Antinociceptive Effect of Dihydroetorphine and Its Tolerance/Dependence Liability in Mice

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The profile of actions of dihydroetorphine (DHE) concerning antinociception, tolerance and dependence was compared with those of morphine in mice. DHE at 1, 5, 10 or 20 μg/kg produced an antinociceptive effect in a dose dependent manner and 10 μg/kg was nearly equipotent to that of 10 mg/kg of morphine. The antinociceptive effect of both drugs was completely suppressed by 1 mg/kg of naloxone, while neither 10 mg/kg of naltrindole nor 1 mg/kg of nor-binaltorphimine had any suppressive effect. Mice tolerant to morphine antinociception were tolerant to DHE and *vice versa*. The naloxone-sensitive, locomotor accelerating activity was progressively enhanced by daily administration of DHE and morphine and a cross reverse tolerance developed between these compounds, suggesting that common mechanisms, especially mediating opioid receptors, underlay the activity enhancement. The development of physical dependence as evidenced by naloxone precipitated withdrawal signs, however, was not observed with daily treatment with DHE, 10, 20 and 100 μg/kg for 6 d. Thus, we demonstrated that DHE produces the antinociceptive effect mediated through μ opioid receptors without causing development of a physical dependence, suggesting that it is safe to use in the clinical therapy of patients suffering severe pain such as that accompanying cancer.

Keywords dihydroetorphine (DHE); morphine; physical dependence; antinociception; tolerance; locomotor activity

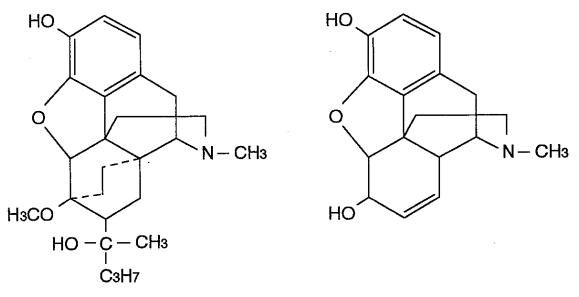
Bentley and Hardy[1] first synthesized dihydroetorphine (DHE) in 1967 and reported the potent antinociceptive effect of this compound. Huang and Qin[2,3] in 1982 used several different test methods and animals and demonstrated that DHE, even in a small dose, produces strong antinociception and causes a relatively minimal physical dependence. Thereafter, they continued studies on DHE employing the pharmacological techniques of binding assay[4] and bioassay,[5] and the drug is now in common clinical use in China as an analgesic.[6] As far as we know, however, there are no reports of DHE use except in China.

The present study provides further evidence to support the unique features of DHE compared with morphine in aspects of antinociception, tolerance and physical dependence.

Materials and Methods

Materials Dihydroetorphine (7,8-dihydro-7α-[1-(*R*)-hydroxy-1-methylbutyl] 6,14-endoethanotetrahydro-oripavine, DHE, a gift from Dr. Qin Bo-Yi, Academy of Military Medical Sciences, China), morphine (Takeda, Osaka), naloxone (Sigma, St. Louis, U.S.A.), naltrindole and nor-binaltorphimine (gifts from Dr. H. Nagase, Toray, Kamakura) were dissolved in saline. They were administered i.p. in a volume of 0.1 ml/10 g of body weight, and doses are expressed in terms of the salts. The chemical structures of DHE and morphine are shown in Fig. 1.

Animals Male mice of the ddY strain weighing 18—20 g (Otsubo Exp. Animals, Nagasaki) were purchased and housed in groups of 20. They were maintained in an ambient temperature (22±1 °C) and relative humidity (55±5%) controlled room with free access to laboratory diet (MF, Oriental Yeast, Tokyo) and tap water. After reaching a weight of 23 to 28 g, they were used for the experiments.



dihydroetorphine (DHE) morphine

Fig. 1. Chemical Structure of Dihydroetorphine (DHE) and Morphine

Assessment of Antinociceptive Effect The antinociceptive effect was measured by the modified Haffner method,[7] which is a tail pinch test (TP), with a cutoff time of 6 s to avoid damage to the tail, done every 15 min after the administration of DHE or morphine for a period of 90 min. Naloxone, naltrindole and nor-binaltorphimine were injected 10 min before the administration of DHE or morphine.

Evaluation of Tolerance and Cross Tolerance DHE 10 μg/kg, or morphine, 10 mg/kg, was injected daily for 5 d. The effect was expressed as area under the curve (*AUC*) by plotting the increase in response time (s) on the ordinate and the time intervals (min) on the abscissa. A significant decrease of *AUC*, compared with that of the 1st day, indicated the development of tolerance. In animals rendered tolerant by 5 daily treatments with one of the drugs, the antinociceptive effect of morphine in DHE tolerant mice, and that of DHE in morphine tolerant animals was estimated on the 6th day to assess the development of cross tolerance.

Measurement of Locomotor Activity Five mice were placed in an apparatus (SCANET animal movement analyzing system, SV-10, Toyo) to measure the locomotor activity: locomotion, rearing, grooming, sniffing and licking. After an adaptation period of 30 min, mice were treated daily with DHE, 10 μg/kg, or morphine, 10 mg/kg, for 5 d. Daily changes in the activity were measured for 90 min following drug administration. One day after the final injection of DHE and morphine, the locomotor activity of DHE in mice treated daily with morphine and that of morphine in DHE-treated animals was estimated to assess the development of cross reverse tolerance.

Evaluation of Physical Dependence Ten, 20 and 100 μg/kg of DHE or 10, 20 and 100 mg/kg of morphine was given daily for 6 d. One hour after the final injection of DHE or morphine, each group was challenged with 1 mg/kg of naloxone, and the precipitated withdrawal signs such as jumping, falling, peeping below, rearing and sniffing were observed for 10 min. The signs were scored as in our previous report with a minor modification.[8]

Influence on Body Weight Gain Following 7 daily treatment of mice with morphine at a dose of 10 mg/kg and DHE at a dose of 10 μg/kg, the changes in body weight gain were measured as an index of toxicity of these compounds.

Statistical Analyses The results were expressed as the mean±S.E. Following analysis of variance for repeated measurements of the overall data to assess statistical significance, differences between the individual mean values in different groups were analyzed by Dunnett's test. For withdrawal scores, significance of the difference was determined by

Student's t-test. A difference was considered significant at $p<0.05$.

Results

Figure 2 shows the antinociceptive effect of DHE, 1, 5, 10 and 20 μg/kg and morphine, 1, 5, 10 and 20 mg/kg. Both compounds produced the effect in a dose dependent manner. The antinociceptive effect of 10 μg/kg of DHE was nearly equipotent to that of 10 mg/kg of morphine, but was somewhat short-lasting and completely disappeared in 90 min, even at a dose of 20 μg/kg.

The antinociceptive effect of 10 μg/kg of DHE was completely suppressed by 1 mg/kg of naloxone as was the effect of 10 mg/kg of morphine. On the contrary, neither 10 mg/kg of naltrindole nor 1 mg/kg of nor-binaltorphimine, doses capable of blocking δ and κ opioid receptors, respectively, affected the antinociceptive effect of DHE or morphine (Fig. 3).

Mice given 10 μg/kg of DHE or 10 mg/kg of morphine injections daily rapidly developed tolerance to the antinociceptive effect (Fig. 4, left). In animals tolerant to morphine, the intensity of DHE antinociception was reduced significantly. Similarly, mice rendered tolerant to DHE were tolerant to morphine antinociception (Fig. 4, right).

As shown in Fig. 5 (left), both DHE, 10 μg/kg, and morphine, 10 mg/kg, produced a significant increase in the locomotor activity compared with the saline control, and this ambulation accelerating effect of DHE was more evident than that of morphine. The hypermotility produced by these compounds was markedly reduced by pretreatment with naloxone, 1 mg/kg.

The repeated administration of DHE and morphine produced a progressive augmentation of the locomotor

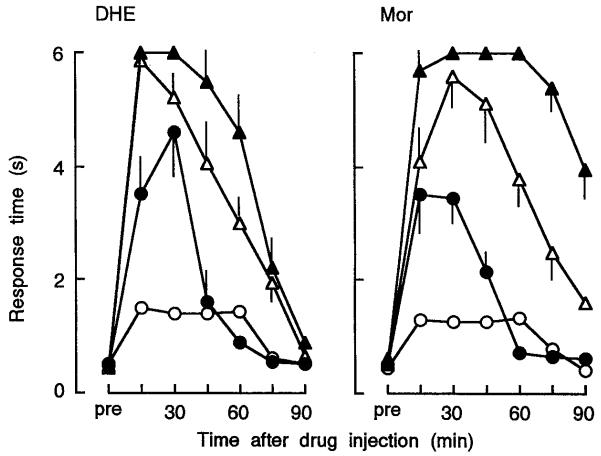


Fig. 2. Antinociceptive Effect of DHE and Morphine

The antinociceptive effect was measured by a modified Haffner method every 15 min after DHE or morphine injection for 90 min. DHE, 1 (○), 5 (●), 10 (△) and 20 (▲) μg/kg, i.p. (left) and morphine, 1 (○), 5 (●), 10 (△) and 20 (▲) mg/kg, i.p. (right). Each point is the mean ± S.E. of the data obtained from 7—14 mice.

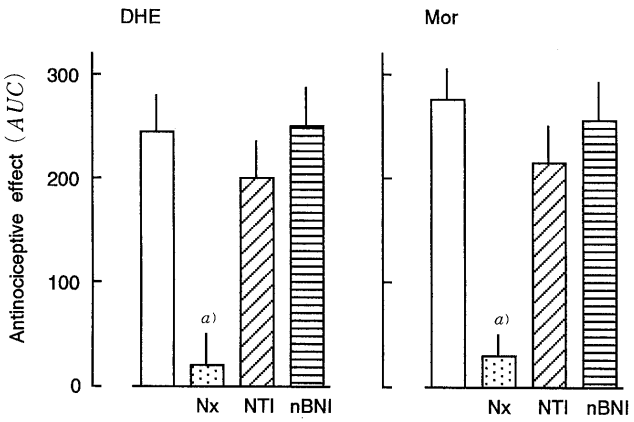


Fig. 3. Effect of Naloxone, Naltrindole or Nor-binaltorphimine on the Antinociceptive Effect of DHE and Morphine

The antinociceptive effect was expressed as the area under the curve (AUC) by plotting the increase in response time (s) on the ordinate and the time intervals on the abscissa. Mice were pretreated with i.p. saline (□), naloxone, 1 mg/kg (Nx, ▨), naltrindole, 10 mg/kg (NTI, ▨) and nor-binaltorphimine, 1 mg/kg (nBNI, ▤) 10 min before DHE, 10 μg/kg, i.p. (left panel) or morphine, 10 mg/kg, i.p. (right panel). Each point is the mean ± S.E. of the data obtained from 14—18 mice. *a)* $p<0.01$, compared with the saline pretreated group (Dunnett's test). For other details, refer to the legend of Fig. 2.

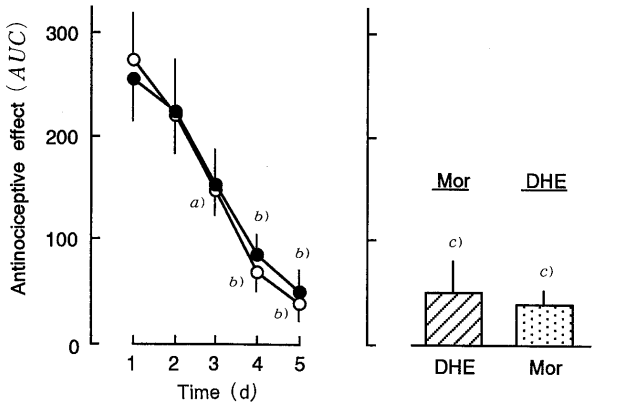


Fig. 4. Development of Tolerance to DHE and Morphine Antinociception and Cross Tolerance between Them

Left: Daily changes in the antinociceptive effect of morphine (○, 10 mg/kg/d, i.p.) and DHE (●, 10 μg/kg/d, i.p.). Each point is the mean ± S.E. of the data obtained from 12—14 mice. *a)* $p<0.05$, *b)* $p<0.01$, compared with the respective value on the 1st day (Dunnett's test). Right: Cross tolerance between morphine and DHE. In the morphine tolerant (Mor) and DHE tolerant (DHE) animals, the antinociceptive effect induced by morphine, 10 mg/kg (Mor, ▨), or DHE, 10 μg (DHE, ▨), was estimated on the 6th day. Values are the mean ± S.E. of the data obtained from 12—14 animals. *c)* $p<0.01$, compared with the respective value on the 1st day (Dunnett's test). For other details, refer to the legend of Fig. 2.

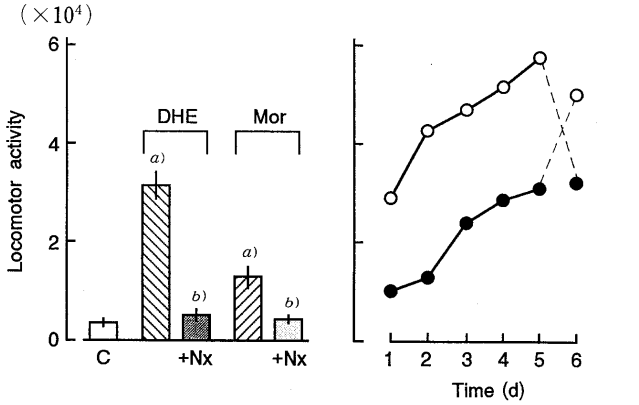


Fig. 5. Naloxone Antagonism of Locomotor Accelerating Effect of DHE and Morphine, Daily Enhancement in the Effect, and Cross Reverse Tolerance between Them

Left: Mice were treated with DHE (DHE, 10 μg/kg) or morphine (Mor, 10 mg/kg) and locomotor accelerating activity was measured for 90 min after drug administration. One mg/kg of naloxone (Nx) was injected 10 min before the administration of test drug. Values are the mean of the data obtained from 3 experiments of 15 animals. *a)* $p<0.01$, compared with the saline treated control (C) group. *b)* $p<0.01$, compared with respective test drug group. Right: Mice were treated daily with DHE (10 μg/kg, ○) or morphine (10 mg/kg, ●) for 5 d. One day after the final injection of DHE and morphine, the locomotor activity of DHE in mice treated daily with morphine (○) and that of morphine in DHE treated animals (●) were measured. Values are the mean of the data obtained from 2 experiments of 10 animals.

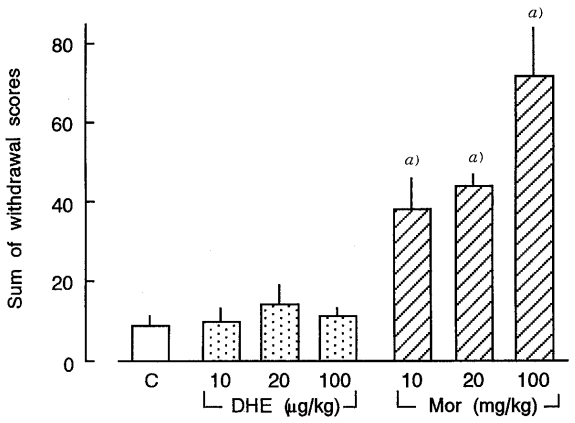


Fig. 6. Development of Dependence on DHE and Morphine

Mice were treated daily with 10, 20 and 100 μg/kg of DHE (DHE) or 10, 20 and 100 mg/kg of morphine (Mor) for 6 d. One hour after the final injection, each group was challenged with 1 mg/kg of naloxone, and the precipitated withdrawal signs were checked for 10 min. Values are the mean ± S.E. of the data obtained from 12—14 animals. *a)* $p<0.01$, compared with the saline treated control (C) group (Student's *t*-test).

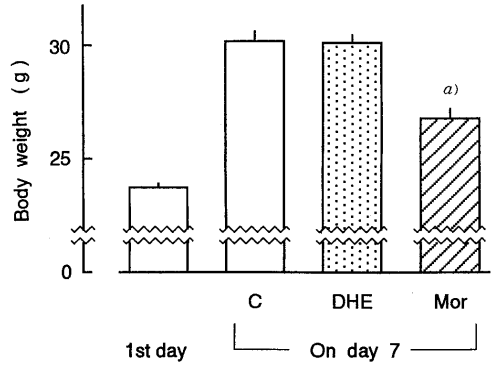


Fig. 7. Body Weight Gain in Mice Treated Daily with DHE and Morphine

Body weight of mice before (1st day) and 7 d after daily treatment with DHE (DHE, 10 μg/kg) and morphine (Mor, 10 mg/kg). Values are the mean ± S.E. of the data obtained from 12—14 animals. *a)* $p<0.05$, compared with the saline treated control (C) group (Dunnett's test).

accelerating effect throughout 5 d, indicative of the development of reverse tolerance, an increase in sensitivity to the effect of DHE and morphine. On the 6th day, the DHE-experienced mice exhibited higher locomotor accelerating activity in response to morphine than did naive animals, as shown after the 1st day of administration of morphine, and likewise, the repeated administration of morphine elicited a significant increase in the sensitivity to DHE when that had been primarily given (Fig. 5, right).

The degree of physical dependence in all groups treated with DHE, 10, 20 and 100 μg/kg, was nearly equal to that of the control group. In contrast, the morphine, 10, 20 and 100 mg/kg, daily-treated group demonstrated higher withdrawal scores than the control group (Fig. 6).

The body weight of the saline control group increased significantly after a 7-d treatment. Compared to the control mice, the weight gain was significantly delayed in the group treated daily with morphine, while the repeated administration of DHE did not affect body weight gain (Fig. 7).

Discussion

We have thus confirmed Huang and Qin's report[2,3] that DHE has a potent antinociceptive effect without developing physical dependence. The effect at a dose of 10 μg/kg was nearly equipotent to that of 10 mg/kg of morphine, indicating that DHE was about 1000 times more potent than morphine by the TP method.

The results that naloxone, a μ opioid receptor antagonist, but not naltrindole, a selective δ opioid receptor antagonist,[9] or nor-binaltorphimine, a selective κ opioid receptor antagonist,[10] significantly antagonized DHE- or morphine-induced antinociception suggest that the μ opioid receptor mechanism is primarily involved in the production of the antinociceptive effect of morphine and DHE. In support of this hypothesis, Wang *et al.*[4] demonstrated that DHE has high affinity for μ receptors in rat brain homogenates.

Daily injection of both DHE and morphine easily caused the development of tolerance to its antinociception. Meanwhile, mice tolerant to morphine also developed tolerance to DHE, and *vice versa*. The formation of two-way cross-tolerance has also suggested that DHE and morphine produce antinociception which is mediated through the μ opioid receptor mechanism.

Likewise, in the course of the measurement of the antinociceptive effect of DHE and morphine, we found that both compounds stimulated the locomotor activity, and that such hypermotility was invalidated by naloxone. This suggests that opioid receptor mechanisms participate in the production of their locomotor accelerating effect. Despite the difference in the enhancement of this effect by DHE and morphine, the formation of cross reverse tolerance between the two compounds indicates that common mechanisms underlie the augmentation of the locomotor accelerating activity they elicit.

Using doses of DHE in which the potency of antinociceptive effect corresponds to those of morphine, the development of physical dependence was assessed by observing the naloxone precipitated withdrawal signs. In spite of the appearance of signs such as falling, peeping below, rearing and sniffing in mice treated with morphine, the withdrawal signs in the DHE-treated mice were seen to the same extent as in control animals treated with saline alone. In this experiment, then, we have demonstrated that DHE is incapable of causing development of physical dependence as stated in Huang and Qin's report.[3] Although DHE produces an antinociceptive effect mediated through μ opioid receptors, the reason for the lack of development of physical dependence remains unclear.

In addition to the beneficial characters of DHE, as a nonphysical dependence-liable analgesic drug, its low toxicity as evidenced by the minimal effect on body weight gain suggests that the substance may be useful in clinical therapy for patients suffering severe pain such as that caused by cancer.

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