ELSEVIER

European Journal of Pharmacology 351 (1998) 189–191

ejp

Short communication

Methadone analgesia in morphine-insensitive CXBK mice

Albert Chang, David W. Emmel, Grace C. Rossi, Gavril W. Pasternak *

The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan–Kettering Cancer Center, New York, NY 10021, USA

Received 4 May 1998; accepted 12 May 1998

Abstract

Methadone, a potent opioid analgesic, has long been considered a μ-opioid, based upon the similarities between its actions and those of morphine. This classification is supported by the sensitivity of methadone analgesia to the highly μ-opioid receptor-selective antagonist β-funaltrexamine. Yet, CXBK mice respond normally to methadone despite their insensitivity to systemic morphine, distinguishing between the receptor mechanisms of the two drugs. β-Funaltrexamine antagonizes methadone analgesia in CXBK mice, implying that the opioid is still acting through a μ-opioid receptor. These results reveal distinct analgesic mechanisms for morphine and methadone and provide further support for multiple subtypes of μ-opioid receptors.

Keywords: Morphine; Strain; Analgesia; μ-Opioid receptor

1. Introduction

Methadone has long been used as an analgesic in the management of drug abuse (Dole, 1988; Ling et al., 1984; Irwin et al., 1951; Kristensen et al., 1994; Bouvier et al., 1994). It traditionally has been classified as a μ-opiate, displaying high affinity for μ-opioid receptors in binding assays. Recent studies have explored the possibility of μ-opioid receptor subtypes (Pasternak and Standifer, 1995; Pasternak, 1993), raising questions regarding the pharmacologies of many traditional μ-opioid analgesics. Recent work has revealed significant differences in the analgesic mechanisms of morphine and a number of drugs long considered μ-opioids, including fentanyl, heroin and 6-acetylmorphine (Brown et al., 1997; Rossi et al., 1995b, 1996, 1997). The CXBK mouse has proven valuable in the classification of these analgesics. Unlike most strains of mice, CXBK mice are insensitive to systemic morphine (Moskowitz and Goodman, 1985; Vaught et al., 1988; Reith et al., 1981; Baron et al., 1975; Pick et al., 1993). In the present study, we have compared the analgesic actions of methadone in CD-1 and CXBK mice.

2. Materials and methods

Male CD-1 mice (Charles River Laboratories, Raleigh, VA) or CXBK mice (Jackson Laboratories, Bar Harbor, ME) were used in the current studies. Drugs were provided by the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). Antinociception was assessed quantally in the mouse tailflick assay and defined as a doubling or greater of baseline latencies for each mouse, which typically ranged from 2 to 3 s. For convenience, this response is referred to as analgesia. (±)-Methadone was administered, subcutaneously (s.c.) and analgesia assessed at peak effect, which was 30 min. Naloxone and β-funaltrexamine were administered 15 min and 24 h prior to the methadone, respectively. Comparison of individual points was performed using the Fisher Exact test. ED_{50} values with 95% confidence limits were calculated using a computerized Litchfield–Wilcoxon-based program (Tallarida and Murray, 1987).

3. Results

Methadone has been considered a μ-analgesic based upon extensive studies in animal models (Dole, 1988; Ling et al., 1984; Irwin et al., 1951; Kristensen et al., 1994; Bouvier et al., 1994). In the current study, methadone

* Corresponding author. Department of Neurology, Memorial Sloan–Kettering Cancer Center 1275 York Avenue, New York, NY 10021, USA. Tel.: +1-212-639-7046; fax.: +1-212-794-4332; e-mail: pasterng@mskmail.mskcc.org

0014-2999/98/$19.00

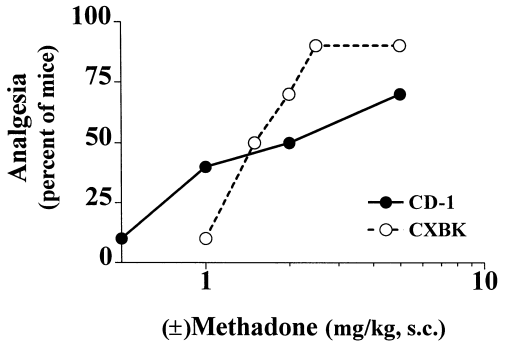

Fig. 1. Methadone analgesia in CD-1 and CXBK mice. Groups of mice ($n \geq 10$) received (±)-methadone at doses from 0.5 to 5 mg/kg, s.c., and were tested for analgesia in the tailflick assay 30 min later. The ED_{50} for the CD-1 and CXBK mice were 2.0 mg/kg, s.c., (1.2, 3.3) and 1.6 mg/kg, s.c., (1.3, 2.0), respectively.

produced a dose dependent analgesia in CD-1 mice (Fig. 1). To assess the receptor selectivity of methadone analgesia in these mice, we examined its sensitivity to the general opioid antagonist naloxone and the μ-selective antagonist β-funaltrexamine (Fig. 2). Both antagonists significantly blocked methadone analgesia, consistent with its prior classification as a μ-opioid analgesic.

CXBK mice are insensitive to systemic morphine (Moskowitz and Goodman, 1985; Vaught et al., 1988; Reith et al., 1981; Baron et al., 1975; Pick et al., 1993). (±)-Methadone, however, retained full potency in CXBK mice with an ED_{50} (1.6 mg/kg, s.c.) slightly lower than in the CD-1 mice (2 mg/kg, s.c.) (Fig. 1). Similar results were seen with (−)-methadone (data not shown). This suggested differences in the mechanisms of methadone and morphine analgesia. The question then arose as to whether the methadone was still acting through μ-opioid receptors in CXBK mice. Again, both naloxone and β-funaltrexamine antagonized (±)-methadone analgesia, implying that

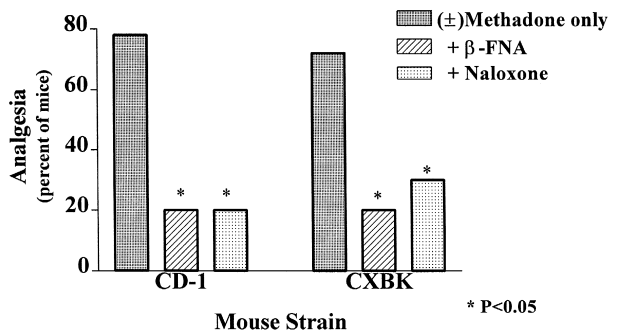

Fig. 2. Effect of opioid antagonists on methadone analgesia. Groups of mice ($n \geq 20$) received (±)-methadone (0.65 mg/kg, s.c.) alone or with naloxone (1 mg/kg, s.c., $n = 10$). The third group received β-funaltrexamine (40 mg/kg, s.c.) 24 h prior to testing with (±)-methadone (0.65 mg/kg, s.c., $n = 10$). Naloxone and β-funaltrexamine significantly decreased the analgesic responses to methadone in both strains of mice ($P < 0.05$).

methadone retains its μ-opioid characteristics in the CXBK mice (Fig. 2). (−)-Methadone analgesia also was equally sensitive to these antagonists (data not shown).

4. Discussion

Our results confirm the μ-opioid receptor classification of methadone analgesia. The sensitivity of methadone analgesia to naloxone indicates an opioid mechanism of action. In addition, the analgesia is readily reversed by β-funaltrexamine, a highly selective μ-opioid receptor antagonist (Ward et al., 1982a,b). Thus, methadone is similar to morphine. Yet, methadone retained its analgesic activity in CXBK mice, a strain which is insensitive to systemic morphine. This difference implies that the actions of methadone are complex and can be dissociated from those of morphine, even if both drugs are within the μ-opioid family.

The receptor systems responsible for methadone analgesia remain unclear. The ability of β-funaltrexamine to antagonize methadone analgesia implies an important role for μ-opioid receptors. Yet, other μ-opioid analgesics also are analgesic in CXBK mice (Rossi et al., 1996). Foremost is morphine-6β-glucuronide, a very potent morphine metabolite (Pasternak et al., 1987; Paul et al., 1989). Morphine-6β-glucuronide analgesia can be distinguished from morphine in several paradigms. As with methadone, morphine-6β-glucuronide retains its analgesic actions in CXBK mice. Antisense mapping of the MOR-1 clone reveals important differences in sensitivity between the 2 agents in both mice and rats, implying different receptor mechanisms of action (Rossi et al., 1995a,b, 1996, 1997). However, the most dramatic difference is seen in a MOR-1 knockout mouse (Schuller et al., data not shown). Disrupting exon 1 in the knockout mouse eliminated morphine analgesia, but morphine-6β-glucuronide retained its analgesic activity in these mice, as did heroin and its active metabolite 6-acetylmorphine. Thus, the CXBK mouse is a valuable model for exploring differences among μ-opioid analgesics. Yet, it is unlikely that methadone acts through the morphine-6β-glucuronide receptor since methadone is not active in the MOR-1 knockout mice (M.A. King, A.G.P. Schuller, J.E. Pintar and G.W. Pasternak, unpublished observations).

Systemically, morphine acts predominantly through μ_1-opioid receptor systems, explaining its inactivity when given systemically in CXBK mice which are deficient in μ_1-opioid receptors (Moskowitz and Goodman, 1985). However, μ_2-opioid receptor analgesic systems are intact in the CXBK mice, as indicated by normal analgesic activity of morphine given spinally (Pick et al., 1993). Thus, it is possible that the actions of methadone may be mediated through a μ_2-opioid receptor system. It will be interesting to define in the future the receptor mechanisms responsible for methadone's actions.

Acknowledgements

This work was supported, in part, by a research grant from the National Institute on Drug Abuse (DA07242) to G.W.P. G.R. is a recipient of a Mentored Scientist Award (DA00310), while G.W.P. is a recipient of a Senior Scientist Award (DA00220) from the National Institute on Drug Abuse.

References

Baron, A., Shuster, L., Elefterhiou, B.E., Bailey, D.W., 1975. Opiate receptors in mice: genetic differences. Life Sci. 17, 633–640.

Bouvier, C., Unteutsch, A., Hagen, S., Zhu, W.Z., Bunzow, J.R., Grandy, D.K., 1994. Agonist properties of methadone at the cloned rat μ opioid receptor. Regul. Pept. 54, 31–32.

Brown, G.P., Yang, K., King, M.A., Rossi, G.C., Leventhal, L., Chang, A., Pasternak, G.W., 1997. 3-Methoxynaltrexone, a selective heroin/morphine-6β-glucuronide antagonist. FEBS Lett. 412, 35–38.

Dole, V.P., 1988. Implications of methadone maintenance for theories of narcotic addiction. J. Am. Med. Assoc. 260, 3025–3029.

Irwin, S., Houde, R.W., Bennett, D.R., Hendershot, L.C., Seevers, M.H., 1951. The effects of morphine, methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. J. Pharmacol. Exp. Ther. 101, 132–143.

Kristensen, K., Christensen, C.B., Christrup, L.L., 1994. The mu_1, mu_2, delta, kappa opioid receptor binding profiles of methadone stereoisomers and morphine. Life Sci. 56, PL45–PL50.

Ling, G.S.F., Tappe, N.S., Inturrisi, C.E., 1984. Methadone induced physical dependence in the rat. Life Sci. 34, 683–690.

Moskowitz, A.S., Goodman, R.R., 1985. Autoradiographic analysis of mu_1, mu_2, and delta opioid binding in the central nervous of C57BL/6BY and CXBK (opioid receptor-deficient) mice. Brain Res. 360, 108–116.

Pasternak, G.W., 1993. Pharmacological mechanisms of opioid analgesics. Clin. Neuropharmacol. 16, 1–18.

Pasternak, G.W., Standifer, K.M., 1995. Mapping of opioid receptors using antisense oligodeoxynucleotides: correlating their molecular biology and pharmacology. Trends Pharmacol. Sci. 16, 344–350.

Pasternak, G.W., Bodnar, R.J., Clark, J.A., Inturrisi, C.E., 1987. Morphine-6-glucuronide, a potent mu agonist. Life Sci. 41, 2845–2849.

Paul, D., Standifer, K.M., Inturrisi, C.E., Pasternak, G.W., 1989. Pharmacological characterization of morphine-6β-glucuronide, a very potent morphine metabolite. J. Pharmacol. Exp. Ther. 251, 477–483.

Pick, C.G., Nejat, R., Pasternak, G.W., 1993. Independent expression of two pharmacologically distinct supraspinal mu analgesic systems in genetically different mouse strains. J. Pharmacol. Exp. Ther. 2265, 166–171.

Reith, M.E.A., Sershen, H., Vadasz, C., Lajtha, A., 1981. Strain differences in opiate receptors in mouse brain. Eur. J. Pharmacol. 74, 377–380.

Rossi, G.C., Pan, Y.-X., Brown, G.P., Pasternak, G.W., 1995a. Antisense mapping the MOR-1 opioid receptor: evidence for alternative splicing and a novel morphine-6β-glucuronide receptor. FEBS Lett. 369, 192–196.

Rossi, G.C., Standifer, K.M., Pasternak, G.W., 1995b. Differential blockade of morphine and morphine-6β-glucuronide analgesia by antisense oligodeoxynucleotides directed against MOR-1 and G-protein α subunits in rats. Neurosci. Lett. 198, 99–102.

Rossi, G.C., Brown, G.P., Leventhal, L., Yang, K., Pasternak, G.W., 1996. Novel receptor mechanisms for heroin and morphine-6β-glucuronide analgesia. Neurosci. Lett. 216, 1–4.

Rossi, G.C., Leventhal, L., Pan, Y.X., Cole, J., Su, W., Bodnar, R.J., Pasternak, G.W., 1997. Antisense mapping of MOR-1 in rats: distinguishing between morphine and morphine-6β-glucuronide antinociception. J. Pharmacol. Exp. Ther. 281, 109–114.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacological Calculations with Computer Programs. Springer, New York, p. 153.

Vaught, J.L., Mathiasen, J.H.R., Raffa, R.B., 1988. Examination of the involvement of supraspinal and spinal mu and delta opioid receptors in analgesia using the mu receptor deficient CXBK mouse. J. Pharmacol. Exp. Ther. 245, 12–16.

Ward, S.J., Portoghese, P.S., Takemori, A.E., 1982a. Pharmacological characterization in vivo of the novel opiate, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220, 494–498.

Ward, S.J., Portoghese, P.S., Takemori, A.E., 1982b. Pharmacological profiles of β-funaltrexamine (β-FNA) and β-chlornaltrexamine (β-CNA) on the mouse vas deferens preparation. Eur. J. Pharmacol. 80, 377–384.