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Short communication

Antinociceptive effect of dihydroetorphine in diabetic mice

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Abstract

The antinociceptive potency of dihydroetorphine in diabetic mice was examined. Subcutaneous administration of dihydroetorphine produced a dose-dependent antinociception in both non-diabetic and diabetic mice. The antinociceptive potency of s.c. dihydroetorphine was less in diabetic mice than in non-diabetic mice. The antinociception induced by i.c.v. dihydroetorphine (0.02 μg) was also significantly less in diabetic mice than in non-diabetic mice. The antinociceptive effects of dihydroetorphine (10 μg/kg i.p.) in both diabetic and non-diabetic mice were significantly antagonized by s.c. administration of β-funaltrexamine, a selective μ-opioid receptor antagonist. Furthermore, the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in non-diabetic mice, but not in diabetic mice, was also significantly antagonized by naloxonazine, a selective μ_1-opioid receptor antagonist. The time course and the potency of the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in diabetic mice were similar to those in naloxonazine-treated non-diabetic mice. Naltrindole, a selective δ-opioid receptor antagonist, or nor-binaltorphimine, a selective κ-opioid receptor antagonist, had no significant effect on the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in both diabetic and non-diabetic mice. These results suggest that dihydroetorphine produces an antinociceptive effect through the activation of both μ_1- and μ_2-opioid receptors in mice. Furthermore, the reduction in dihydroetorphine-induced antinociception in diabetic mice, as compared with non-diabetic mice, may be due to the hyporesponsive to supraspinal μ_1-opioid receptor-mediated antinociception in diabetic mice.

Keywords: Dihydroetorphine; Diabetes; Antinociceptive effect; Opioid receptor; (Mouse)

1. Introduction

The development of naloxonazine (Hahn et al., 1982), a selective μ_1-opioid receptor antagonist, has allowed the identification of at least two μ-opioid receptor subtypes; μ_1- and μ_2-opioid receptors (Heyman et al., 1988; Kamei et al., 1993; Pasternak and Wood, 1986; Paul et al., 1989). We recently reported that the naloxonazine-sensitive pharmacological actions of morphine, such as supraspinal antinociception (Kamei et al., 1994a) and Straub tail reaction (Kamei et al., 1994b), in diabetic mice were significantly less than those in non-diabetic mice. However, there is no significant difference in the naloxonazine-insensitive pharmacological actions of morphine, such as spinal antinociception (Kamei et al., 1994a) and antitussive effect (Kamei et al., 1994c), between diabetic mice and non-diabetic mice. Based on this evidence, we proposed that diabetic mice are selectively hyporesponsive to μ_1-opioid receptor-mediated pharmacological action, but not to that mediated by μ_2-opioid receptors.

Bentley and Hardy (1967) synthesized dihydroetorphine and reported that its equivalent antinociceptive dose was 12000 times lower than that of morphine and one-fourth of that of etorphine. Recently, Tokuyama et al. (1993) reported that dihydroetorphine produced a potent antinociceptive effect through the activation of μ-opioid receptors. However, there is little information available regarding the involvement of μ-opioid receptor subtypes in this μ-opioid receptor-mediated antinociceptive effect of dihydroetorphine. Thus, the first aim of the present study was to investigate the influence of naloxonazine, a selective μ_1-opioid receptor antagonist, on dihydroetorphine to determine the role of the μ-opioid receptor subtypes in the antinociceptive effect of dihydroetorphine. An additional aim of this study was to investigate the effect of diabetes on dihydroetorphine to clarify our hypothesis that diabetic

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mice are selectively hyporesponsive to μ_1-opioid receptor-mediated pharmacological action, but not to that mediated by μ_2-opioid receptors.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science Co., Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at 22 ± 1°C with a 12-h light-dark cycle. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with the vehicle alone. The experiments were conducted 2 weeks after injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. Studies in this report were performed in accordance with the guide for the care and use of laboratory animals as adopted by Hoshi University.

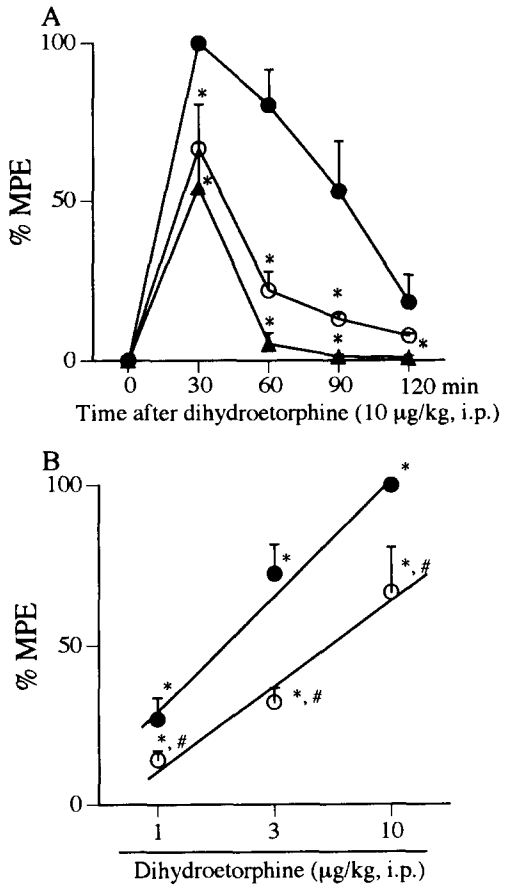

Fig. 1. A: Time course of the antinociceptive effects of dihydroetorphine in diabetic (open circle), non-diabetic (closed circle) and naloxonazine-treated non-diabetic (closed triangle) mice. The antinociceptive effects of dihydroetorphine (10 μg/kg s.c.) were measured in the tail-flick test 30, 60, 90 and 120 min after injection of dihydroetorphine. Each point represents the mean ± S.E. for 7 mice in each group. Analysis of variance (ANOVA) revealed a significant relation ($P < 0.01$): $F(11,72) = 15.7$. * $P < 0.05$ versus the values for non-diabetic mice. B: Dose-response relationship of the antinociceptive effect of dihydroetorphine in diabetic (open circle) and non-diabetic (closed circle) mice. The antinociceptive effects of dihydroetorphine were measured in the tail-flick test 30 min after injection. Each column represents the mean ± S.E. for 7–8 mice in each group. ANOVA revealed a significant relation ($P < 0.01$): $F(7,53) = 30.6$. * $P < 0.05$ versus the respective saline-treated group (non-diabetic mice, 1.6 ± 0.2%MPE, $n = 8$; diabetic mice, 1.0 ± 0.2%MPE, $n = 8$). # $P < 0.05$ versus the values for non-diabetic mice.

2.2. Antinociceptive assay

The antinociceptive response was evaluated by recording the latency in the tail-flick test using radiant heat as a stimulus. The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 2–3 s. A cut-off latency of 15 s was used to prevent injury to the tail. Animals which did not respond within 15 s were removed and assigned a score of 15 s. The percent maximum possible effect (%MPE) was calculated for each animal using the formula: %MPE = 100 × (post-drug latency − pre-drug latency)/(15 − pre-drug latency).

2.3. Drugs

Dihydroetorphine was synthesized and kindly provided by Dr. T. Iwamura (Gifu Pharmaceutical University). β-Funaltrexamine, naloxonazine, naltrindole and nor-binaltorphimine were synthesized by Dr. H. Nagase (Toray Industries, Kamakura, Japan). All of the drugs were dissolved in saline. β-Funaltrexamine (20 mg/kg s.c.) and naloxonazine (35 mg/kg s.c.) were injected 24 h before testing. Naltrindole (1 mg/kg s.c.) was injected 15 min before dihydroetorphine injection. Nor-binaltorphimine (20 mg/kg s.c.) was injected 3 h before dihydroetorphine injection. The i.c.v. injections, in a volume of 5 μl, were performed according to the method of Haley and McCormick (1957).

2.4. Statistical analysis

The data are expressed as the mean ± S.E. The statistical significance of differences was assessed with the Newman-Keuls test. A level of probability of 0.05 or less was accepted as significant. The slope of the linear portion of the dose-response line determined by plotting each individual point was calculated using Program 11 of the Pharmacological Calculations System of Tallarida and Murray (1987).

3. Results

3.1. Effect of i.p. and i.c.v. administration of dihydroetorphine on the tail-flick response

Fig. 1A shows the time courses of the antinociception produced by dihydroetorphine in both non-diabetic and diabetic mice. Dihydroetorphine at a dose of 10 μg/kg i.p. significantly increased the tail-flick latency in both non-diabetic and diabetic mice. This effect reached its peak at 30 min after the administration of dihydroetorphine and then gradually decreased. The peak effect of the antinociception induced by dihydroetorphine (10 μg/kg i.p.) was significantly less in diabetic mice than in non-diabetic mice.

The dependency of the antinociceptive effect on the amount of dihydroetorphine in both non-diabetic and diabetic mice is shown in Fig. 1B. Dihydroetorphine at doses of 1–10 μg/kg i.p. produced a dose-dependent inhibition of the tail-flick response in both non-diabetic and diabetic mice. The potency of dihydroetorphine to inhibit the tail-flick response in diabetic mice was less than that in non-diabetic mice. Indeed, the ED_{50} values (mg/kg with 95% confidence limits) were 2.0 (0.5–8.1) and 6.3 (2.4–16.4) for non-diabetic and diabetic mice, respectively. As shown in Fig. 1B, diabetes produced a marked rightward shift of the dose-response curve of the antinociceptive effect of dihydroetorphine ($Y = 73.1X + 30.4$ for non-diabetic mice and $Y = 52.8X + 11.5$ for diabetic mice, 3.1-fold right shift).

Dihydroetorphine at a dose of 0.02 μg i.c.v., when assessed 15 min after administration, produced a significant ($F(3,24) = 257.1$, $P < 0.01$) antinociception in both non-diabetic (saline, 0.8 ± 0.5%MPE, $n = 7$; dihydroetorphine, 96.2 ± 3.8%MPE, $n = 7$) and diabetic (saline, 0.7 ± 0.5%MPE, $n = 7$; dihydroetorphine, 45.2 ± 4.1%MPE, $n = 7$) mice. The antinociception induced by i.c.v. dihydroetorphine (0.02 μg) was also significantly less in diabetic mice than in non-diabetic mice.

3.2. Effects of β-funaltrexamine, naloxonazine, naltrindole and nor-binaltorphimine on the antinociception induced by dihydroetorphine

The effects of selective opioid receptor antagonists on the antinociceptive effect of dihydroetorphine are summarized in Fig. 2. Pretreatment with β-funaltrexamine, a selective μ-opioid receptor antagonist, 24 h before testing, antagonized the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in both non-diabetic and diabetic mice. When naloxonazine, a selective μ_1-opioid receptor antagonist, was injected 24 h before testing, the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in non-diabetic was significantly reduced. In diabetic mice, naloxonazine had no significant effect on the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.). However, the antinociceptive effect of a higher dose of dihydroetorphine (20 μg/kg i.p.) in diabetic mice was significantly ($F(1,14) = 4.6$, $P < 0.05$) reduced by naloxonazine (without naloxonazine, 95.2 ± 3.2%MPE, $n = 8$; with naloxonazine, 61.7 ± 13.2%, $n = 8$). Furthermore, the time course and the potency of the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) in diabetic mice were similar to those in naloxonazine-treated non-diabetic mice (Fig. 1A). On the other hand, administration of naltrindole, a selective δ-opioid receptor antagonist, 15 min before administration of dihydroetorphine, did not significantly affect the antinociceptive effects of dihy-

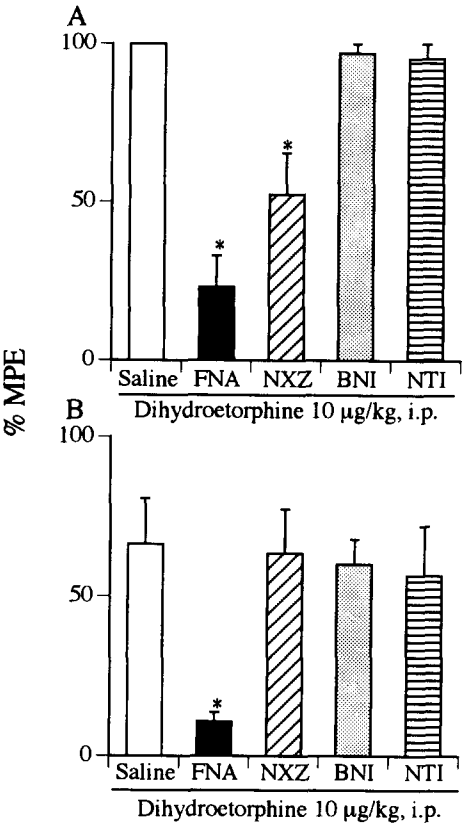

Fig. 2. Blockade of the antinociceptive effects of dihydroetorphine (10 μg/kg s.c.) by opioid receptor antagonists in non-diabetic (A) and diabetic (B) mice. Naltrindole (NTI, 1 mg/kg) was injected s.c. 15 min before administration of dihydroetorphine. Nor-binaltorphimine (BNI, 20 mg/kg s.c.) was injected 3 h before administration of dihydroetorphine. β-Funaltrexamine (FNA, 20 mg/kg) was injected s.c. 24 h before administration of dihydroetorphine. Naloxonazine (NXZ, 35 mg/kg), a selective μ_1-opioid receptor antagonist, was injected s.c. 24 h before administration of dihydroetorphine. The mice were tested 30 min after the injection of dihydroetorphine using the tail-flick test. Each column represents the mean ± S.E. for 7 mice in each group. ANOVA revealed a significant relation ($P < 0.01$): $F(4,30) = 16.6$ in non-diabetic mice (A) and $F(4,30) = 15.2$ in diabetic mice (B). * $P < 0.05$ versus the saline-treated group.

droetorphine (10 μg/kg i.p.) in either diabetic or non-diabetic mice. Moreover, when nor-binaltorphimine (20 mg/kg s.c.), a selective κ-opioid receptor antagonist, was injected 3 h before the administration of dihydroetorphine, the antinociceptive effect of dihydroetorphine (10 μg/kg i.p.) was not significantly reduced in either non-diabetic or diabetic mice.

4. Discussion

The results of the present study demonstrate that i.p. administration of dihydroetorphine increased tail-flick latency in a dose-dependent manner in both non-diabetic and diabetic mice. The results of the present experiments also clearly indicate that the antinociceptive potency of dihydroetorphine is significantly lower in diabetic mice than in non-diabetic mice. Dihydroetorphine-induced antinociception in both non-diabetic and diabetic mice was markedly antagonized by s.c. pretreatment with β-funaltrexamine, a selective μ-opioid receptor antagonist, but not by naltrindole, a selective δ-opioid receptor antagonist, or nor-binaltorphimine, a selective κ-opioid receptor antagonist, indicating that the antinociceptive effect of dihydroetorphine results from the activation of μ-opioid receptors. These results suggest that diabetic mice are hyporesponsive to the μ-opioid receptor-mediated antinociceptive effect of dihydroetorphine. The results of this study agree with our previous findings that the antinociceptive effects of μ-opioid receptor agonists are significantly lower in diabetic mice than in non-diabetic mice (Kamei et al., 1992a,b, 1994a,c), and support our hypothesis that diabetic mice are selectively hyporesponsive to μ-opioid receptor-mediated pharmacological actions.

The present study also used naloxonazine, a selective μ_1-opioid receptor antagonist (Hahn et al., 1982), to compare the sensitivity of the antinociceptive effect of dihydroetorphine in diabetic and non-diabetic mice. Naloxonazine partially, but significantly, antagonized the antinociceptive effect of i.p. dihydroetorphine in non-diabetic mice. In diabetic mice, although naloxonazine had no significant effect on the antinociceptive effect of a low dose of dihydroetorphine (10 μg/kg i.p.), the sensitivity of the antinociceptive effect of dihydroetorphine to naloxonazine appeared when naloxonazine was tested against a high dose of dihydroetorphine (20 μg/kg i.p.) that produced approximately 100%MPE. The sensitivity of the antinociceptive effect of dihydroetorphine to naloxonazine indicates that dihydroetorphine produces antinociception, at least in part, through the activation of μ_1-opioid receptors, i.e., the antinociceptive effect of dihydroetorphine may be mediated through the activation of both μ_1- and μ_2-opioid receptors.

We recently demonstrated that the antinociceptive effect of i.c.v. morphine, which is mediated mainly by μ_1-opioid receptors, in diabetic mice was significantly less than that in non-diabetic mice (Kamei et al., 1994a). However, there were no significant differences in the antinociceptive effects of i.t.-administered morphine, which is mediated mainly by μ_2-opioid receptors, in diabetic and non-diabetic mice (Kamei et al., 1994a). Based on these results, we proposed that mice with diabetes are selectively hyporesponsive to μ_1-opioid receptor-mediated pharmacological action, but not to that which is mediated by μ_2-opioid receptors. This hypothesis is further supported by the present results that (1) the antinociceptive effect of dihydroetorphine is significantly less in diabetic mice than in non-diabetic mice, (2) naloxonazine, a selective μ_1-opioid receptor antagonist, significantly reduced the antinociceptive effect of dihydroetorphine (10 μg/kg) in non-diabetic, but not in diabetic mice, (3) the sensitivity of the antinociceptive effect of dihydroetorphine to naloxonazine in diabetic mice appeared when naloxonazine was tested against a high dose of dihydroetorphine (20 μg/kg i.p.) that produced approximately 100%MPE, and (4) the time course and the potency of the antinociceptive effect of dihydroetorphine (10 μg/kg) in diabetic mice were similar to those in naloxonazine-treated non-diabetic mice. On the other hand, the results obtained in the present study indicate that the antinociceptive effect of i.c.v. dihydroetorphine is smaller in diabetic mice than in non-diabetic mice. This attenuation cannot be explained by pharmacokinetic considerations since the dihydroetorphine was administered directly into the brain.

We previously demonstrated that transfer of mononuclear spleen cells or the supernatant of spleen cell homogenate from diabetic mice to naive mice significantly reduced the antinociception mediated by μ-opioid receptor agonists in these mice (Kamei et al., 1992b, 1994c). Furthermore, Brase et al. (1987) have suggested that the number and the affinity of μ-opioid receptors in diabetic animals are not significantly different from those in non-diabetic animals. Therefore, we proposed that factor(s) derived from spleen cells of diabetic mice reduced the antinociceptive effect mediated by μ-opioid receptor agonists by modifying the μ-opioid receptor-effector coupling mechanisms or by modifying the binding of μ-opioid receptor agonists to μ-opioid receptors (Kamei et al., 1992b, 1994c).

In conclusion, the present results suggest that dihydroetorphine produces an antinociceptive effect through the activation of both μ_1- and μ_2-opioid receptors in mice. Furthermore, the reduction in dihydroetorphine-induced antinociception in diabetic mice, as compared with non-diabetic mice, may be due to the hyporesponsive to supraspinal μ_1-opioid receptor-mediated antinociception in diabetic mice.

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References

Bentley, K.W. and D.G. Hardy, 1967, Novel analgesics and molecular rearrangements in the morphine-the baine group. III. Alcohols of the 6,14-endo-ethenotetrahydrooripavine series and derived analogs of *N*-allylmorphine and -narcodeine, J. Am. Chem. Soc. 89, 3281.

Brase, D.A., T.H. Han and W.L. Dewey, 1987, Effects of glucose and diabetes on binding of naloxone and dihydromorphine to opiate receptors in mouse brain, Diabetes 36, 1173.

Hahn, E.F., M. Carroll-Buatti and G.W. Pasternak, 1982, Irreversible opiate agonists and antagonists: The 14-hydroxydihydromorphinone azines, J. Neurosci. 5, 572.

Haley, T.J. and W.G. McCormick, 1957, Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse, Br. J. Pharmacol. 12, 12.

Heyman, J.S., C.L. Williams, T.F. Burks, H.I. Mosberg and F. Porreca, 1988, Dissociation of opioid antinociception and central gastrointestinal propulsion in the mouse: studies with naloxonazine, J. Pharmacol. Exp. Ther. 245, 238.

Kamei, J., Y. Ohhashi, T. Aoki, N. Kawashima and Y. Kasuya, 1992a, Streptozotocin-induced diabetes selectively alters the potency of analgesia produced by μ-opioid agonists, but not by δ- and κ-opioid agonists, Brain Res. 571, 199.

Kamei, J., N. Kawashima and Y. Kasuya, 1992b, Role of spleen or spleen products in the deficiency in morphine-induced analgesia in diabetic mice, Brain Res. 576, 139.

Kamei, J., Y. Iwamoto, T. Suzuki, M. Misawa, H. Nagase and Y. Kasuya, 1993, The role of the μ_2-opioid receptor in the antitussive effect of morphine in μ_1-opioid receptor-deficient CXBK mice, Eur. J. Pharmacol. 240, 99.

Kamei, J., Y. Iwamoto, H. Hitosugi, M. Misawa, H. Nagase and Y. Kasuya, 1994a, Streptozotocin-induced diabetes selectively reduces antinociception mediated by μ_1-opioid receptors, but not that mediated by μ_2-opioid receptors, Neurosci. Lett. 165, 141.

Kamei, J., M. Ohsawa, M. Misawa, H. Nagase and Y. Kasuya, 1994b, Effects of diabetes on the morphine-induced Straub tail reaction in mice, Neurosci. Lett. 178, 66.

Kamei, J., Y. Iwamoto, M. Misawa, H. Nagase and Y. Kasuya, 1994c, Evidence for differential modulation of μ-opioid receptor-mediated antinociception and antitussive activities by spleen-derived factor(s) from diabetic mice, Neuropharmacology (in press).

Pasternak, G.W. and P.L. Wood, 1986, Multiple mu opiate receptors, Life Sci. 38, 1889.

Paul, D., R.J. Bodnar, M.A. Gistrak and G.W. Pasternak, 1989, Different μ receptor subtypes mediate spinal and supraspinal analgesia in mice, Eur. J. Pharmacol. 168, 307.

Tallarida, R.J. and Murray, R.B., 1987, Manual of Pharmacological Calculations with Computer Programs (Springer-Verlag, New York).

Tokuyama, S., M. Takahashi and H. Kaneto, 1993, Antinociceptive effect of dihydroetorphine and its tolerance/dependence liability in mice, Biol. Pharm. Bull. 16, 774.