A Potent Mu-Opioid Receptor Agonist, Dihydroetorphine, Fails to Produce the Conditioned Place Preference in Mice

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Received April 30, 1996 Accepted June 15, 1996

ABSTRACT—Reinforcing effects of dihydroetorphine (DHE) and morphine were evaluated by the conditioned place preference paradigm. Both DHE and morphine produced an antinociceptive effect in a dose-dependent manner. On the other hand, DHE (0.1, 1, 3 and 10 µg/kg, i.p.) failed to induce the conditioned preference, while morphine (0.1, 1, 3 and 10 mg/kg, i.p.) caused a dose-dependent preference for the drug-paired place. Thus, these characteristic properties of DHE make it attractive for development as a novel potent analgesic compound that has less dependence liability.

Keywords: Dihydroetorphine, Morphine, Conditioned place preference

Dihydroetorphine (DHE) has been reported to possess an extraordinarily potent antinociceptive effect at least 1,000 times more potent than morphine, a µ-opioid receptor agonist, without the development of physical dependence (1, 2). Furthermore, we have also shown that DHE has a potent antinociceptive effect that is mediated through µ-opioid receptors with a minimum physical dependence liability at the equipotent antinociceptive dose compared to morphine (3, 4). However, it is still not clear whether DHE has a reinforcing effect or not. The conditioned place preference paradigm reliably establishes a measurement of the reinforcing properties of drugs and is particularly useful for evaluating the reinforcing effects of narcotics and psychostimulants (5). A variety of µ-opioid receptor agonists, such as morphine, fentanyl and etorphine, have been shown to produce a place preference in rats (6, 7). It has been believed that this property of narcotics is one of the critical factors leading to the abuse of and dependence on these drugs. Accordingly, in this study, the reinforcing effect of DHE in the conditioned place paradigm was evaluated and compared with that of morphine.

Male ddY mice, weighing 18 to 20 g (Otsubo Exp. Animals, Nagasaki), were purchased and kept in a room maintained at 21±2°C under a natural day/night regime with free access to a standard laboratory diet (MF; Oriental Yeast, Tokyo) and tap water ad libitum. After reaching 23 to 25 g, they were used for experiments. 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 Sciences, China) and morphine (Takeda Pharm. Co., Osaka) 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 antinociceptive effect was evaluated by a modified Haffner method (a cutoff time of 6 sec) for 90 min after the administrations of DHE or morphine and is expressed as area under the curve (AUC) by plotting the increase in response time (sec) on the ordinate and time interval (min) on the abscissa.

The place preference conditioning procedure was conducted in a chamber consisting of three compartments made of acryl-resin board: two end compartments (15 × 15 × 15 cm: W × L × H) and a middle compartment (10 × 10 × 12 cm: W × L × H), which was separated from the others by removable guillotine doors. One end compartment was white with a textured floor; the other was black with a smooth floor. One side of both the white and black compartments consisted of transparent plexiglas so that the movement of the animals placed inside could be observed. The middle compartment was gray and served as a small, neutral region between the black and white compartments (Fig. 1). Animals were immediately confined for 30 min to one compartment after the injection of DHE (0.1, 1, 3 and 10 µg/kg, i.p.) or morphine (0.1, 1, 3 and 10 mg/kg, i.p.) and to the other compartment after the injection of saline. Animals that had been injected with drugs were confined to one end compartment on one day and to the other compartment after the injection of saline (0.1 ml/10 g) on the following day. This
conditioning cycle was performed 3 times (6 days). The kind of injection (drug or saline) and the type of compartment (white or black) were counterbalanced across subjects. The control mice were injected with saline instead of drugs during each of the conditioning sessions. Tests of conditioning were conducted on day 7. Preference for a particular place was assessed in the drug-free state, after placing the animals in the neutral middle compartment and allowing them free access to each compartment. The time spent in each compartment during a 30 min-session was measured. Conditioning scores represent the time spent in the drug-paired place minus the time spent in the saline-paired place. The results are expressed as means ± S.E.M. following a two-way analysis of variance (ANOVA) for repeated measurements with the overall data to assess statistical significance. Differences between the individual mean values in various groups were analyzed with the Wilcoxon test for the conditioned place preference method. A difference was considered significant at P < 0.05.

As shown in Fig. 2, DHE (0.1, 1, 3 and 10 µg/kg, i.p.) and morphine (0.1, 1, 3 and 10 mg/kg, i.p.) produced an antinociceptive effect in a dose dependent manner. The effect, as shown by AUC, of DHE was nearly 1,000 times more potent than those of morphine.

In the experiment using the conditioned place prefer-
ence paradigm, DHE (0.1, 1, 3 and 10 µg/kg, i.p.)-conditioned mice exhibited no place preference, the same as the saline-conditioned control mice, in the conditioning scores evaluated by the time spent in the drug-paired place minus the time spent in the saline-paired place. On the other hand, morphine (0.1, 1, 3 and 10 mg/kg, i.p.) produced a place preference for the drug-conditioned place in a dose dependent manner; that is, the time spent in the drug-paired place was increased and the time spent in the saline-paired place was decreased (Fig. 3). Meanwhile, the time spent in the middle compartment (neutral region) throughout all the experiments was not changed significantly (data not shown).

The present results were consistent with previous reports (1, 3) that i.p. injection of DHE produced a potent antinociceptive effect in a dose-dependent manner, and the efficacy ratio of the antinociceptive effect between DHE and morphine was approximately 1,000 : 1 by the i.p. administration of these drugs.

DHE has been shown to possess a selective affinity for µ-opioid receptors (8). We have also reported that the antinociceptive effect of DHE is suppressed by naloxone (a µ-opioid receptor antagonist), but by neither naltrindole (a δ-opioid receptor antagonist) nor nor-binaltorphimine (a κ-opioid receptor antagonist), suggesting that the effect of DHE is mediated through µ-opioid receptors (3). It is widely accepted that µ-opioid receptor agonists have a reinforcing effects, as evaluated by the conditioned place preference paradigm (6, 7). In the present study, however, DHE failed to induce the conditioned place preference, while morphine induced the place preference for the drug-paired place in a dose dependent manner. These results indicate that only morphine, but not DHE, possesses a reinforcing effect. Since the reinforcing effect of narcotics has been believed to be one of the critical factors leading to the abuse of and dependence on these drugs, DHE may have a large therapeutic range in the clinical field.

Our previous data (9) have shown that by direct application of DHE into the CNS (i.c.v. or i.t.), the antinociceptive effect is about 10 to 20 times higher than that of morphine, and the duration of the effect of DHE is much shorter than that of morphine, suggesting that the accumulation of DHE in the CNS seems to be quite low. Hence, one possibility is that the lack of place preference of DHE might be due to a rapid elimination of this drug from the CNS, namely, less chance to modulate biochemical or physiological functions of opioid receptors.

Presently, µ-opioid receptors are divided into two subtypes of: µ1 and µ2, based on binding studies (10). It has been reported that µ1-receptors mediate supraspinal analgesia and feeding behaviors, while respiratory depression and dopamine turnover are regulated by µ2-receptors (11–13). Recently, Suzuki et al. (14) have shown that morphine induces the conditioned place preference in µ1-receptor deficient CXBK mice and have suggested that the
morphine-induced place preference is closely related to a μ2-receptor-mediated system. In this experiment, although DHE produced a potent antinociceptive effect involving μ1-receptors, DHE failed to induce the place preference involving μ2-receptors. These findings suggest that DHE might preferentially act on μ1-opioid receptors, and it might be applicable to pharmacological research as a suitable μ1-receptor agonist.

Interestingly, in addition to the lack of the reinforcing effect of DHE, it has also been reported that the physical dependence liability of DHE is low; that is, at the dose to produce a sufficient degree of antinociception, the development and/or expression of physical dependence on DHE could not be detected (2–4). The probability of separating the antinociceptive effect from the physical and/or psychic dependence, as proposed by Kaneto et al. (15), is strongly supported by these findings.

Thus, we have demonstrated in this study that DHE produces a strong antinociceptive effect without possessing a reinforcing effect, as evaluated by the conditioned place preference paradigm, suggesting that it could be useful for the clinical therapy of patients suffering from chronic severe pain. In addition, these characteristic properties of DHE would make it suitable for development as a novel analgesic.

Acknowledgment
The authors are grateful to Professor Qin Bo-Yi (Academy of Military Medical Sciences, China) for the generous supply of DHE.

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