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Analgesic effect of μ- and κ-opioid agonists in beige and CXBK mice

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Abstract—The analgesic effects of μ- and κ-opioid agonists, including morphine, FK33,824, U50,488H, tifluadom and bremazocine, have been determined in C57BL/6J-bg^{J} (beige) and CXBK mice which are hyporesponsive to μ-opioid receptor-mediated analgesia compared with those of control mice (C57BL/6J (C6J), C57BL/6By (C6By), BALB/cBy (BALB)) using an abdominal constriction assay. The analgesic effect of subcutaneously administered morphine and FK33,824 in both beige and CXBK mice was significantly reduced compared with the controls and the analgesic effect of U50,488H and tifluadom in beige mice was significantly reduced compared with the wild strain (C6J). No reduction of analgesic effect of U50,488H and tifluadom was seen in CXBK compared with its progenitor strains, C6By and BALB, except for a reduction of the effect of tifluadom in CXBK compared with C6By. There was no strain difference in the bremazocine-induced analgesia. These results suggest that the beige mouse has a deficit in analgesia mediated by both μ- and κ-opioid receptors, whereas the CXBK is deficient only in the μ-opioid receptor-mediated analgesia.

The effects of opioids in mice depend on the strain (Oliverio et al 1983; Muraki & Kato 1986; Muraki et al 1988). The CXBK and C57BL/6J-bg^{J} (beige) strains of mice are reported to be less sensitive to the analgesic effect of morphine (Oliverio et al 1975; Moskowitz et al 1985; Mathiasen et al 1987). These mice may offer an animal model for study of the functions of opioid receptor subtypes. The CXBK strain, which is derived from a cross between C57BL/6By (C6By) and BALB/cBy (BALB), is deficient in μ- but not δ-opioid receptors (Baran et al 1975; Reith et al 1981; Moskowitz & Goodman 1985). The beige strain is a coisogenic strain of the C57BL/6J line and the homozygotes have characteristics that resemble Chediak-Higashi syndrome in man, an autosomally inherited defect in lysosome-containing cells (Oliver 1976). The beige mouse was less responsive to the analgesic effect mediated by μ- but not δ-opioid receptors (Mathiasen et al 1987). The analgesic defect of the beige mouse would not be the result of an insufficient number of μ-opioid receptors as is the case in the CXBK strain because there is no decrease in the number of μ-opioid binding sites in the brain of beige mice (Raffa et al 1988a). In addition to the μ-opioid receptor, there is good evidence for the role of κ-receptors in the mediation of analgesia (Millan 1990). κ-Agonists have attracted interest in view of the possibility of developing analgesics which lack the potential for abuse. It is not known whether CXBK and beige strains of mice show a decreased response to the antinociceptive effect of κ-agonists. In this paper, we have characterized the analgesic effect of κ-agonists in CXBK and beige strains.

Materials and methods

Animals. Both male and female mice, 8–11 weeks of age, of the C57BL/6J-bg^{J}/bg^{J} (beige), C57BL/6J (C6J), CXBK, C57BL/6By (C6By) and BALB/cBy (BALB) strains (National Institute of Genetics, Mishima) were used. Mice were housed in an air-conditioned room (22 ± 2°C) with 14 h light–10 h dark cycle (lights on at 0600 h). Water and standard laboratory chow were freely available. Analgesia was tested using the abdominal constriction method. Mice were pretreated subcutaneously with 0·9% NaCl (saline) (10 mL kg^{-1}) or the analgesics. Fifteen min after drug treatment, the mice were challenged intraperitoneally with 0·7% acetic acid (10 mL kg^{-1}) and the number of abdominal contrictions was counted over 30 min. Antinociceptive action was expressed as the percentage decrease in the number of abdominal constrictions observed in drug-treated animals compared with the mean number of abdominal constrictions in saline-injected animals of the same strain.

Drugs. Analgesics were dissolved in saline and were given to mice subcutaneously in a volume of 10 mL kg^{-1}. Morphine HCl was purchased from Sankyo Co. (Tokyo), U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide) from Upjohn Co. (Kalamazoo, MI). FK33,824 (D-Ala^{2}, MePhe^{4},Met(O)ol^{5}-enkephalin) and tifluadom were provided by Sandoz Ltd (Basle), bremazocine by ACF Chemiefarma NV (Maarssen).

Statistics. The results were evaluated statistically by the Kruskal-Wallis test followed by the Wilcoxon Rank Sum test.

Results

The number of abdominal constrictions elicited by intraperitoneal administration of acetic acid in the saline control mice of the 5 strains is shown in Table 1. The difference in the number of abdominal constrictions among strains was not significant. The analgesic effect of morphine (0·5 mg kg^{-1}) and FK33,824 (0·1 mg kg^{-1}) in the beige mouse was significantly less than its wild type (C6J). The CXBK strain was less sensitive to the analgesic effect of morphine (1 mg kg^{-1}) and FK33,824 than its progenitor strains, C6By and BALB, though the difference between CXBK and C6By in the FK33,824-induced analgesia was not statistically significant (Fig. 1). These results are consistent with the previous observations that both beige and CXBK strains have a deficiency in the analgesic response to μ-agonists (Moskowitz et al 1985; Mathiasen et al 1987). The beige mouse was more sensitive to morphine (1 mg kg^{-1})-induced analgesia than was CXBK. A similar but not significant difference in the sensitivity to FK33,824 was seen between the beige and CXBK strains.

Table 1. Abdominal constriction response to acetic acid in control mice. Mice were injected subcutaneously with saline (10 mL kg^{-1}) followed by the intraperitoneal injection of 0·7% acetic acid (10 mL kg^{-1}) 10 min later. The number of abdominal constrictions was counted over 30 min.

Strain	Number of abdominal constrictions* (Mean ± s.e.m., n = 6)
Beige	27·8 ± 3·0
C6J	28·7 ± 3·8
CXBK	46·8 ± 8·7
C6By	36·3 ± 2·4
BALB	32·8 ± 3·1

* No significant difference among strains (Kruskal-Wallis test).

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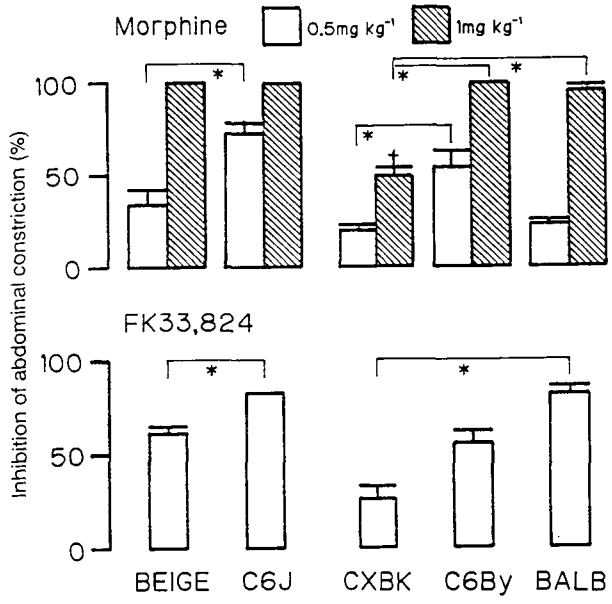

FIG. 1. Analgesic effects of μ-specific agonists, morphine (0·5, 1 mg kg^{-1}) and FK33,824 (0·1 mg kg^{-1}) in 5 strains of mice. * $P < 0.05$, † $P < 0.05$ vs beige (Wilcoxon test).

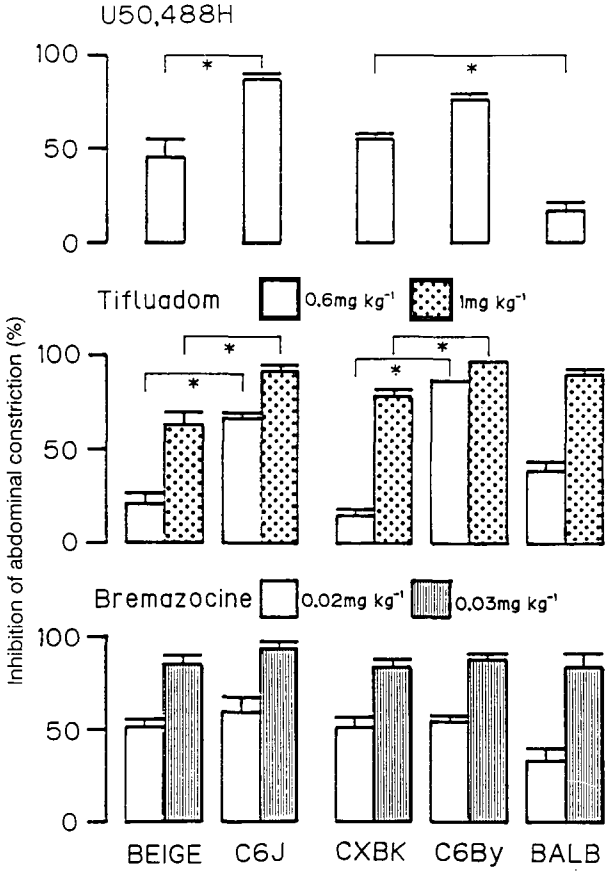

FIG. 2. Analgesic effects of κ-specific agonists, U50,488H (3 mg kg^{-1}), tifluadom (0·6, 1 mg kg^{-1}) and bremazocine (0·02, 0·03 mg kg^{-1}) in 5 strains of mice. * $P < 0.05$ (Wilcoxon test).

The BALB strain was the least responsive to the analgesic effect of U50,488H among the five strains of mice examined. The sensitivity of CXBK strains to the U50,488H-induced analgesia was intermediate between C6By and BALB, the progenitor strains of CXBK. The CXBK strain was less sensitive than C6By to the analgesia induced by tifluadom; however, the analgesic effect of tifluadom in the CXBK strain was not very different from the effect in the BALB strain. The sensitivity of the beige mouse to the analgesia induced by U50,488H and tifluadom was less than its wild strain, C6J, although it was not very different from that of CXBK. On the other hand, we found that the analgesic effect of bremazocine, another putative κ-agonist, was almost the same among the 5 strains (Fig. 2).

Discussion

The analgesic effect of subcutaneous morphine and FK33,824, relatively μ-specific agonists (Moyse et al 1986), measured by the abdominal constriction test was less in the beige and CXBK strains than in the control strains. This result confirms previous observations that beige and CXBK strains are hyporesponsive to morphine-induced analgesia as determined by the tail flick and hot plate tests (Oliverio et al 1975; Mathiasen et al 1987). Although both strains are defective in μ-opioid receptor-mediated analgesia, we found that the CXBK strain was much less responsive to morphine than the beige strain.

The analgesic effect elicted by the κ-specific agonist U50,488H (VonVoigtlander et al 1983) and the relatively κ-specific agonist tifluadom (Römer et al 1982) in the CXBK strain was significantly reduced compared with a progenitor strain, C6By, but was not less responsive than another progenitor strain, BALB. Therefore, we consider that the recombinant inbred strain CXBK is not hyporesponsive to the κ-receptor-mediated analgesia compared with the progenitor strains. The BALB strain was the least responsive to the analgesic effect of U50,488H among the five strains examined. It may be that the BALB strain has a defect in the function of κ-opioid receptors. We demonstrated that the beige strain was less responsive to the analgesic effect of κ-agonists, U50,488H and tifluadom compared with the wild strain C6J. Therefore, it seems that the beige mouse is defective in the analgesic effect mediated by both μ- and κ-opioid receptors. On the other hand, we could not show a significant difference between beige and C6J strains in the analgesic effect of another κ-agonist, bremazocine (Römer et al 1980). This might be because the dose-response curve for the analgesic effect of bremazocine does not readily fit a single straight line as was shown in the rat: Petrillo et al (1984) found a plateau of the dose-response curve at the higher doses of bremazocine than that which elicited 60% of the maximal response and they suggested this may be due to bremazocine's μ- and δ-antagonist properties.

Previous studies indicated that the CXBK strain displays a low number of whole brain opioid binding sites (Baran et al 1975), especially a deficiency in μ_1 binding in areas associated with nociception (Moskowitz & Goodman 1985). The μ_1 site was suggested to be important in mediating the analgesic effect of opioids (Pasternak 1986). The defect in the analgesia induced by μ-agonists in the CXBK strain may relate to the deficiency in μ_1 binding; however, a defect of brain κ-receptor in the CXBK strain has not been shown. On the other hand, the beige mouse has no decrease in brain opioid binding sites (Raffa et al 1988a). Although the locus of the beige gene and the basic defect in Chediak-Higashi syndrome are not known, it was found that the analgesic defect in the beige mouse was corrected by carbachol treatment and by splenectomy (Raffa et al 1987, 1988b). Raffa et al (1988b) proposed that the low efficacy of morphine in the beige mouse is not the result of a decrease in the number of opioid receptors of the beige mouse brain, but of some

circulating factor of splenic origin or elicited by some factor of splenic origin.

In conclusion, our study suggests that the CXBK may be specifically defective in the μ-receptor-mediated analgesia, whereas, the beige mouse is defective in analgesia mediated by both μ- and κ-opioid receptors due to an unknown cause. Although the analgesic defect in the beige mouse lacks opioid receptor subtype specificity, this strain may be useful for understanding the mechanism of analgesia at the molecular level, because the defect is associated with one gene mutation.

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Antidiuretic effect of bremazocine and U-50,488 in rats after α_2-adrenoceptor blockade

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Abstract—The role of α_2-adrenoceptors and κ-opioid receptors in urination was studied in rats. In water-loaded rats (40 mL kg^{-1} p.o.) the κ-opioid agonist bremazocine (0·05–0·2 mg kg^{-1} i.p.) induced a dose-related diuretic response in the second hour after administration, but had no effect in the first hour. When rats were pretreated with the α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1} s.c.), bremazocine induced a dose-related antidiuretic response in the first hour; thereafter the rats showed an increase of urination similar to that with bremazocine alone. The antidiuretic effect of bremazocine was dependent on the dose of idazoxan with maximal response after 1–3 mg kg^{-1}. Similar results were obtained with bremazocine in the presence of yohimbine (1 mg kg^{-1} s.c.). The antidiuretic profile of bremazocine after idazoxan was shared by U-50,488 (2·5–10 mg kg^{-1} i.p.), although this compound alone at the high dose reduces urine output in the first hour. The antidiuresis induced by bremazocine in the presence of idazoxan in water-loaded rats was completely antagonized by 10 but not 2 mg kg^{-1} i.p. of the opioid antagonist naloxone. Thus, κ-opioid agonists, in addition to their diuretic effect, also produce an antidiuretic response which may be mediated by α_2-adrenoceptors.

κ-Opioid agonists cause marked diuresis in normally hydrated and water-loaded rats (Slizgi & Ludens 1982; Leander 1983a, b; Huidobro-Toro & Parada 1985; Blackburn et al 1986; Leander et al 1987). This effect has been ascribed to inhibition of vasopressin release with a mechanism different from that of the α_2-adrenoceptor agonist clonidine (Slizgi & Ludens 1982; Leander et al 1985, 1987; Blackburn et al 1986; Oiso et al 1988). However, it was recently reported that the diuresis induced by κ-opioid agonists could be antagonized by the α_2-adrenoceptor antagonist idazoxan (Birch & Hayes 1988), indicating that the noradrenergic system may also be involved. Since α_2-adrenoceptors play a significant role in modulating the activity of vasopressin in the kidney (Pettinger et al 1987) and an interaction between α_2-adrenoceptors and κ-opioid receptors has been reported (Jackisch et al 1986; Limberger et al 1986; Ramme et al 1986; Adamson et al 1988), it was of interest to further investigate the role of these receptors in urination in the rat.