid receptor antagonists, naltrindole, TIPP and TIPP[ψ], effectively decreased the amount of time spent teeth chattering and writhing during the precipitated withdrawal syndrome, while having no effect on general behaviours such as ambulating, rearing, grooming and resting. Both naltrindole and TIPP[ψ] attenuated the severity of eye twitch, while TIPP[ψ] also decreased the severity of salivation. However, none of the i.c.v. δ-opioid receptor antagonist treatments affected the frequency of jumps or wet dog shakes (data not shown). Jumps and wet dog shakes have been shown to be mediated by brain areas around the fourth ventricle (Laschka et al., 1976). Because we infused very small volumes into the lateral ventricle, the δ-opioid receptor antagonists may have been absorbed by brain areas around the lateral ventricle, and therefore not have been able to diffuse adequately to the fourth ventricle. In TIPP[ψ]-treated rats, the ED_{50} for morphine in the tail-flick test following 6 days of morphine treatment was unchanged from the pretreatment value. Thus, chronic inhibition of δ-opioid receptors in the brain with TIPP[ψ] was effective in suppressing the development of both tolerance and dependence during chronic systemic morphine treatment.

Even though we used equipotent doses, TIPP[ψ] was more effective than either naltrindole or TIPP in attenuating the development of tolerance and dependence. TIPP[ψ] may have been more effective because it is more selective than naltrindole, and more stable in physiological medium than TIPP (Schiller et al., 1993). Unlike previous reports in mice, we did not find an attenuation of analgesic tolerance with naltrindole in rats, suggesting a possible species difference.

It remains to be determined whether activation of δ-opioid receptors is critical only to the development of *morphine* tolerance and dependence, or whether this activation is important to the development of *opioid* tolerance and dependence in general. If this is a phenomenon relating to opioids in general, the development of opioid tolerance and dependence may be attenuated by use of opioid compounds with mixed μ-opioid receptor agonist/δ-opioid receptor antagonist properties.

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