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Tolerance to analgesia and dependence liability by topical application of dihydroetorphine to hairless rats

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Abstract

The tolerance to analgesia and dependence liability of dihydroetorphine following topical application were investigated in hairless rats with and without formalin-induced inflammation. The analgesic effect of dihydroetorphine (s.c.) was 4600- to 7200-fold more potent than that of morphine. In non-inflamed rats, the analgesic effect of 24-h topical application of dihydroetorphine tape (35 μg) and 4-day repeated tape applications (20 μg/5 h/day) decreased with time after the start of application, even though the plasma dihydroetorphine concentrations did not decrease. In formalin-inflamed rats, however, the tolerance to analgesia diminished. Naloxone-precipitated weight loss was observed after 24-h infusion of dihydroetorphine but not after the tape application in non-inflamed rats. A significant rewarding effect was found in the non-inflamed rats conditioned by s.c. injection and tape application but not in the formalin-inflamed rats. These results indicate that topical application of dihydroetorphine has a tolerance and dependence liability when there is no pain, and therefore, it should be used only for pain relief.

Keywords: Dihydroetorphine; Hairless, rat; Topical application; Tolerance to analgesia; Physical dependence; Rewarding effect

1. Introduction

Dihydroetorphine (7,8-dihydro-7α-[1-(*R*)-hydroxy-1-methylbutyl]-6,14-*endo*-ethanotetrahydro-oripavine) produces extraordinarily strong analgesia through the activation of μ-opioid receptors (Katsumata et al., 1995; Wang et al., 1995; Kamei et al., 1996), with a potency ratio to morphine of 1000 to 12,000 (Bentley and Hardy, 1967; Huang and Qin, 1982; Tokuyama et al., 1996). In China, dihydroetorphine began to be clinically used for pain relief in 1981. Although dihydroetorphine was registered as an analgesic in 1992, it was also used for suppressing opiate withdrawal syndrome. However, the Government of China restricted the clinical use of dihydroetorphine in 1993, because the number of dihydroetorphine abusers increased quickly as soon as it was commercialized (WHO Expert Committee on Drug Dependence, thirty-first report, 1999). Epidemiological studies showed that the majority of abusers took dihydroetorphine to avoid the withdrawal syndrome of heroin or other opiates, whereby potent psychic- and/or mild physical-dependence developed (Liu et al., 1995). In March 1999, the United Nations decided to include dihydroetorphine in Schedule I of the Single Convention on Narcotic Drugs of 1961 and that Convention as amended by the 1972 Protocol (Commission on Narcotic Drug, Report on the forty-second session, 1999). Up to now, dihydroetorphine has been clinically used for pain relief under restricted control in China (Wang et al., 1999; Zang 1999).

In experiments on the dependence liability of dihydroetorphine, it was shown that dihydroetorphine induced a potent reinforcing effect and discriminative stimulus effect in the drug self-administration test in rats and monkeys (Martin et al., 1997; Beardsley and Harris, 1997) and a rewarding effect in the conditioned place preference test in rats (Liu and Zhang, 1999a,b). Furthermore, dihydroetorphine produced physical dependence in the case of continuous exposure by infusion (Zhang and Qin, 1994; Aceto et al., 2000) and short-term repeated injections (Tokuyama et al., 1994) in rats. Tokuyama et al. (1993) reported the development of tolerance to analgesia with dihydroetorphine and cross-tolerance with morphine. These studies indicate that dihydroetorphine has significant potential for abuse like other opioids such as morphine, fentanyl and heroin. However, it has been reported that the tolerance and dependence liability of morphine are reduced in ani-

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mals suffering from formalin-induced pain (Vaccarino et al., 1993; Vaccarino and Couret, 1993; Suzuki et al., 1996) and in humans with chronic pain (Poter and Jick, 1980). It has not been investigated whether the tolerance to and dependence on dihydroetorphine develops under the influence of inflammatory nociception.

Dihydroetorphine has been clinically administered as sublingual tablets in China (Wang et al., 1999). Because the sublingual dose exhibits a short duration of analgesic effect (within 1.5–5.3 h), it has to be administered several times a day for continuous pain relief (Wu and Sun, 1991). In our previous study, transdermal delivery of dihydroetorphine was shown to produce a prolonged analgesic effect for more than 24 h in hairless rats (Ohmori et al., 2000b). However, the potential to promote dependence by continuous exposure to dihydroetorphine is of concern and requires clarification. The objectives of this study are (1) to compare the potency of the analgesic effect and dependence liability of dihydroetorphine and morphine in hairless rats, (2) to compare the tolerance to analgesia and dependence liability of continuous infusion or repeated s.c. injection and topical application of dihydroetorphine, and (3) to compare the tolerance and dependence liability of dihydroetorphine in rats with and without formalin-induced nociception.

2. Materials and methods

2.1. Animals

The male hairless rats (WBN/ILA-Ht strain) weighing 200–250 g (10–12 weeks old) used in all experiments were supplied by the Ishikawa Experimental Animal Laboratory (Saitama, Japan). Animals were kept in a room that was maintained at 24 ± 1 °C under a 12-h light–dark cycle and had free access to a standard rodent chow and clean drinking water. These experiments were performed in accordance with the *Guide for Laboratory Animal Experiment* adopted by Josai University.

For the treatment of formalin-inflamed rats, formalin (2.5%, 50 μl) was injected into the plantar surface of the hind paw just once on the initial day. Suzuki et al. (1996) reported that, in Sprague–Dawley rats, the formalin-treated (2.5%, 50 μl) paw continued to swell for 11 days, and a pronounced reduction in the formalin-treated paw pressure threshold was observed for 9 days. In this study, the hairless rats showed aversive behavior, and the formalin-treated paw continued to swell for 6 days (data not shown). For evaluation of tolerance development, the administration of opioids was begun 1 day after the formalin injection. For the evaluation of the rewarding effect in the conditioned place preference test, animal screening was carried out 2 days after and then conditioning following administration of opioids was begun 3 days after the formalin injection.

In all experiments, individual rats received only a single dosing schedule.

2.2. Preparation and topical application of dihydroetorphine tape

The dihydroetorphine tape was prepared according to our previous report (Ohmori et al., 2000b). Briefly, dihydroetorphine, styrene-isoprene-styrene block co-polymer, rosin ester and isopropyl myristate were dissolved in chloroform. The mixture was cast onto backing film, and then dried. The tape was covered with a release liner, and then the tape was pulled out circularly to give an area of 0.13, 0.28, 0.38 and 0.50 cm^2. The dihydroetorphine content of the tape was 70 $\mu g/cm^2$. A placebo tape was prepared by the same method excluding the dihydroetorphine.

The 0.13-, 0.28- and 0.38-cm^2 tapes were applied to the abdominal skin, and the 0.5-cm^2 tape was applied to the dorsal skin of rats. The abdominal and dorsal applications provide steady-state plasma concentrations of the drug for 8 and 24 h, which are appropriate for repeated application and 24-h continuous exposure, respectively (Ohmori et al., 2000b). Because the absorption rate of dihydroetorphine through the abdominal and dorsal skin was different, the area of the tape was adjusted to maintain the same plasma concentration. During the repeated application, the tape was re-applied to different sites on the abdominal skin each day, because the skin was slightly damaged when the tape was removed (Ohmori et al., 2000b).

2.3. Intravenous infusion of drugs

Rats were cannulated with polyethylene tubing in a femoral vein under diethylether anesthesia. The tip of the cannula was drawn through the skin on the back of the neck. Rats were placed in a Bollman cage and left for 2 h after surgery to recover from anesthesia. Morphine (8 mg/kg/h), dihydroetorphine (1.8 μg/kg/h) or saline (0.5 ml/h) was infused for 24 h through the venous cannula.

2.4. Blood sampling for the determination of plasma dihydroetorphine concentration

To withdraw blood samples for the determination of the plasma dihydroetorphine concentration during the 24-h infusion, rats were cannulated with polyethylene tubing in a femoral artery, in addition to a femoral vein for the infusion of dihydroetorphine, under diethylether anesthesia. Individual rats were placed in the Bollman cage and left for 2 h after surgery to recover from anesthesia. Dihydroetorphine (1.8 μg/kg/h) was infused for 24 h through the venous cannula. Blood (0.5 ml) was withdrawn from the arterial cannula at 2, 4, 8, 16 and 24 h after the start of infusion.

To withdraw blood samples during the 4-day repeated s.c. injections (2 μg/kg/day) and the abdominal tape application, rats were cannulated with polyethylene tubing in a femoral artery on day 4, immediately before s.c. injection and tape application under diethylether anesthesia. The tip of the cannula was drawn through the skin on the back of the neck. After being left for 2 h, dihydroetorphine was administered by s.c. injection or tape application. Blood (0.5–1 ml) was withdrawn at 15, 45, 90 and 180 min after the s.c. injection and at 1, 2, 3, 4 and 5 h after the tape was applied.

Blood samples were placed in heparinized tubes and the plasma was separated by centrifugation. Plasma samples were stored at −20 °C until analysis.

2.5. Measurement of the plasma dihydroetorphine concentration

Plasma dihydroetorphine concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS-MS) (Ohmori et al., 2000a). Briefly, an exact volume less than 0.5 ml of the sample was mixed with buprenorphine as an internal standard and 50 mM phosphate buffer (pH 6.0). The mixture was applied to a solid-extraction cartridge (Bond Elut Certify®, Varian, Harbor City, CA), and the cartridge was washed with 100 mM acetic acid and methanol. The eluent with 2% ammonium hydroxide in ethyl acetate was collected and redissolved in acetonitrile–water (80:20). An aliquot was applied to the LC-MS-MS system (API-300®, Perkin Elmer-SCIEX, Foster City, CA) connected to an Inertsil ODS-2 column (5 μm, 2.1 mm i.d. × 150 mm, GL Science, Tokyo, Japan) filled with acetonitrile—50 mM ammonium acetate (95:5) at 0.2 ml/min. DHE and the internal standard were detected at m/z 414:414 and 468:468, respectively.

2.6. Measurement of analgesic effect

The analgesic effect was determined in the tail-immersion test (Ouellet and Pollack, 1995). In the infusion study, the tail of the rat was allowed to emerge from the Bollman cage. In the tape application study, rats were loosely wrapped in a cloth so that the tail emerged from the cloth. The distal two-thirds of the tail was immersed in hot water (50–55 °C), and the latency time before a flick of the tail or struggle was measured. The basal latency to heat stimuli was slightly different between formalin-inflamed rats and non-inflamed rats and during repeated administration. Therefore, the temperature of the water was adjusted in each group, in order that the basal latency would be approximately 2 s and not exceed 3 s. A cut-off time of 10 s was adopted to prevent damage to the tail. The analgesic effect is expressed as % of the maximum possible effect (%MPE): %MPE = [post-drug latency − pre-drug latency]/[cut-off latency − pre-drug latency] × 100. The area under the %MPE–time curve (AUC) was calculated from the %MPE vs. time curve by trapezoidal rule.

2.7. Naloxone-precipitated weight loss

Physical dependence was assessed by measuring the naloxone-precipitated loss of weight (Zhang and Qin, 1994). After the infusion of morphine (8 mg/kg/h), dihydroetorphine (1.8 μg/kg/h), saline (0.5 ml/h), dorsal application of dihydroetorphine tape (35 μg/0.5 cm^2) or placebo tape (0.5 cm^2) for 24 h, the rats were weighed and injected with naloxone (5 mg/kg, i.p.). The rats were then placed individually in a plastic box for 1 h, and then the rats were weighed again.

2.8. Conditioned place preference

Conditioned place preference test was performed according to the method reported by Suzuki (1999) with a minor modification. The apparatus consisted of an acryl-resin shuttlebox (22 × 44 × 22 cm ($w \times l \times h$)), which was divided into two compartments of equal size by a removable guillotine door (10 × 14 cm ($l \times h$)). One compartment was white with a carpeted floor and the other was black with a slippery floor. The front sides of both compartments were made of transparent acryl-resin board to observe the movement of the animals placed inside. The shuttlebox was covered with a blind-box to prevent light and noise from entering, and the brightness inside was set at 27 lx.

Prior to place conditioning, rats were placed in the shuttlebox after removal of the guillotine door, and the time spent in each compartment over a period of 900 s was measured. The rats which showed no difference in the time spent in each compartment (less than 200 s) were used for the test. On day 1, rats were given morphine (10 mg/kg), dihydroetorphine (2 μg/kg), saline (1 ml/kg) by s.c. injection, or the dihydroetorphine tape (9 μg/0.13 cm^2, 20 μg/0.28 cm^2 or 27 μg/0.38 cm^2) was applied to the abdominal skin. The rats were conditioned in one compartment for 60 min, but in the case of tape application, a 60-min interval before the conditioning period was needed to produce the analgesic effect (Ohmori et al., 2000b). On day 2, rats were conditioned to the other compartment for 60 min after s.c. injection of saline (1 ml/kg) or application of a placebo tape (0.5 cm^2). On days 3 and 4, the conditioning was conducted in the same procedure as on days 1 and 2, respectively. The order of administration (drug or vehicle) and the compartment (white and black) were counterbalanced across the subjects. On day 5, the guillotine door was removed, and a transparent platform was inserted along the joint separating the compartments. The rats were gently placed on the platform, and the time spent in each compartment during 900 s was measured. Conditioning scores represented the time spent in the

drug-paired place minus the time spent in the vehicle-paired place.

2.9. Statistical analysis

Data are expressed as means ± S.E.M. The dose that produced 50% analgesia, ED_{50}, was calculated from the log-dose vs. %MPE or AUC by linear regression techniques. Statistical analysis was performed using one- or two-way analysis of variance followed by Dunnett's test to assess significant differences between the drug-treatment group and the control group. A difference was considered significant at $P < 0.05$.

2.10. Chemicals

Free base dihydroetorphine was synthesized from codeine by reported procedures (Bentley and Hardy, 1967; Barber and Rapoport, 1975). Morphine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Naloxone hydrochloride was purchased from Sigma (St. Louis, MO). Formaldehyde solution was purchased from Wako (Osaka, Japan). These drugs and chemicals were dissolved in saline for injection. Styrene-isoprene-styrene block co-polymer (Cariflex TR-1107, Shell Chemical, Tokyo, Japan), rosin ester (KE-311, Arakawa Chemical, Osaka, Japan) and isopropyl myristate (Tokyo Chemical Industry, Tokyo, Japan) were used for the preparation of dihydroetorphine tape.

3. Results

3.1. Analgesic effect of morphine and dihydroetorphine in hairless rats

Fig. 1 shows the analgesic effects after s.c. injection of morphine and dihydroetorphine in hairless rats. The %MPE of morphine increased gradually after s.c. injection and reached a maximum at 45–60 min after s.c. injection. The %MPE induced by s.c. injection of dihydroetorphine increased rapidly and reached a maximum at 15–30 min, and then it decreased more rapidly in comparison with that of morphine. Morphine and dihydroetorphine produced dose-dependent analgesic effects. The ED_{50} calculated from the maximum value of %MPE was 10.1 mg/kg for morphine and 1.4 μg/kg for dihydroetorphine. The ED_{50} calculated from the AUC was 19.4 mg/kg for morphine and 4.2 μg/kg for dihydroetorphine. The ED_{50} ratio of dihydroetorphine to morphine was 4600 to 7200 in hairless rats.

3.2. Plasma dihydroetorphine concentration

Fig. 2 shows the plasma dihydroetorphine concentrations in rats during infusion and tape application for 24 h

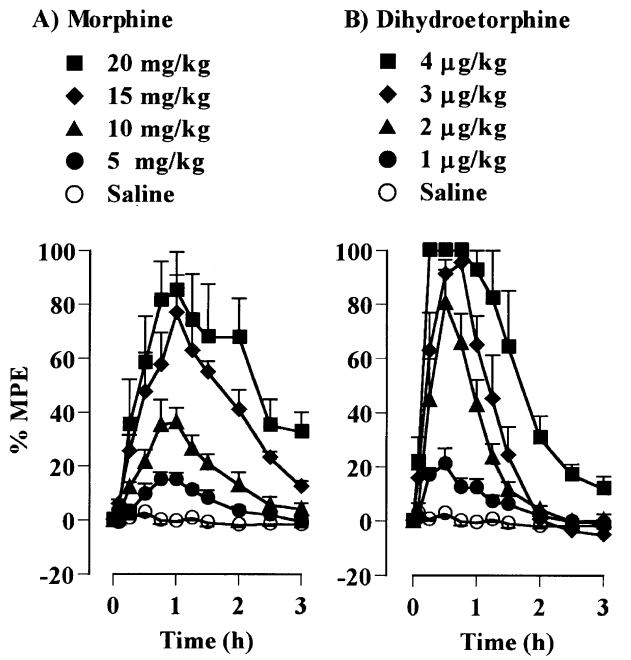

Fig. 1. Analgesic effects after s.c. injection of (A) morphine (closed symbols, 5, 10, 15 and 20 mg/kg), dihydroetorphine (closed symbols, 1, 2, 3 and 4 μg/kg) and saline (open symbols in panels (A) and (B), 1 ml/kg) in hairless rats. Each data point represents the mean ± S.E.M. for five rats.

(panel A) and on days 1 and 4 during the repeated s.c. injections and tape application for 4 days (panel B). The dorsal application of the 35 μg/0.5 cm^2 tape and the abdominal application of the 20 μg/0.28 cm^2 tape provided steady state concentrations for 24 h and for 5 h, respectively, as reported by other authors (Ohmori et al., 2000b). The steady state concentration during the 1.8 μg/kg/h infusion of dihydroetorphine was equivalent to that obtained with the dorsal application of the 35 μg/0.50 cm^2 tape, though the time lag to reach the steady state concentration was shorter (panel A). The peak concentration after 2 μg/kg s.c. injection of dihydroetorphine, which was reported in a previous study (Ohmori et al., 2001), was slightly higher than that during the abdominal application of the 20 μg/0.28 cm^2 tape for 5 h (day 1 in panel B). Furthermore, the concentration profile on day 4 was similar to that on day 1 during the repeated administration (panel B).

3.3. Tolerance to analgesia during 24-h continuous exposure

Analgesic effects in formalin-inflamed and non-inflamed hairless rats treated with 24-h continuous infusion of dihydroetorphine and morphine, and topical application of dihydroetorphine tape are shown in panels A and B in Fig. 3, and their AUCs are shown in panel C. The analgesic effect induced by continuous infusion of dihydroetorphine (1.8 μg/kg/h) and morphine (8 mg/kg/h) in-

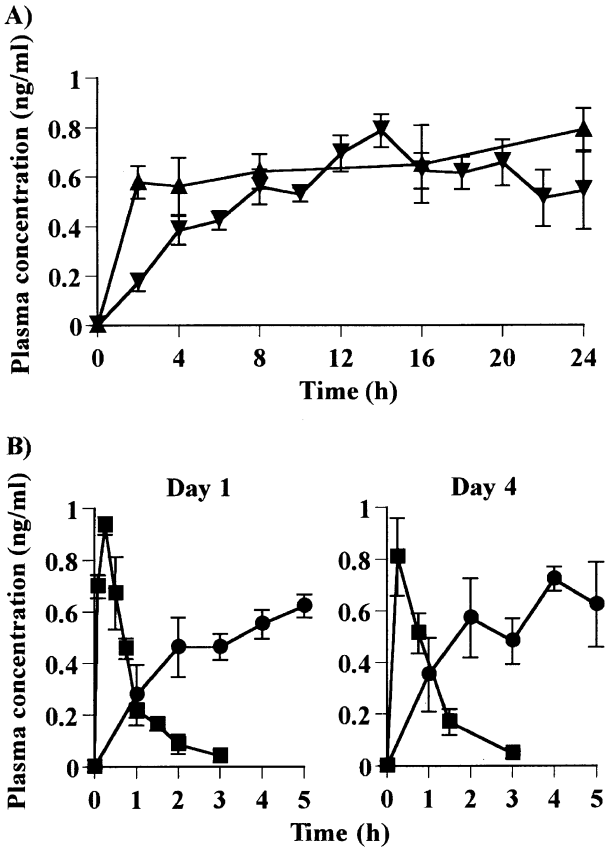

Fig. 2. Plasma dihydroetorphine concentrations in rats during infusion (▲, 1.8 μg/kg/h) and tape application (▼, 35 μg/0.50 cm^2 to dorsal skin) for 24 h (panel A) and on days 1 and 4 during the 4-day repeated s.c. injections (■, 2 μg/kg/day) and tape application (●, 20 μg/0.28 cm^2 to abdominal skin for 5 h/day) (panel B). Each data point represents the mean ± S.E.M. for five rats. The data for 24-hour dorsal application of the 35 μg/0.5 cm^2 tape and for day 1 of abdominal application of the 20 μg/0.28 cm^2 tape are taken from our previous report (Ohmori et al., 2000b). The data for day 1 after 2 μg/kg s.c. injection are taken from our previous study (Ohmori et al., 2001).

creased to more than 50 %MPE at 4 h, and then %MPE decreased to the basal level at 14 h in non-inflamed rats. In the formalin-inflamed rats, although the peak effect was the same degree as that in the non-inflamed rats, %MPE during infusion was maintained at a higher level. Consequently, the AUCs in the formalin-inflamed rats were 1.8- to 2.2-fold greater than those in non-inflamed rats ($P < 0.05$). Topical application of the tape onto the dorsal skin (35 μg/0.5 cm^2) produced prolonged analgesia, of which the AUC was 10-fold greater than that by s.c. injection (2 μg/kg). However, there was no significant difference between the AUCs in the formalin-inflamed and non-inflamed rats.

3.4. Tolerance to analgesia during 4-day repeated exposure

Fig. 4 shows the analgesic effect during repeated s.c. injection of dihydroetorphine or topical application of dihydroetorphine tape for each of 4 days in panels A and B, and their AUCs in panel C. The analgesic effect during the 4-day repeated s.c. injections of dihydroetorphine (2 μg/kg) was reduced with the number of injections in the non-inflamed rats, and the AUC of the analgesic effect on day 4 was significantly decreased compared with that on

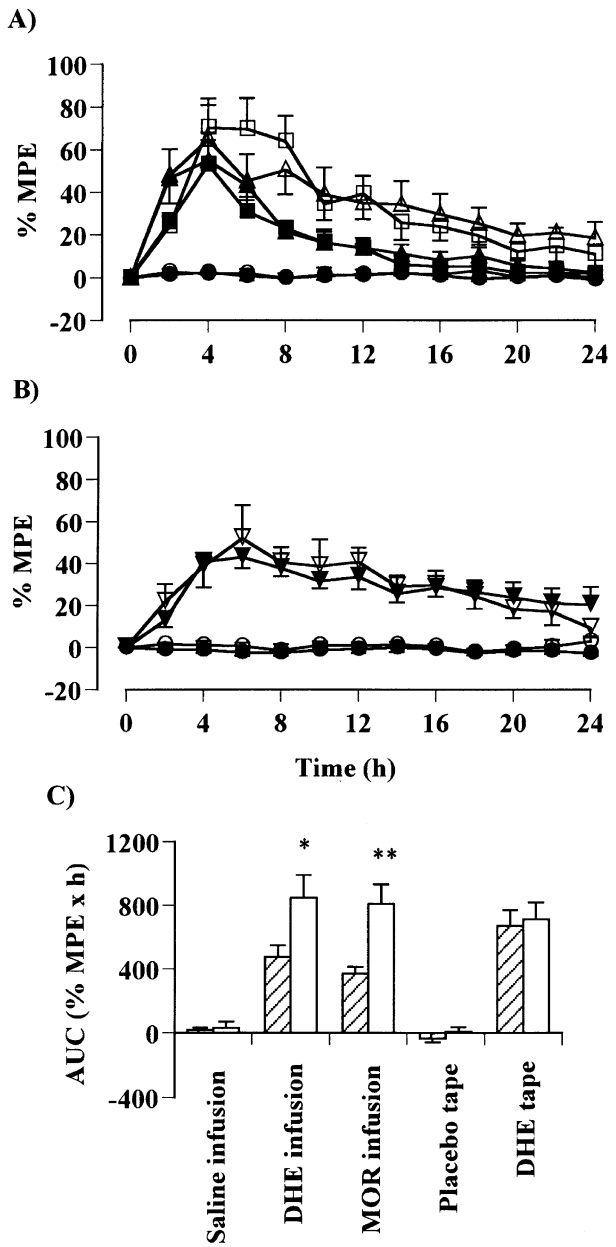

Fig. 3. Analgesic effects in non-inflamed (closed symbols and hatched bars) and formalin-inflamed (open symbols and open bars) hairless rats treated with (A) the infusion of dihydroetorphine (DHE infusion, ▲ △, 1.8 μg/kg/h), morphine (MOR infusion, ■ □, 8 mg/kg/h) or saline (Saline infusion, ● ○, 0.5 ml/h), (B) topical application of dihydroetorphine tape to dorsal skin (DHE tape, ▼ ▽, 35 μg/0.5 cm^2) and placebo tape to dorsal skin (Placebo tape, ● ○, 0.5 cm^2), and (C) the AUC of analgesic effects following these applications. Each data point represents the mean ± S.E.M. for five rats. $^*P < 0.05$, $^{**}P < 0.01$ vs. saline infusion group (Dunnett's test).

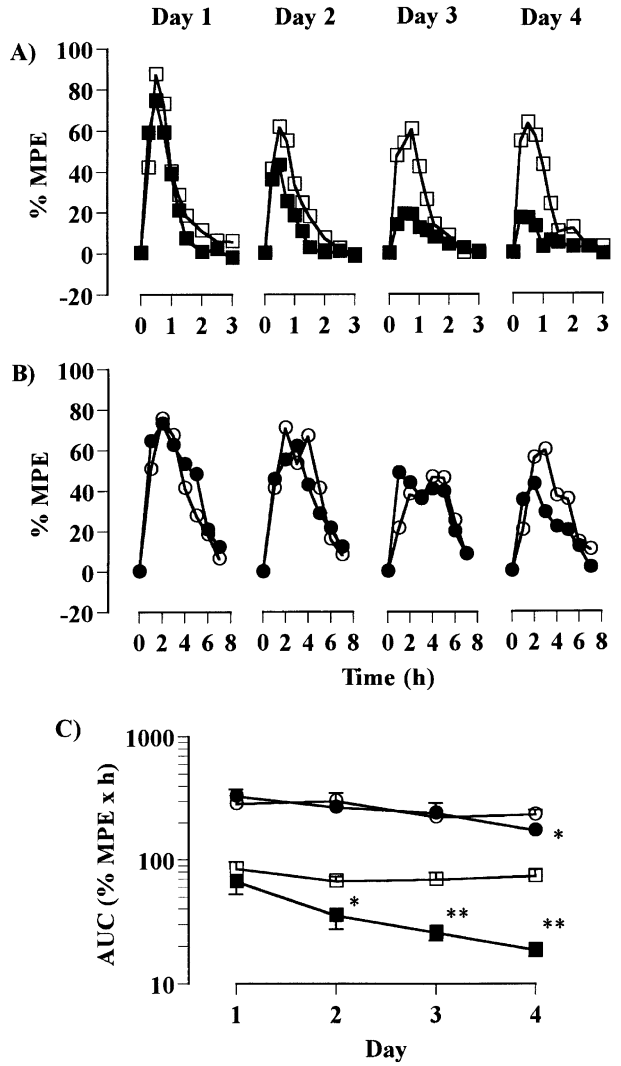

Fig. 4. Analgesic effects in non-inflamed (closed symbols) and formalin-inflamed (open symbols) hairless rats treated with (A) repeated s.c. injections of dihydroetorphine (■ □, 2 μg/kg/day), (B) topical application of dihydroetorphine tape to abdominal skin (●○, 20 μg/0.28 cm^2, 5 h/day) for 4 days, and (C) the AUC of analgesic effects following these applications. Each data point represents the mean ± S.E.M. for five rats. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. day 1 (Dunnett's test).

day 1 ($P < 0.01$). However, in the formalin-inflamed rats, the decrease in AUC was less marked during the 4-day repeated injections. Topical application of dihydroetorphine tape on the abdominal skin (20 μg/0.28 cm^2 for 5 h) also resulted in a decrease in the AUC of analgesic effect in both formalin-inflamed and non-inflamed rats, but a significant difference was only detected on day 4 in non-inflamed rats ($P < 0.05$), unlike that of repeated s.c. injection.

3.5. Naloxone-precipitated weight loss

Fig. 5 shows the naloxone-precipitated weight loss in hairless rats treated with the 24-h infusion of dihydroetorphine and morphine or topical application of dihydroetorphine tape. The continuous infusion of morphine (8 mg/kg/h) and dihydroetorphine (1.8 μg/kg/h) for 24 h induced a significant weight loss ($P < 0.01$). However, the topical application of dihydroetorphine tape to the dorsal skin (35 μg/0.5 cm^2) had no effect on body weight.

3.6. Place conditioning

Fig. 6 shows the place conditioning produced by s.c. injection of morphine, dihydroetorphine or abdominal application of dihydroetorphine tape in formalin-inflamed and non-inflamed hairless rats. The non-inflamed rats conditioned with saline did not exhibit a significant preference for either compartment (−212 to 155 s for one compartment, $n = 8$). Rats conditioned by s.c. injection of morphine (10 mg/kg, $P < 0.01$) and dihydroetorphine (2 μg/kg, $P < 0.05$) exhibited a significant preference for the drug-paired place. Topical application of dihydroetorphine tape produced a dose-dependent place preference and significant conditioning was observed with the application of the 27 μg/0.38 cm^2 tape ($P < 0.01$).

The formalin-inflamed rats conditioned with saline did not exhibit a significant preference for either compartment (−254 to 194 s for one compartment, $n = 8$). Subcutaneous injection of morphine (10 mg/kg), dihydroetorphine (2 μg/kg) and topical application of dihydroetorphine

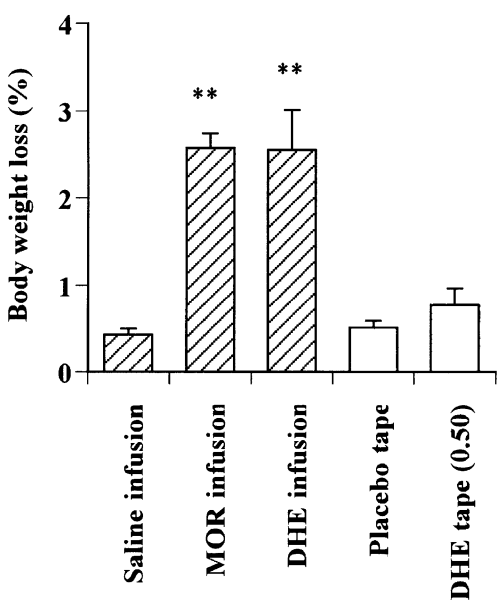

Fig. 5. Naloxone-precipitated (5 mg/kg, i.p.) weight loss in hairless rats treated with an infusion (hatched bars) of morphine (MOR infusion, 8 mg/kg/h), dihydroetorphine (DHE infusion, 1.8 μg/kg/h), saline (saline infusion, 0.5 ml/h) or dorsal application (open bars) of dihydroetorphine tape (DHE tape, 35 μg/0.5 cm^2), placebo tape (placebo tape, 0.5 cm^2) for 24 h. Each data point represents the mean ± S.E.M. for six rats. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. saline infusion group (Dunnett's test).

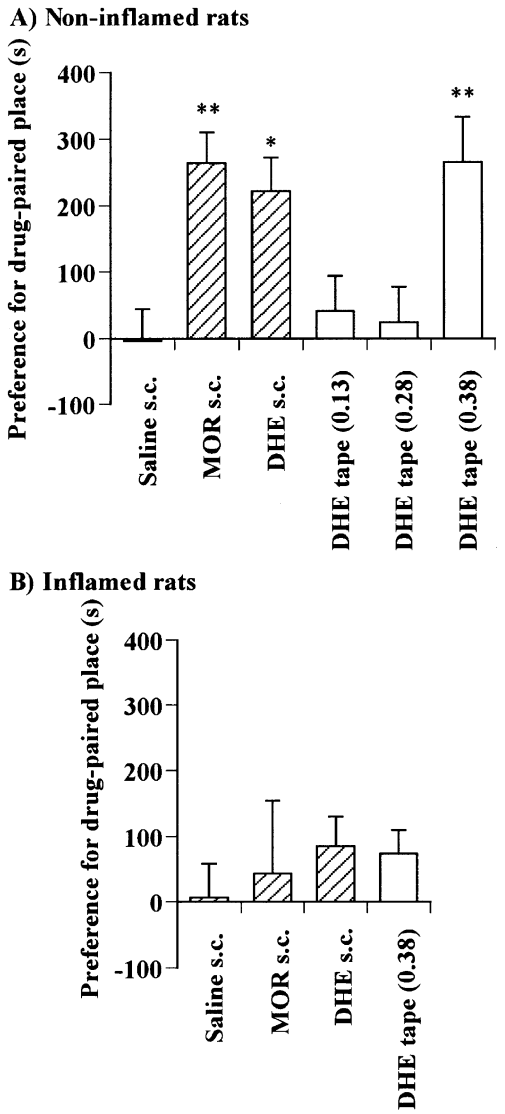

Fig. 6. Place conditioning produced by s.c. injection (hatched bars) of morphine (MOR, s.c., 10 mg/kg), dihydroetorphine (DHE, s.c., 2 μg/kg), saline (saline, s.c., 1 ml/kg) or topical application (open bars) of dihydroetorphine tape (DHE tape, 9 μg/0.13 cm^2, 20 μg/0.28 cm^2 and 27 μg/0.38 cm^2) in (A) non-inflamed and (B) formalin-inflamed hairless rats. Each data point represents the mean ± S.E.M. for eight rats. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. saline s.c. group (Dunnett's test).

tape (27 μg/0.38 cm^2) did not produce a significant preference for the drug-paired compartment.

4. Discussion

The WBN/ILA-Ht strain hairless rat, which is the dominant mutant derived from the Wistar strain, has been used to evaluate drug skin permeation (Sato et al., 1991). There are few reports regarding the nociceptive response and dependence behavior in hairless rats (Sugibayashi et al., 1989). In this study, hairless rats sensitively responded to heat stimulus as phasic pain in the tail-immersion test. The ED_{50} ratio of morphine to dihydroetorphine was 4600 to 7200 in the hairless rat, which is within the range of previous reports (Bentley and Hardy, 1967; Huang and Qin, 1982; Tokuyama et al., 1996). Moreover, dihydroetorphine and morphine induced a physical dependence and rewarding effect in hairless rats. The dose ratios for morphine to dihydroetorphine which produced the same extent of physical dependence and rewarding effect were 4400 and 5000, respectively. The analgesic effect and rewarding effect were also observed when dihydroetorphine was applied to the hairless rat skin. This indicates that hairless rats show suitable properties to evaluate the analgesic effects and dependence liability of transdermally delivered analgesics.

In this study, the tolerance to analgesia and the dependence liability following topical application of dihydroetorphine tape were compared with those of continuous infusion or repeated s.c. injections of dihydroetorphine and morphine. The tolerance and physical dependence were evaluated for 35 μg/0.50 cm^2 dorsal application of dihydroetorphine tape, 1.8 μg/kg/h infusion of dihydroetorphine and 8 mg/kg/h infusion of morphine, dosages which produced the same analgesic effect (maximum value of % MPE-time course in Fig. 3). The tolerance and rewarding effect were evaluated for repeated application of 20 μg/0.28 cm^2 dihydroetorphine tape abdominally, 2 μg/kg dihydroetorphine s.c. and 10 mg/kg morphine s.c.; a single application of these dosages produced the same extent of analgesic effect (Figs. 1 and 4).

In many cases, the analgesic effects of dihydroetorphine or morphine were reduced with the increase in time after the start of drug exposure and with increasing number of applications in normal rats (Figs. 3 and 4). The decrease in plasma dihydroetorphine concentrations was not found during the 24-h continuous infusion and 4-day repeated administrations (Fig. 2). In both cases, the dihydroetorphine concentrations in the central nervous system were found to be 3- to 5-fold higher than the plasma concentrations (Ohmori et al., 2000b). These results indicate that tolerance to analgesia occurred and increased time-dependently during dihydroetorphine and morphine infusion in non-inflamed rats, as previously reported (Gardmark et al., 1993; Ouellet and Pollack, 1995). To examine tolerance during the 4-day repeated application of the tape, the tape was applied to the abdominal skin for 5 h each day, because the analgesic effect decreased after a 5-h application (Ohmori et al., 2000b). The 2-fold increase in the AUC during the 24-h continuous exposure in the formalin-inflamed rats was due to attenuation of the time-dependent decrease of %MPE, in comparison to that in non-inflamed rats. However, the analgesic effect elicited by both infusion and topical application of dihydroetorphine in the formalin-inflamed rats tended to decrease with increasing time and number of applications (Figs. 3 and 4). Formalin injection is reported to delay the appearance of tolerance to analgesia during repeated morphine dosing in mice, and

the time-lag depended on the formalin dose (Rahman et al., 1994). In hairless rats, the inflammatory nociception elicited by formalin injection (2.5%, 50 μl) was too small to induce the chronic pain stimulus.

The 24-h continuous infusion of dihydroetorphine (1.8 μg/kg/h) and morphine (8 mg/kg/h) induced a significant weight loss precipitated by naloxone (Fig. 5). In this study, the weight loss of the rats was caused by urination, evacuation and diarrhea, which are induced by the withdrawal syndrome in dependent animals (Vaccarino and Couret, 1993). Total doses of dihydroetorphine and morphine infused for 24 h were 43.2 μg/kg and 192 mg/kg, which were 31- and 19-fold of their ED_{50}, respectively. Zhang and Qin (1994) reported that 24-h infused dihydroetorphine and morphine at more than 10-fold of their ED_{50} (0.46 μg/kg and 5 mg/kg, respectively) induced a significant reduction of the analgesic effect and naloxone-precipitated weight loss. Aceto et al. (2000) described that a typical withdrawal syndrome was observed after continuous infusion but not intermittent doses of dihydroetorphine. Tokuyama et al. (1994) clearly demonstrated that naloxone-precipitated withdrawal behaviors were observed following five repeated injections of dihydroetorphine at intervals of less than 2 h. In the case of morphine, the weight loss was correlated with the total amount of infused drug but not with the steady-state concentration of the drug (Nakaki et al., 1981). These reports show that continuous exposure to dihydroetorphine is liable to lead to physical dependence. However, the dorsal application of dihydroetorphine tape (35 μg/0.50 cm^2 for 24 h) did not induce weight loss in this study (Fig. 5). The total amount of dihydroetorphine, which entered through the skin from the tape, was about 40 μg/kg (Ohmori et al., 2000b)—which was the same amount as 1.8 μg/kg/h infused for 24 h. During the topical application of the tape, the plasma dihydroetorphine concentration increased gradually and was maintained in the range of effective concentrations, but it was more variable than that after intravenous infusion (Fig. 2). These results indicate that the topical application of dihydroetorphine by means of the tape did not induce physical dependence. Additionally, the spontaneous withdrawal syndrome and aversive behavior were not found after the dihydroetorphine infusion was stopped and the tape was removed.

The non-inflamed rats conditioned by s.c. injection of dihydroetorphine (2 μg/kg) and morphine (10 mg/kg) exhibited a significant preference for the drug-paired place (Fig. 6). Liu and Zhang (1999a) reported that dihydroetorphine induced a dose-related place preference in rats in the range of 0.0005–5 μg/kg s.c. Differently from physical dependence, the rewarding effect developed even when the drug was administered in intermittent doses. In other words, it is important in the conditioned place preference test that the rats expressing the analgesic effect were always placed in one compartment. There have been few reports regarding place preference conditioned by a route of drug administration other than i.v., i.p., s.c., p.o. and direct injection into the brain (Tzschentke, 1998). It is difficult to condition animals by topical application of dihydroetorphine tape, because the analgesic effect after topical application had a time lag and then continued for a long time (Figs. 3 and 4). For this reason, a 60-min interval before conditioning period was used. After the 60-min conditioning, the tape was immediately removed, and the rats were returned to the cage. The analgesic effect remained for some time after removal of the tape, therefore, the rats might be confused in the conditioning session. However, dihydroetorphine tape (27 μg/0.38 cm^2) elicited significant place preference under these conditioning procedures (Fig. 6). The AUC of plasma dihydroetorphine concentration (0.51 ng · h/ml) during the conditioning period up to 2 h after the abdominal application of the 20 μg/0.28 cm^2 tape was equivalent to that (0.59 ng · h/ml) up to 1 h after 2 μg/kg s.c. injection (Fig. 2). These results indicate that dihydroetorphine tape has a potent rewarding effect, similar to that of an acute injection, and therefore, the dihydroetorphine tape should be used under restricted conditions, as is the case for other narcotic drugs such as morphine and fentanyl.

The significant place preference induced by repeated s.c. injection of dihydroetorphine and morphine and the topical application of dihydroetorphine tape diminished in the formalin-inflamed rats. This result agrees with the report on the reduction of place preference induced by morphine in formalin-inflamed rats (Suzuki et al., 1996). However, it has been reported that the reduction of place preference induced by morphine was attenuated with time after formalin injection, in relation to recovery of the swelling of the formalin-treated paw and the reduction in the formalin-treated paw pressure threshold (Suzuki et al., 1996). In this study, animal screening was carried out 2 days after and conditioning was begun 3 days after formalin injection, because the hairless rats moved little in the shuttlebox for 1 day after the formalin injection. From our results, the inflammatory nociception induced by formalin pretreatment was enough to diminish the rewarding effect of dihydroetorphine. This result strongly indicated that dihydroetorphine is a useful and safe analgesic for the relief of chronic pain. If this drug were to have been clinically used only for pain relief, then the abuse of dihydroetorphine would not have occurred. Although there are some reports regarding a reduction of the tolerance and dependence liability of morphine in formalin-inflamed rats (Vaccarino et al., 1993; Vaccarino and Couret, 1993), this report is the first with regard to the safety of dihydroetorphine when used for pain relief.

Topical application of dihydroetorphine by the tape revealed the potential significance of a lack of dependence and place preference. The plasma dihydroetorphine concentrations during the dorsal and abdominal applications of the tape increased gradually and changed more at steady state, in comparison with those during the acute s.c. injec-

tion and the continuous infusion of dihydroetorphine (Fig. 2). It is suggested that dependence liability is related to the rate of drug exposure. Therefore, it is possible to prevent dependence and place preference by a suitable control of the rate of release of dihydroetorphine from the tape. Furthermore, it has been reported that place preference induced by dihydroetorphine and morphine are suppressed by pretreatment with a dopamine receptor antagonist (Liu and Zhang, 1999b) and by pretreatment with a δ-opioid receptor antagonist or *N*-methyl-D-aspartate receptor antagonist in normal rats and mice. (Suzuki et al., 1999a,b). It is possible to prevent abuse by the concomitant application of dihydroetorphine and dependence-suppressors in this tape formulation.

In conclusion, we fulfilled the three objectives of this study regarding tolerance to analgesia and dependence liability following topical application of dihydroetorphine in hairless rats. First, the analgesic potency and dependence liability of dihydroetorphine were 4600–7200 and 4400–5000 times stronger than those of morphine in hairless rats. Secondly, the topical application of dihydroetorphine tape produced tolerance and a rewarding effect, but not physical dependence in non-inflamed rats. Thirdly, the tolerance and rewarding effects were diminished in formalin-inflamed rats. From these results, dihydroetorphine tape has a potent dependence liability, and therefore it should be used only for the relief of pain under restricted control. Information regarding the safe use of dihydroetorphine and its tape formulation will prevent its abuse and contribute to the cure of severe pain and improve the quality of life of terminal-care patients.

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