

## Antinociceptive Effect of Dihydroetorphine Following Various Routes of Administration: a Comparative Study with Morphine

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Using various routes of administration, the antinociceptive effects of dihydroetorphine (DHE) and morphine were measured in mice. Regardless of the route of systemic and local administration, DHE (1—20  $\mu\text{g}/\text{kg}$ , i.p.; 1—10  $\mu\text{g}/\text{kg}$ , s.c.; 1—10  $\mu\text{g}/\text{kg}$ , i.v.; 10—1000  $\mu\text{g}/\text{kg}$ , p.o.; 10—100 ng/mouse, intracerebroventricularly (i.c.v.); and 10—100 ng/mouse, intrathecally (i.t.)) and morphine (1—20 mg/kg, i.p.; 1—10 mg/kg, s.c.; 1—10 mg/kg, i.v.; 10—100 mg/kg, p.o.; 1—10  $\mu\text{g}/\text{mouse}$ , i.c.v.; and 0.5—3  $\mu\text{g}/\text{mouse}$ , i.t.) produced an antinociceptive effect in a dose-dependent manner, as evaluated by the tail pinch method. However, the duration of the antinociceptive effect of DHE was shorter than that of morphine. The efficacy ratio of the antinociceptive effect between DHE and morphine was approximately 1000 to 1500: 1 by parenteral administration (i.p., s.c., or i.v.) and about 100:1 by the oral route. Meanwhile, using direct application into the central nervous system (CNS) (i.c.v. or i.t.), the effect of DHE was only 10 to 20 times that of morphine. These data suggest that DHE has an ideal quality as an analgesic by systemic administration which is a more convenient application than local injection, since only a minimum dose of DHE is needed to induce suitable potency of antinociception, and the duration of the effect is short. Further, these unique characteristics of DHE might lead to the prevention of the development of dependence by avoiding accumulation of the drug in the CNS.

**Key words** dihydroetorphine (DHE); morphine; antinociception

The development of tolerance to and dependence on opioids through repeated use is a characteristic of all the opioids represented by morphine, a  $\mu$ -opioid receptor agonist. The possibility of developing drug dependence is one of the major limitations of their clinical use. Although efforts have been made to develop new drugs possessing a strong antinociceptive effect but lacking dependence liability, the purpose has not yet been achieved. Bently and Hardy<sup>1)</sup> have synthesized dihydroetorphine (DHE) and have reported its extraordinarily potent antinociceptive effect, at least 1000 times more potent than morphine. Unfortunately, further studies have been prevented because of the traditional concept of its narcotic nature: that dependence liability always corresponds to antinociceptive potency. Hung and Qin have reexamined the pharmacological profile of DHE<sup>2)</sup> and have found that DHE causes minimal physical dependence<sup>3)</sup> in spite of its potent antinociceptive effect. Furthermore, it has been demonstrated that DHE possesses a high affinity for  $\mu$ -opioid receptors in an *in vitro* binding assay.<sup>4)</sup> We also have confirmed the findings that DHE has a potent antinociceptive effect which is mediated through  $\mu$ -opioid receptors with a minimum physical dependence liability at an equipotent antinociceptive dose compared to morphine.<sup>5,6)</sup> In this study, to further clarify the pharmacological characteristics of DHE, the antinociceptive effect of this opioid induced by various routes of administration was examined and compared with that of morphine.

Male ddY mice, weighing 18 to 20 g (Otsubo Exp. Animals, Nagasaki) were purchased and were kept in a room maintained at  $21 \pm 2^\circ\text{C}$  under a natural day/night regime with free access to a standard laboratory diet (MF; Oriental Yeast, Tokyo) and tap water. After reaching 23 to 25 g, they were used for the experiments. Dihydroetorphine (7,8-dihydro-7 $\alpha$ -[1-(R)-hydroxy-1-methylbutyl]-6,14-endoethanotetrahydro-orphavine, DHE, M.W.

450.02, a gift from Dr. Qin Bo-Yi, Academy of Military Sciences, China) and morphine (Takeda Pharm. Co., Osaka) were dissolved in saline or distilled water. They were administered in a volume of 0.1 ml/10 g of body weight for intraperitoneal (i.p.), subcutaneous (s.c.), intravenous (i.v.), and oral (p.o.) administration and in 10  $\mu\text{l}$  for intracerebroventricular (i.c.v.) or intrathecal (i.t.) injection. The i.c.v. injection was carried out according to the method of Haley and McCormick<sup>7)</sup> and the i.t. injection by the method of Hylden and Wilcox.<sup>8)</sup> The antinociceptive effect was evaluated by a modified Haffner method<sup>9)</sup> (a cutoff time of 6 s) for 90 min after i.p., s.c., and p.o. administrations and for 60 min after i.v., i.c.v., and i.t. injections. The effect was expressed by plotting the response time (s) on the ordinate and the time interval (min) on the abscissa. The antinociceptive effects of DHE and morphine were compared as follows: Area B [area under the curve (AUC) of test drug] enclosed by the response time curve (ordinate) and the time after injection (abscissa) and area A ( $AUC_{\text{max}}$ ) enclosed by the maximum response time (6 s) and the time after injection (60 or

Table 1. Comparison of Antinociceptive Effect of Dihydroetorphine and Morphine

	DHE	Mor	Mor/DHE
i.p.	8.2 $\mu\text{g}/\text{kg}$	8.9 mg/kg	1092
s.c.	5.8	8.2	1421
i.v.	5.3	6.7	1250
p.o.	818.0	78.1	96
i.c.v.	68.5 ng/mouse	1.2 $\mu\text{g}/\text{mouse}$	18
i.t.	87.3	0.9	10

Figures indicate the 50%  $AUC_{\text{max}}$  ( $= AUC_{\text{max}}/2$ ), which was obtained by plotting the  $B/A \times 100$  (%) where B is the area under the curve of test drug enclosed by the response time curve (ordinate) and the time after injection (abscissa) and A is the  $AUC_{\text{max}}$  enclosed by the maximum response time (6 s) and the time after injection. Mor/DHE is calculated by the comparison of the  $AUC_{\text{max}}/2$ .

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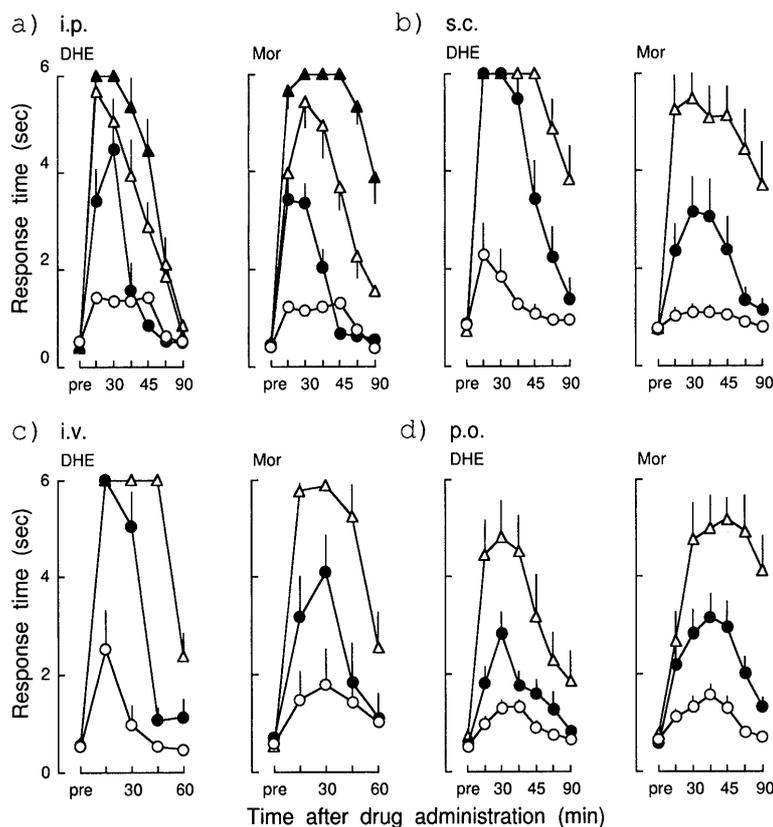


Fig. 1. Antinociceptive Effect of Dihydroetorphine and Morphine by Systemic Route Administration

The antinociceptive effect was measured by a modified Haffner method every 15 min for 90 min after i.p., s.c., and *p.o.* administration and for 60 min after i.v. injection of DHE or morphine at doses in the order of  $\circ$ ,  $\bullet$ ,  $\triangle$ ,  $\blacktriangle$ . a) i.p.: DHE 1, 5, 10, 20  $\mu\text{g}/\text{kg}$  and Mor 1, 5, 10, 20 mg/kg, b) s.c.: DHE 1, 5, 10  $\mu\text{g}/\text{kg}$  and Mor 1, 5, 10 mg/kg, c) i.v.: DHE 1, 3, 10  $\mu\text{g}/\text{kg}$  and Mor 1, 3, 10 mg/kg, d) *p.o.*: DHE 10, 30, 1000  $\mu\text{g}/\text{kg}$  and Mor 10, 30, 100 mg/kg. Each point is the mean  $\pm$  S.E.M. of the data obtained from 7–8 mice. Data from i.p. injection was shown in our previous report (reference 5).

90 min) were calculated. By plotting the  $B/A \times 100$  (%) on the ordinate and the dose of the drug on the abscissa, the 50%  $AUC_{\text{max}}$  ( $=AUC_{\text{max}}/2$ ) was obtained (Table 1).

Figure 1 shows that regardless of the route of systemic administration, DHE at the dose of 1, 5, 10, and 20  $\mu\text{g}/\text{kg}$ , i.p.; 1, 5, and 10  $\mu\text{g}/\text{kg}$ , s.c.; 1, 3, and 10  $\mu\text{g}/\text{kg}$ , i.v.; 10, 30, and 1000  $\mu\text{g}/\text{kg}$ , *p.o.* and morphine at the dose of 1, 5, 10, and 20 mg/kg, i.p.; 1, 5, and 10 mg/kg, s.c.; 1, 3, and 10 mg/kg, i.v.; 10, 30, and 100 mg/kg, *p.o.* produced antinociception in a dose-dependent manner. Furthermore, the potency of DHE was 1000 to 1500 times stronger than that of morphine by i.p., s.c., and i.v. routes and nearly 100 times stronger by oral administration (Table 1).

The local injection of DHE at doses of 10, 30, and 100 ng/mouse, i.c.v.; 10, 30, and 100 ng/mouse, i.t., and morphine at doses of 1, 3, and 10  $\mu\text{g}/\text{mouse}$ , i.c.v.; 0.5, 1, and 3  $\mu\text{g}/\text{mouse}$ , i.t., also induced an antinociceptive effect in a dose-dependent manner. Although the effect of DHE disappeared within 30 to 45 min, the antinociception induced by morphine continued over 60 min (Fig. 2). In this case, the efficacy of DHE by direct application into the central nervous system (CNS), that is, i.c.v. and i.t. injection, was only 10 to 20 times more potent than that of morphine (Table 1).

In accordance with previous reports, i.p.<sup>2,5)</sup> and i.c.v.<sup>6)</sup> injections of DHE produced a potent antinociceptive effect. In addition, in the present study, we found that other systemic and local administrations, s.c., i.v., *p.o.*,

and i.t., of DHE also induced antinociception in a dose-dependent manner. However, the attribute produced by each administration appears to be somewhat different. The efficacy ratio of the antinociceptive effect between DHE and morphine was approximately 1000 to 1500:1 by parenteral administration (i.p., s.c., or i.v.) and about 100:1 by the oral route, suggesting that DHE is rapidly degraded in the gastrointestinal tract and/or is influenced easily by first-pass metabolism in the liver compared to morphine, although it is easy for DHE to be absorbed by the gastrointestinal tract. On the other hand, by direct application into the CNS (i.c.v. or i.t.), the effect of DHE was about 10 to 20 times that of morphine, and the duration of the antinociceptive effect of DHE was shorter than that of morphine. These results suggest that DHE is able to cross the blood brain barrier and/or to be eliminated from the CNS more easily than morphine because of its lipophilicity, which seems to be closely involved with penetration into the brain. Furthermore, Weinberg *et al.*<sup>10)</sup> have shown that increased lipid solubility maximizes concentration-independent drug absorption through buccal mucosa. Interestingly, in our preliminary data, DHE also induced antinociception even through transdermal application, indicating the great lipid solubility of DHE.

DHE has been shown to possess a selective affinity for  $\mu$ -opioid receptors.<sup>4)</sup> We have also reported that the antinociceptive effect of DHE is suppressed by naloxone (a  $\mu$ -opioid receptor antagonist), but not naltrindole (a

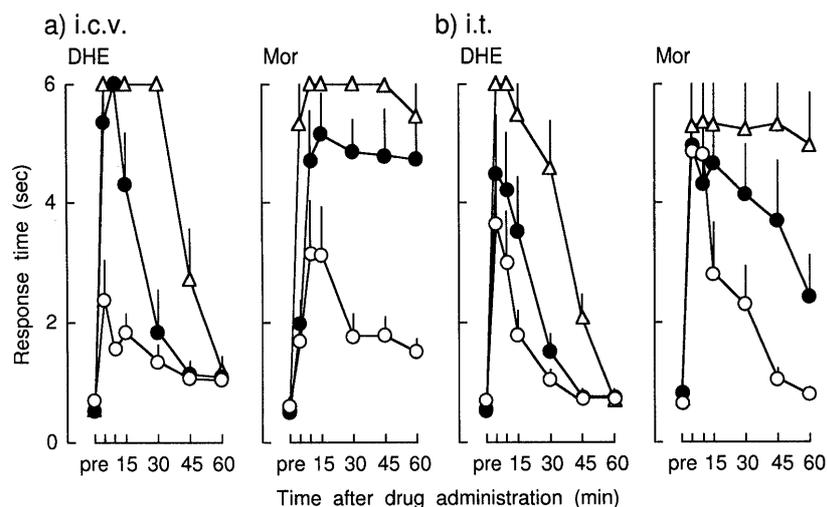


Fig. 2. Antinociceptive Effect of Dihydroetorphine and Morphine by Direct CNS Administration

The antinociceptive effect was measured by a modified Haffner method every 15 min for 60 min after i.c.v. and i.t. injection of DHE or morphine at doses in the order of ○, ●, △. a) i.c.v.: DHE 10, 30, 100 ng/mouse and Mor 1, 3, 10  $\mu$ g/mouse, b) i.t.: DHE 10, 30, 100 ng/mouse and Mor 0.5, 1, 3  $\mu$ g/mouse. Each point is the mean  $\pm$  S.E.M. of the data obtained from 7–8 mice. Data from i.c.v. injection was shown in our previous report (reference 6).

$\delta$ -opioid receptor antagonist) or nor-binaltorphimine (a  $\kappa$ -opioid receptor antagonist), suggesting that the effect is mediated through  $\mu$ -opioid receptors.<sup>5</sup> It is widely believed that the mechanisms mediated through  $\mu$ -opioid receptors play an essential role in the production of a potent antinociceptive effect and physical dependence.<sup>11,12</sup> Nevertheless, there are some reports that DHE has an extremely potent antinociceptive effect with minimal physical dependence liability; that is, at the dose required to produce a sufficient degree of antinociception, the development of physical dependence on DHE could not be detected.<sup>2,3,5</sup> These findings, together with the present study, suggest that the lack of dependence liability of DHE might be due to its short-lasting CNS action, which lessens the accumulation of the drug. In fact, five repeated injections of DHE at 1 or 2 h (but no greater) intervals, the concentration of which in the CNS seems to be constant under this condition, have been demonstrated to produce physical dependence on DHE, as evaluated by naloxone precipitated withdrawal signs.<sup>6</sup>

In conclusion, the data obtained from this study suggest that DHE has an ideal quality as an analgesic by systemic administration which is a more convenient application than local injection, since only a minimum dose of DHE is needed to induce suitable potency of antinociception, and the duration of the effect is short. Furthermore, characteristic properties of DHE, an extremely potent antinociceptive effect but of minimal dependence liability

due to its short-lasting CNS action, might lead to the development of a novel compound safely used clinically in the treatment of patients suffering from severe pain.

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