Physical Dependence Produced by Dihydroetorphine in Mice

Shogo TOKUYAMA, Fumihiro NAKAMURA, Masakatsu TAKAHASHI, and Hiroshi KANETO*

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852, Japan.
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Using various administration schedules, the physical dependence produced by dihydroetorphine (DHE) was compared with that of morphine in mice. Physical dependence, evaluated by naloxone-precipitated withdrawal signs, did not develop following daily treatment with DHE (10, 20, 100 and 1000 μg/kg, i.p. or 30, 100 and 1000 ng/mouse, i.c.v.) for 6 d. However, 5 repeated injections of DHE (10 μg/kg, i.p.) at 1 or 2 h intervals did produce physical dependence and the dependent state disappeared after 2 h. Accordingly, it was demonstrated that a sufficient degree of antinociceptive activity needed to be maintained, longer than several hours, for the development of physical dependence on DHE and that the duration of the dependent state was very short. In the single dose suppression test, a single dose of DHE completely suppressed the natural withdrawal signs that appeared following abstinence in morphine-dependent animals without reappearance of significant withdrawal signs, indicating the suitability of DHE as a substitute for morphine. The characteristic properties of DHE, the extremely potent antinociceptive effect and minimal dependence, indicate the separation of the antinociceptive effect from dependence, and suggest that it may be possible to develop a novel drug which may be safely used in clinical situations.

Keywords dihydroetorphine; morphine; naloxone; physical dependence; withdrawal sign

Hung and Qin[1,2] have reported that, in spite of its potent antinociceptive effect, dihydroetorphine (DHE) causes minimal physical dependence. Furthermore , in an *in vitro* binding assay Wang *et al.*[3] have demonstrated that DHE possesses high affinity for μ opioid receptors. We have confirmed that DHE has a potent antinociceptive effect without producing physical dependence at the antinociceptive dose equipotent with morphine (Mor) and shown that the effect is mediated through μ opioid receptors.[4] However, these observations raise a serious question in terms of the mechanism of action of opioids since μ opioid receptor-mediated mechanisms play an essential role in the production of the potent antinociceptive effect, as well as the physical dependence, of Mor.[5,6]

The aim of the present study is to ascertain the degree of dependence produced by DHE in comparison with that of Mor.

MATERIALS AND METHODS

Materials DHE, (7,8-dihydro-7α-[1(*R*)-hydroxy-1-methylbutyl]-6,14-endoethanotetrahydro-oripavine, a gift from Dr. Qin Bo-Yi, Academy of Military Sciences, China), Mor-HCl (Mor, Takeda Pharm. Co., Osaka) and naloxone–HCl (Sigma, St. Louis) were dissolved in saline. They were administered in a volume of 0.1 ml/10 g body weight by i.p. injection, and in a volume of 10 ml/mouse by intracerebroventricular (i.c.v.) injection. The i.c.v. injection was carried out according to the method described by Haley and McCormick.[7]

Animals Male ddY mice, weighing 18 to 20 g (Otsubo Exp. Animals, Nagasaki) were housed in groups of 20 animals to a cage. They were kept in a room maintained at 22±1 °C and were given a standard laboratory diet (MF, Oriental Yeast, Tokyo) and tap water *ad libitum*. On reaching 23 to 28 g, they were used for the experiments.

Evaluation of Antinociceptive Effect The antinociceptive effect was measured by the modified Haffner method,[8] with a cut-off time of 6 s to avoid damage to the tail, at 5, 10 and 15 min and then at intervals of 15 min for the following 45 min. The effect was expressed as the area under the curve (*AUC*), which was obtained by plotting the increase in response time (second) on the ordinate and the time interval (min) on the abscissa. Then the 50% AUC_{max}, half the area of the theoretical maximum *AUC* (AUC_{max}) of the maximum response time (6 s) *versus* the time after injection (60 min), was calculated and compared.

Schedule of Drug Administration 1) Daily Injection: Ten, 20, 100 and 1000 μg/kg DHE or 10, 20 and 100 mg/kg Mor were given i.p. for 6 d. In the case of i.c.v. injection, 30, 100, 1000 ng/mouse DHE or 3 μg/mouse Mor were used.

2) Hourly Injection: Repeated i.p. injections of 10 μg/kg DHE or 10 mg/kg Mor was made at 1 h intervals.

3) Repeated Injection at Various Intervals: i.p. injection of 10 μg/kg DHE or 10 mg/kg Mor was repeated 5 times at 1, 2, 3 and 6 h intervals.

4) Injection of Daily Increasing Doses: Mice were treated twice a day (around 9 a.m. and 6 p.m.) with daily increasing doses of i.p. Mor (10, 20, 40, 60, 80 and 100 mg/kg, i.p.) for 6 d and with a maintenance dose (100 mg/kg) for the following 3 d; then, on the 10th day, around 7 a.m., the final maintenance dose of Mor was given. The control group was given saline instead of Mor.[9]

Evaluation of Physical Dependence One hour after the final injection of DHE or Mor, each group was challenged with 1 mg/kg i.p. naloxone. According to the method of Kaneto *et al.*,[10] with a minor modification in the scoring system, withdrawal signs, such as jumping, falling, backward locomotion (score 3), peeping below, rearing (score 2), sniffing, urination and defecation (score 1), were observed for a 10 min period immediately after the naloxone injection.

Single Dose Suppression Test After confirming the appearance of natural withdrawal signs in the animals made dependent by twice daily administrations of pro-

gressively increasing doses of Mor, 50 μg/kg DHE or 50 mg/kg Mor was given i.p. and the suppressive effect on the natural withdrawal signs was observed. Details of the test procedure have been described elsewhere.[9]

Statistical Analysis The results were expressed as the means ±S.E. Following analysis of variance for repeated measurements using the overall data to assess statistical significance, differences between individual mean values in various groups were analyzed by Dunnett's test. A difference was considered significant at $p<0.05$.

RESULTS

Antinociceptive Effect by i.c.v. Injection The i.c.v. injection of both DHE (10, 30 and 100 ng/mouse) and Mor (1, 3 and 10 μg/mouse) produced an antinociceptive effect in a dose-dependent manner. DHE, at 30 ng/mouse, produced maximum antinociception 10 min after injection, but the effect disappeared within 30 to 45 min. On the other hand, Mor at 3 μg/mouse, produced a nearly maximum effect 10 min after injection and maintained this level over 60 min (Fig. 1).

Development of Physical Dependence 1) Daily Injection: In the animals treated daily with i.p. Mor for 6 d, naloxone precipitated various withdrawal signs indicating the development of physical dependence and the intensity of the withdrawal scores was dependent on the dose of Mor. Daily i.c.v. injection of Mor at a dose of 3 μg/mouse also produced severe physical dependence. In contrast, i.p. and i.c.v. administration of DHE, at an antinociceptive dose equipotent with that of Mor, produced no withdrawal symptoms precipitated by naloxone, although in the animals given i.p. DHE, 1000 μg/kg/d, for 6 d, a slight, but statistically insignificant increase in withdrawal signs was precipitated by naloxone (Fig. 2).

2) Hourly Injection: In parallel with the number of hourly injections, Mor produced severe physical dependence; however, in the case of DHE at least 5 injections are required for the development of a significant degree of physical dependence (Fig. 3).

3) Repeated Injection at Various Intervals: After 5 repeated injections of 10 μg/kg DHE at 1 or 2 h intervals,

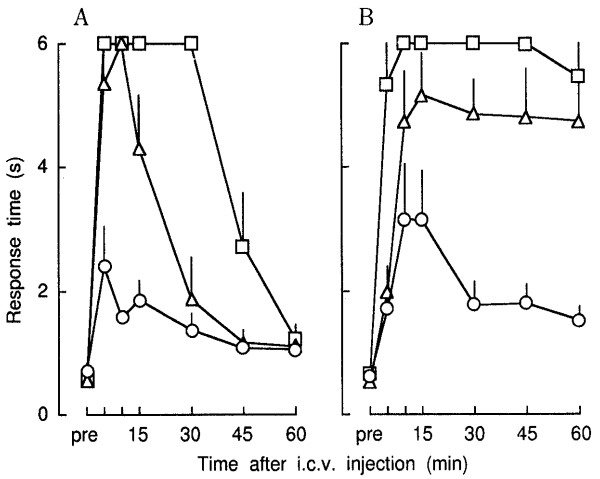

Fig. 1. Antinociceptive Effect Following i.c.v. Injection

The antinociceptive effect was measured by a modified Haffner method at 5, 10 and every 15 min after i.c.v. injection of DHE (A) or Mor (B) for 60 min. A: DHE; 10 (○), 30 (△) and 100 (□) ng/mouse (left). B: Mor; 1 (○), 3 (△) and 10 (□) μg/mouse (right). Each point is the mean ±S.E. of data obtained from 7—14 mice.

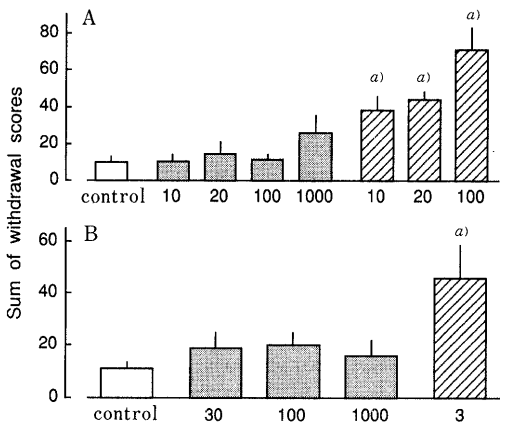

Fig. 2. Development of Physical Dependence Following Daily Injections

Mice were treated daily with (A) 10, 20, 100 and 1000 μg/kg i.p. DHE (▩) or 10, 20, 100 mg/kg of i.p. Mor (▨) and with (B) 30, 100, 1000 ng/mouse i.c.v. DHE (▩) or 3 μg/mouse i.c.v. Mor (▨) for 6 d. Values are the means ±S.E. of data obtained from 12—14 mice. Significantly different from control, *a*) $p<0.01$.

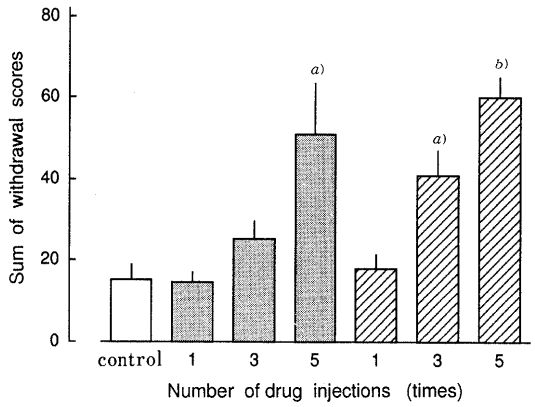

Fig. 3. Development of Physical Dependence Following Hourly Injections

Mice were treated hourly 1, 3 or 5 times with 10 μg/kg DHE i.p. (▩) or 10 mg/kg Mor i.p. (▨). Values are the means ±S.E. of data obtained from 12—14 mice. Significantly different from control, *a*) $p<0.05$, *b*) $p<0.01$.

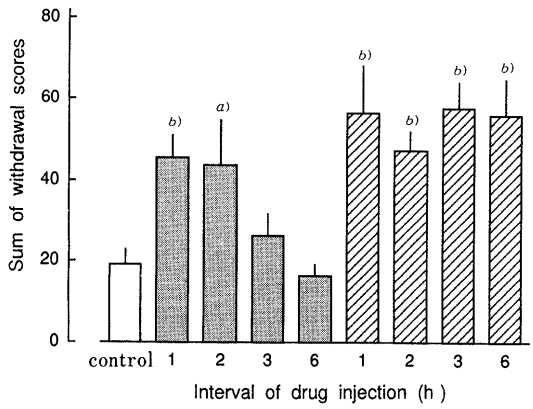

Fig. 4. Development of Physical Dependence Following Repeated Injections at Various Intervals

Mice were treated 5 times with 10 μg/kg of i.p. DHE (▩) or 10 mg/kg of i.p. Mor (▨) at intervals of 1, 2, 3 and 6 h. Values are the means ±S.E. of the data obtained from 12—14 mice. Significantly different from control, *a*) $p<0.05$, *b*) $p<0.01$.

naloxone precipitated withdrawal signs; however, when the interval between the repeated injections of DHE was extended to 3 h or more, naloxone failed to precipitate withdrawal signs indicating the abolition of the dependent state after 2 to 3 h. Meanwhile, in the case of Mor, regardless of the interval between the repeated injections, 1, 2, 3 or 6 h, marked withdrawal signs were precipitated by naloxone (Fig. 4).

4) Abolition of the Dependent State: To confirm the rapid abolition of the DHE-dependent state, naloxone was given 1, 2 or 3 h after the final dose of 5 hourly injections of 10 μg/kg DHE. Naloxone precipitated withdrawal signs when given 1 h after the final dose of DHE, but when the interval between the final DHE dose and naloxone was extended to 2 h or more no withdrawal signs were precipitated. On the other hand, the naloxone challenge, 1, 2, or 3 h after the final dose of Mor, precipitated significant withdrawal signs of similar sevirity (Fig. 5).

Single Dose Suppression Test In the animals treated with daily increasing doses of Mor, signs starting with restlessness, urination and defecation, then various withdrawal signs, such as peeping below, backward locomotion, rearing and sniffing, began to emerge around 3 h after the final injection and reaching maximum severity around 5 to 6 h. Administration of 50 μg/kg DHE or 50 mg/kg Mor completely suppressed these withdrawal signs. All the signs reappeared with maximum intensity after 3 or 4 h in the Mor-treated group; however, the withdrawal scores in the DHE-treated group tended to increase but only temporally and never exceeded the level of the natural withdrawal scores after Mor abstinence (Fig. 6).

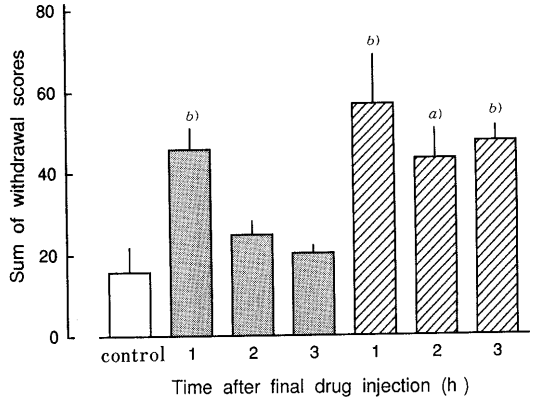

Fig. 5. Abolition of the Dependent State

Mice were treated 5 times with 10 μg/kg DHE i.p. (▩) or 10 mg/kg Mor i.p. at 1 h interval (▨). Naloxone was given 1, 2 or 3 h after the final DHE or Mor injection. Values are the means ± S.E. of data obtained from 7—8 mice. Significantly different from control, *a)* $p<0.05$, *b)* $p<0.01$.

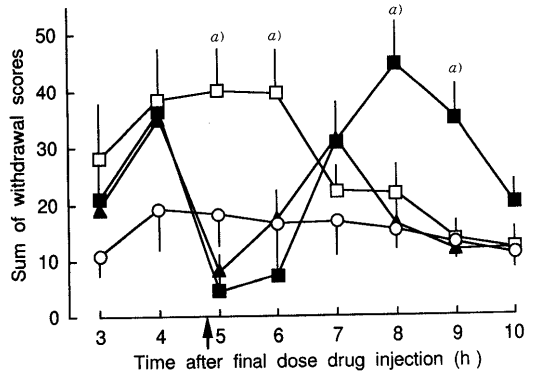

Fig. 6. Single-Dose Suppression Test

Mice were treated twice daily with daily increasing doses of Mor 10, 20, 40, 60, 80 and 100 mg/kg (▲, □, ■) for 6 d and with a maintenance dose (100 mg/kg) of i.p. Mor for a further 3 d and on the 10th day, in the morning, the animal was given the final maintenance dose of Mor. The control group (○) was treated with saline instead of Mor. Observation of natural withdrawal signs was performed from 3 to 10 h after the final injection. At the time indicated by the arrow, saline (□), 50 μg/kg DHE (▲), or 50 mg/kg Mor (■) was administered. Values are the means ± S.E. of data obtained from 8—10 mice. Significantly different from control, *a)* $p<0.05$.

DISCUSSION

We have previously reported that the efficacy ratio of the antinociceptive effect of DHE compared with Mor is approximately 1000 : 1 following i.p. administration.[4] Likewise, administration of DHE and Mor by other parenteral routes produced antinociception in a dose-dependent manner, and Mor doses of 8.2 mg/kg, s.c. and 6.7 mg/kg, i.v. and DHE 5.8 μg/kg, s.c. and 5.3 μg/kg, i.v. were equipotent; however, the efficacy ratios of these compounds were 1420 : 1, s.c. and 1250 : 1, i.v. (data not shown). In the present study, following direct i.c.v. application, the effect of DHE was found to be more potent than that of Mor, but the efficacy ratio of DHE to Mor was approximately 20 : 1. Furthermore, the duration of the antinociceptive effect of DHE was shorter than that of Mor. These results may be attributable to the greater lipophilicity of DHE, being able to cross the blood-brain barrier more readily and also to its rapid elimination from the central nervous system.

The antinociceptive effect of DHE was suppressed by naloxone, a μ opioid antagonist, but not by naltrindole, a δ opioid antagonist or nor-binaltorphimine, a κ opioid antagonist, suggesting that the effect is mediated through μ opioid receptors.[4] It is widely accepted that the mechanisms mediated by μ opioid receptors play an essential role in the production of potent antinociceptive effects and physical dependence.[5,6] In fact, daily treatment with DHE at a very high dose, 1000 μg/kg for 5 d, tended to produce physical dependence and even at a low dose, 10 μg/kg, when the antinociceptive effect was maintained for longer than 5 h by hourly injections, withdrawal signs were precipitated by naloxone. Furthermore, the physical dependence produced by acute repeated injections of DHE disappeared within 2 h. These results suggest that DHE pruduces physical dependence mediated by μ receptors; however, the production of physical dependence requires a sustained antinociceptive effect and disappears rapidly after withdrawal.

Methadone is used as a substitute for Mor and heroin in the clinical treatment of opioid addicts.[11] Natural withdrawal signs appearing in Mor-dependent animals are completely suppressed by DHE and no relapse in withdrawal signs is observed. These results suggest the possibility that DHE might be useful in the treatment of opioid abusers and, indeed, DHE has been tested in heroin addicts and its effect in alleviating the abstinence syndrome has been reported.[11]

Tolerance and physical dependence are well known as the inevitable consequences of repeated injection of pharmacologically-effective doses of opioids. However, the characteristic properties of DHE, its exceedingly potent antinociceptive effect associated with minimal dependence, indicate the possibility of separating the antinociceptive effect from the physical dependence, as suggested by Kaneto *et al.*[12,13] This suggests that it might be possible to develop a novel drug which could be safely used in the treatment of the patients suffering from severe pain.

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