ELSEVIER

European Journal of Pharmacology 300 (1996) 75–78

ejp

Short communication

Antinociceptive and antitussive effects of morphine in the DA-bg/bg (Beige) rat

Junzo Kamei [a,*], Kayo Morita [a], Akiyoshi Saitoh [a], Tsutomu Suzuki [b], Hiroshi Nagase [c]

[a] *Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo 142, Japan*
[b] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo 142, Japan*
[c] *Basic Laboratories, Toray Industries Inc., Kamakura 248, Japan*

Received 23 November 1995; accepted 9 January 1996

Abstract

Antinociceptive and antitussive effects of morphine were studied in DA-bg/bg (Beige) rats. Intraperitoneal administration of morphine (10 mg/kg) produced a marked antinociceptive effect, in the tail-flick test, in Beige rats and DA rats, a progenitor strain rats. There was no significant difference in the peak antinociceptive effect of morphine between Beige rats and DA rats. The antinociceptive effect of morphine in both Beige rats and DA rats was significantly reduced following pretreatment with a low dose (0.3 mg/kg, i.p.) of naloxone or naltrexonazine (1 mg/kg, s.c.), a selective μ_1-opioid receptor antagonist. Morphine suppressed coughs dose dependently at doses between 0.3–3 mg/kg, i.p., in Beige rats and between 0.1–1.0 mg/kg, i.p., in DA rats. The antitussive potency of morphine in Beige rats was less than that in DA rats. The antitussive effect of morphine was significantly antagonized by pretreatment with naloxone (0.3 mg/kg, i.p.) in both Beige rats and DA rats. However, pretreatment with naltrexonazine (1 mg/kg, s.c.), a selective μ_1-opioid receptor antagonist, had no effect on the antitussive effect of morphine. These results suggest that Beige rats are hyporesponsive to the μ_2-opioid receptor-mediated antitussive effect, but not to the μ_1-opioid receptor-mediated antinociceptive effect.

Keywords: (Beige rat); Morphine; μ-Opioid receptor, subtype; Antitussive effect; Cough reflex

1. Introduction

Recently, at least two μ-opioid receptor subtypes have been proposed, i.e., μ_1- and μ_2-opioid receptors (Pasternak et al., 1980; Pasternak and Wood, 1986). In recent experiments, naloxonazine (Ling et al., 1986) and naltrexonazine (Kamei et al., 1995; Yokoyama et al., 1992), another type of selective μ_1-opioid receptor antagonist, had no significant effect on μ-opioid receptor-mediated antitussive effects in ICR mice (Kamei et al., 1993a) or μ_1-opioid receptor-deficient CXBK mice (Kamei et al., 1993c). Furthermore, there was no significant difference in the antitussive effect of morphine between ICR and CXBK mice. Based on these results, we previously suggested that μ_2-opioid receptors, but not μ_1-opioid receptors, are involved in the antitussive effects of morphine (Kamei et al., 1993a, c).

C57BL/6J-bg^J/bg^J (Beige-J) mice are known to be an animal model of Chediak-Higashi syndrome, an autosomal recessive genetic disease (Gallin et al., 1974; Lutzner et al., 1967). Raffa et al. (1993) proposed that Beige-J mice are considerably less sensitive than their normal littermates to the antinociceptive effects of μ-opioid receptor agonists. Recently, Nishimura et al. (1989) found an autosomal recessive mutant rat (DA-bg/bg rat) in an inbred colony of the DA strain rat. The pathological features of these DA-bg/bg (Beige) rats are comparable to those of human Chediak-Higashi syndrome patients and Beige-J mice (Nishimura et al., 1989). Thus, it is reasonable to speculate that Beige rats are less sensitive to μ-opioid agonist than DA rats, a progenitor strain rat, as are Beige-J mice. However, little information is available regarding the changes in μ-opioid receptor function in Beige rats. In the present study, therefore, we examined the antinociceptive and antitussive effects of morphine in Beige rats, as compared with those in DA rats, to investigate the possible changes in the function of μ-opioid receptors, especially μ_2-opioid receptors, in Beige rats.

* Corresponding author. Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142, Japan. Fax: (81) (3) 5498-5029.

PII S0014-2999(96)00032-5

2. Materials and methods

2.1. Animals

Inbred male and female DA-bg/bg (Beige) and DA rats were purchased from the Institute of Experimental Animals, Hamamatsu University School of Medicine, Shizuoka, Japan, and were maintained in our university. Male and female rats weighing 300–350 g were used. The rats were housed under a 12-h light-dark cycle, at a temperature of 22 ± 1°C. Food and water were given ad libitum. These studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antinociceptive assay

The antinociceptive effect 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 4–5 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. Percent antinociception was calculated for each animal by using the formula: 100 × (post-drug latency − pre-drug latency)/(15 − pre-drug latency).

2.3. Antitussive assay

The cough reflex was induced by a previously described technique (Kamei et al., 1990). Animals were exposed to a nebulized solution of capsaicin (30 μmol/l) under conscious and identical conditions using a body plethysmograph. The coughs produced during a 5-min exposure period were counted. Capsaicin was dissolved to a concentration of 30 mg/ml in a 10% ethanol and 10% Tween 80 saline solution. The solution was diluted with saline. The rats were exposed for 5 min to capsaicin 30 min before injection of drugs to determine the frequency of control coughs. The animals were exposed to capsaicin aerosol again for 5 min at 30, 60 and 120 min after morphine injection. The number of coughs after morphine injection (Ct) was compared to the number of control coughs (Cc). The antitussive effect was expressed as the percent inhibition of the number of control coughs ((Cc − Ct)/Cc × 100).

2.4. Drugs

Naltrexonazine was synthesized by Dr. H. Nagase (Toray Industries, Kamakura, Japan). Morphine hydrochloride and naloxone hydrochloride were purchased from Sankyo Co., Tokyo, Japan and Sigma Chemical Co., St. Louis, MO, USA, respectively. Naloxone (0.3 mg/kg, i.p.) was injected 15 min before injection of morphine. Naltrexonazine (1 mg/kg, s.c.) was injected 3 h before injection of morphine, as previously described (Kamei et al., 1995; Yokoyama et al., 1992).

2.5. Statistics

Data are expressed as the means ± S.E. The statistical significance of differences was assessed with the Dunnett's test for the antinociceptive effect and with the Mann-Whitney *U*-test for the antitussive effect. A probability of 0.05 or less was considered significant.

3. Results

3.1. Antinociceptive effect

Fig. 1A shows the time course of the antinociception produced by morphine in both Beige rats and DA rats. Morphine at a dose of 10 mg/kg, i.p., significantly increased the tail-flick latency. This effect reached its peak at 30 min after the administration of morphine and then gradually decreased.

The effects of naloxone and naltrexonazine on the antinociceptive effect of morphine in both Beige rats and DA rats are summarized in Table 1. Pretreatment with a low dose of naloxone (0.3 mg/kg, i.p.) reduced the antinociceptive effect of morphine (10 mg/kg, i.p.) in both Beige rats and DA rats. Pretreatment with naltrexonazine, a selective μ_1-opioid receptor antagonist, also reduced the antinociceptive effect of morphine (10 mg/kg, i.p.) in both Beige rats and DA rats.

3.2. Antitussive effect

A 5-min exposure to capsaicin induced 17.9 ± 1.6 coughs (n = 27) in Beige rats and 13.7 ± 0.8 coughs (n = 27) in DA rats. I.p. injection of saline had no significant effect on the stability or reproducibility of coughs in either Beige rats or DA rats (Fig. 1B).

The time course of the effects of i.p. injection of morphine, at a dose of 1 mg/kg, on the number of coughs is shown in Fig. 1B. The cough-depressant effects reached a peak 30 min after the administration of morphine in both Beige and DA rats. At 120 min, the number of coughs had returned to the level in rats that had received saline. Thus, a time interval of 30 min after injection was chosen for experiments designed to quantify the effects of morphine on the cough reflex. As shown in Fig. 1C, morphine suppressed coughs dose dependently at doses between 0.3–3 mg/kg, i.p., in Beige rats and 0.1–1.0 mg/kg, i.p., in DA rats. The antitussive potency of morphine in Beige rats was less than that in DA rats. Indeed, the ED_{50} (95%

confidence limits) of morphine for the inhibition of capsaicin-induced coughs in Beige rats (1.1 (0.8–1.5) mg/kg) was significantly higher than that in DA rats (0.2 (0.1–0.4) mg/kg).

The effects of naloxone and naltrexonazine on the antitussive effects of morphine in both Beige rats and DA rats are summarized in Table 1. These opioid antagonists, by themselves, had no significant effects on the number of coughs (data not shown). When naloxone (0.3 mg/kg, i.p.) was injected 15 min before injection of morphine, the antitussive effect of morphine 30 min after i.p. injection was significantly reduced in both Beige rats and DA rats (Table 1). However, naltrexonazine had no significant effect on the antitussive effect of morphine in both Beige rats and DA rats.

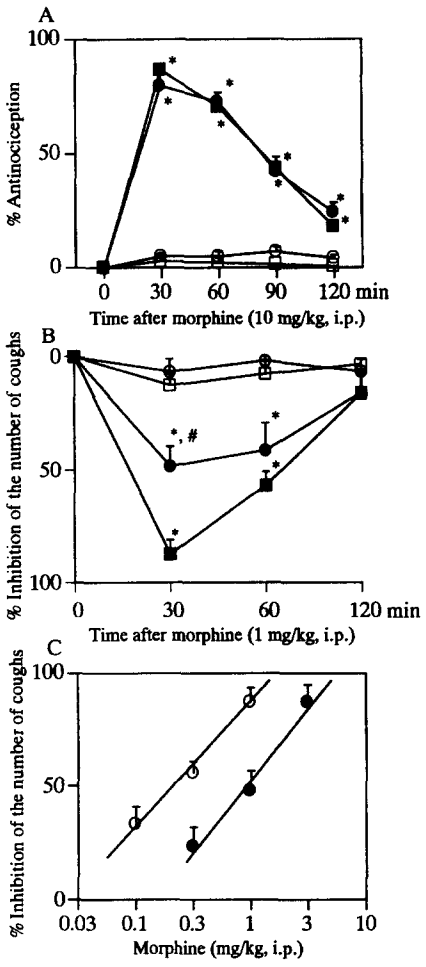

Fig. 1. Time course of the antinociceptive (A) and the antitussive (B) effect of morphine in Beige rats (circle) and DA rats (square). The antinociceptive effect of morphine (10 mg/kg, i.p.) was measured in the tail-flick test 0.5, 1, 1.5 and 2 h after injection. The antitussive effect of morphine (1 mg/kg, i.p.) was measured 0.5, 1 and 2 h after injection. Each point represents the mean ± S.E. from 5 rats for the antinociceptive effect and from 6-7 rats for the antitussive effect in each group. * $P < 0.05$ versus the respective saline-treated group (open symbols). # $P < 0.05$ versus the values for DA rats. (C) Dose-response curves for the antitussive effect of i.p. administered morphine in Beige (closed circle) and DA (open circle) rats. Each point represents the mean ± S.E. for 7 animals in each group.

Table 1
Effects of naloxone and naltrexonazine on the antinociceptive and the antitussive effect of morphine in DA and Beige rats

(a) Antinociceptive effect	% Antinociception (*n*)		
	Saline	Naloxone	Naltrexonazine
DA rats	86.3 ± 8.5 (5)	53.9 ± 7.2 (6) [a]	37.4 ± 13.0 (6) [a]
Beige rats	79.5 ± 8.8 (5)	49.8 ± 9.3 (7) [a]	36.6 ± 13.2 (6) [a]
(b) Antitussive effect	% Inhibition of the number of coughs (*n*)		
	Saline	Naloxone	Naltrexonazine
DA rats	87.2 ± 6.3 (7)	45.6 ± 8.1 (8) [a]	76.8 ± 5.9 (8)
Beige rats	54.2 ± 4.5 (6) [b]	23.3 ± 7.3 (6) [a]	54.2 ± 12.0 (7) [b]

The antinociceptive and antitussive effects were evaluated 30 min after i.p. administration of morphine. Naloxone (0.3 mg/kg, i.p.) was injected 5 min before administration of morphine. Naltrexonazine (1 mg/kg, s.c.) was administered 3 h before testing. Data represent means ± S.E. [a] Significantly different from vehicle (saline)-treated group. [b] Significant difference from the respective value of DA rats.

4. Discussion

The results of the present study demonstrated that i.p. administration of morphine produced a marked antinociceptive effect in both Beige rats and DA rats. Furthermore, the antinociceptive effect of morphine in Beige rats was identical to that in DA rats. The antinociceptive effects of morphine in both Beige rats and DA rats were antagonized by pretreatment with a low dose (0.3 mg/kg, i.p.) of naloxone or naltrexonazine, a selective μ_1-opioid receptor antagonist. Naloxone acts as an antagonist at μ-, δ- and κ-opioid receptors, but a higher dose of naloxone is required to antagonize the effects of δ- and κ-opioid receptor agonists than is required to antagonize the effects of μ-opioid receptor agonists (Gilbert and Martin, 1976; Kosterlitz et al., 1981). Thus, it appears possible that the antitussive action of morphine in both Beige rats and DA rats is mediated mainly via stimulation of μ-opioid receptors, especially μ_1-opioid receptors. It is, therefore, suggested from our results that Beige rats are normally responsive to the μ (μ_1)-opioid receptor-mediated antinociceptive effect of morphine.

In the present study, we also found that the antitussive potency of morphine in Beige rats was significantly less than that in DA rats. The antitussive effect of morphine in DA rats was identical to that in Sprague-Dawley rats (Kamei et al., 1990). Furthermore, the antitussive effects of morphine in both Beige rats and DA rats were antagonized by a low dose (0.3 mg/kg, i.p.) of naloxone, whereas naltrexonazine, a selective μ_1-opioid receptor antagonist, had no effect on the antitussive effect of morphine in either Beige rats or DA rats. We previously reported that the antitussive effects of morphine in rats or

mice are antagonized by μ-opioid receptor antagonists, such as naloxone and β-funaltrexamine (Kamei et al., 1990, 1993a, c). We also reported that δ-opioid receptors were not involved in the antitussive effects of opioids (Kamei et al., 1993b). Indeed, [D-Pen^{2,5}]enkephalin, a selective δ-opioid agonist, had no effect on capsaicin-induced coughs in rats or mice (Kamei et al., 1993b). Moreover, we demonstrated that naltrindole, a selective δ-opioid receptor antagonist, had no significant effect on the antitussive effects of morphine (Kamei et al., 1993c). We also demonstrated that κ-opioid receptors were not involved in the antitussive effects of morphine, since the antitussive effects of morphine were not antagonized by nor-binaltorphimine, a selective κ-opioid receptor antagonist (Kamei et al., 1990). In view of these findings, it is possible that the antitussive effect of morphine is mediated mainly by the activation of μ-opioid receptors, but not of δ- or κ-opioid receptors. We recently suggested that μ_1-opioid receptors were not involved in μ-opioid receptor-mediated antitussive effects, since the antitussive effects of μ-opioid receptor agonists, such as morphine and DAMGO, were not antagonized by either naloxonazine or naltrexonazine, a selective μ_1-opioid receptor antagonist (Kamei et al., 1993a, c). Thus, it seems likely that the antitussive effects of morphine in Beige rats and DA rats may be primarily mediated by μ_2-opioid receptors. Therefore, the results of the present study suggest that Beige rats are hyporesponsive to the μ_2-opioid receptor-mediated antitussive effects of morphine. The attenuation of the antitussive effect of morphine in Beige rats compared to that in DA rats might not involve a pharmacokinetic consideration, since attenuation of several pharmacological effects of morphine in Beige-J mice was observed when morphine was administered directly into the brain (Raffa et al., 1993). However, further studies are necessary before this possibility can be established with greater certainty.

In summary, the present results indicate that Beige rats are hyporesponsive to the μ (μ_2)-opioid receptor-mediated antitussive effect, but not to the μ (μ_1)-opioid receptor-mediated antinociceptive effect. Although the importance of μ-opioid receptors in the development of Chediak-Higashi syndrome is unclear, the present results suggest that Beige rat may be suitable for the study of μ-opioid receptor functions.

Acknowledgements

We thank Ms. T. Takahashi and Ms. M. Sodeyama for their technical assistance.

References

Gallin, J.L., J.S. Bujak, E. Patten and S.M. Wolff, 1974, Granulocyte function in the Chediak-Higashi syndrome of mice, Blood 43, 201.

Gilbert, P.E. and W.R. Martin, 1976, The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog, J. Pharmacol. Exp. Ther. 198, 66.

Kamei, J., Y. Iwamoto, N. Kawashima, T. Suzuki, H. Nagase, M. Misawa and Y. Kasuya, 1993a, Possible involvement of μ_2-mediated mechanisms in μ-mediated antitussive activity in the mouse, Neurosci. Lett. 149, 169.

Kamei, J., Y. Iwamoto, T. Suzuki, M. Misawa, H. Nagase and Y. Kasuya, 1993b, Antitussive effects of μ- and κ-agonists in diabetic mice, Neurosci. Lett. 154, 65.

Kamei, J., Y. Iwamoto, T. Suzuki, M. Misawa, H. Nagase and Y. Kasuya, 1993c, 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., A. Saitoh, T. Suzuki, M. Misawa, H. Nagase and Y. Kasuya, 1995, Buprenorphine exerts its antinociceptive activity via m1-opioid receptors, Life Sci. 56, PL285.

Kamei, J., H. Tanihara, H. Igarashi and Y. Kasuya, 1990, Antitussive effects of two specific κ-opioid agonists, U-50,488H and U-62,066E, in rats, Eur. J. Pharmacol. 187, 281.

Kosterlitz, H.W., S.J. Paterson and L.E. Robson, 1981, Characterization of the κ-subtype of the opiate receptor in the guinea-pig brain, Br. J. Pharmacol. 73, 939.

Ling, G.S.F., K. Spiegel, S.H. Lockhart and G.W. Pasternak, 1986, Naloxonazine actions in vivo, Eur. J. Pharmacol. 129, 33.

Lutzner, M.A., C.T. Lowrie and H.W. Jordan, 1967, Giant granules in leukocytes of the beige mouse, J. Hered. 58, 299.

Nishimura, M., M. Inoue, T. Nakano, T. Nishikawa, M. Miyamoto, T. Kobayashi and Y. Kitamura, 1989, Beige rat: a new animal model of Chediak-Higashi syndrome, Blood 74, 270.

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

Pasternak, G.W., S.R. Childers and S.H. Snyder, 1980, Opiate analgesia: evidence for mediation by a subpopulation of opiate receptors, Science 208, 514.

Raffa, R.B., J.R. Mathiasen, E.S. Kimball and J.L. Vaught, 1993, The combined immunological and antinociceptive defects of beige-J mice: the possible existence of a 'μ-repressin', Life Sci. 52, 1.

Yokoyama, H., K. Onodera, T. Suzuki, K. Iinuma and T. Watanabe, 1992, Opioid μ-deficient CXBK mouse and the role of μ_1-receptors in electrically induced convulsions, Brain Res. 595, 137.