JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1109–1115

Received December 21, 2006
Accepted April 24, 2007
DOI 10.1211/jpp.59.8.0008
ISSN 0022-3573

Involvement of kappa opioid receptors in formalin-induced inhibition of analgesic tolerance to morphine in mice

Shogo Tokuyama, Ryuji Nagae, Emiko Mashida and Wakako Hamabe

Abstract

This study examined the role of kappa opioid receptors (KOR) in the mechanism underlying tolerance to the analgesic effects of morphine induced by chronic pain. The analgesic effect of morphine (10 mg kg^{-1}), estimated by the tail-flick test in mice, gradually decreased during repeated daily morphine treatment. A significant decrease in the analgesic effect of morphine was seen on the fifth day of repeated morphine treatment compared with the first day. Chronic pain was induced by subcutaneous administration of 2% formalin into the dorsal part of the left hind paw, which significantly inhibited development of tolerance to morphine analgesia. The effect of formalin-induced pain on inhibition of morphine tolerance was reversed by the KOR antagonist nor-binaltorphimine. Furthermore, an antisense oligodeoxynucleotide, but not a missense oligodeoxynucleotide, against KOR completely suppressed the inhibitory effect of formalin-induced pain on morphine tolerance. Naltrindole, an antagonist of delta opioid receptor, did not affect chronic-pain-induced tolerance to morphine. Our findings show that the inhibitory effect of chronic pain on analgesic tolerance to morphine is mediated by KOR rather than delta opioid receptors.

Introduction

The narcotic analgesic morphine is used to ameliorate cancer pain. Prolonged use of morphine is limited by the development of tolerance and dependence induced by chronic administration (Ellison 1993). However, Suzuki (2001) proposed that patients with cancer who are in a state of chronic pain do not develop tolerance to or dependence on the effect of morphine. The results of basic research have also shown that the development of morphine tolerance can be inhibited in an in-vivo chronic pain model (Javan et al 2005), although the mechanism by which tolerance to and dependence on morphine are avoided under these conditions is as yet unclear. The well-established animal model of persistent somatic pain is considered to be a model of clinical inflammatory pain and nerve sensitization (Mochizucki, 2004). It consists of subcutaneous injection of formalin into a hind paw of mice or rats. Here, we have developed a chronic pain model that involves moderate continuous pain generated by injured tissue after subcutaneous injection of formalin into the hind paw of the mouse (Iyengar et al 2004).

Opioid receptors are classified as mu, delta or kappa (Kieffer 1995; Kieffer & Evans 2002). Mu opioid receptors (MOR) are the primary site of action of morphine, contributing to most opioid-induced effects, including analgesia and the development of tolerance and dependence (Kieffer & Evans 2002). Delta opioid receptors (DOR) play a role in analgesia and the modulation of emotions. Kappa opioid receptors (KOR) are involved in analgesia, sedation and aversion (Reisine & Pasternak 1996; Snyder & Pasternak 2003). Many reports have shown functional interactions between these opioid receptors (Jordan & Devi 1999; Gray et al 2006; Snook et al 2006). In particular, KOR and MOR are known to interact to mediate various opioid effects. Specifically, the development of tolerance to morphine analgesia, naloxone-precipitated morphine withdrawal symptoms (an indicator of physical dependence) and the morphine-induced rewarding effect (an indicator of psychological dependence) are inhibited by KOR agonists. Furthermore, behavioural studies have shown that MOR and KOR play distinct roles in learning and memory, as well as in synaptic

Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, 1-1-3, Minatojima, Chuo-ku, Kobe, 650-8586, Japan

Shogo Tokuyama, Ryuji Nagae, Emiko Mashida, Wakako Hamabe

Correspondence: Shogo Tokuyama PhD, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, 1-1-3, Minatojima, Chuo-ku, Kobe, 650-8586, Japan. E-mail: stoku@pharm.kobegakuin.ac.jp

plasticity (Suzuki et al 1992; Pan 1998; Jamot et al 2003). These findings suggest that the opposing actions of KOR agonists on the MOR effect may play an important role in the inhibition of morphine tolerance.

In this study we investigate the role of KOR in the mechanism underlying the formalin-induced inhibition of morphine tolerance using pharmacological approaches in-vivo. We used the KOR agonist U-50488H in combination with repeated morphine to clarify whether stimulation of KOR suppresses the development of tolerance to morphine analgesia.

Materials and Methods

Animals

Male ddY mice weighing 18–20 g (purchased from Saitama Experimental Animals, Saitama, Japan) were housed eight per group in plastic bracket cages maintained at a constant temperature of 22 ± 1°C. They had access to food and tap water ad libitum and were used in experiments at a body weight of 23–28 g. All experimental procedures conformed with the Guiding Principles for the Care and Use of Laboratory Animals adopted by the Japanese Pharmacological Society.

Chemicals

The following drugs and substances were used: morphine hydrochloride (Takeda, Osaka, Japan), U-50488H (trans-3, 4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl) cyclohexyl)-benzeneacetamide methanesulfonate hydrate), nor-binaltorphimine (nor-BNI), naltrindole hydrochloride (all Sigma Chemicals, St Louis, MO, USA); formalin (37%; Wako, Osaka, Japan), antisense (AS-ODN) and missense oligodeoxynucleotides (MS-ODN) against KOR (Chien et al 1994) (5′-GGTGCCTCCAAGGACTATCGC-3′ and 5′-GGGTCCCTCAAGGCATACTGC-3′ respectively; Sawadee, Tokyo, Japan).

Morphine hydrochloride, U-50488H, nor-BNI and naltrindole hydrochloride were dissolved in physiological saline, and administered subcutaneously (s.c.) or intraperitoneally (i.p.) in a volume equivalent to 0.1 mL per 10 g body weight. A 2% solution of formalin was prepared with ultrapure water and 20 μL administered s.c. AS-ODN and MS-ODN were dissolved in physiological saline, and 20 μL administered intracerebroventricularly (i.c.v.) according to the method of Haley & McCormick (1957).

Pain inducement and evaluation of oedema

Formalin (20 μL 2% solution) was administered s.c. into the dorsal part of the left hind paws of the mice. To determine the degree of swelling at the application site, the thickness of the induced oedema was measured using a thickness gauge (Teclock, Nagano, Japan). Oedema was calculated from the following formula: increment of footpad thickness (oedema of foot in mm) = (thickness of left foot at the indicated time after formalin administration − thickness of right foot at the same time) − (thickness of left foot before formalin administration − thickness of right foot before formalin administration). Control mice were injected with saline.

Measurement of pain threshold

Pain threshold was assessed using an analgesy meter (Ugo Basile, Comerio VA, Italy) according to the modified Randall–Selitto method (Randall & Selitto 1957). Pressure was gradually applied to the oedematous paw, and the weight at which the mouse removed the paw was recorded. The pain threshold before formalin administration was taken as 100%. Pain threshold were determined daily.

Measurement of analgesic effect

The analgesic effect of morphine was measured using a tail-flick analgesia meter (MK-330B, Muromachi Kikai, Tokyo, Japan). Thermal stimulation was applied to the base of the tail and the time until the tail was flicked was measured. To avoid tissue damage, thermal stimulation was never applied for longer than 10 s. Measurements were taken every 30 min for 90 min after administration of morphine. To test the development of tolerance to morphine analgesia, the analgesic effect of morphine (10 mg kg^{-1} s.c.) was measured once daily for 5 or 14 days. The difference in tail-flick time on each day compared with the first day was plotted against time (day of treatment) and the area under the curve (AUC) calculated. In the formalin-pretreated mice (and saline controls), repeated morphine administration (for 5 or 14 days) was started 24 h after formalin treatment.

For experiments with naltrindole, nor-BNI and U-50488H, the agonist/antagonist was injected daily for 5 days, starting 24 h after the first dose of morphine. Naltrindole (2 mg kg^{-1} i.p.) or nor-BNI (1 mg kg^{-1} i.p.) were injected 10 min before administration of morphine; U-50488H (2 mg kg^{-1} i.p.) was injected 5 min after administration of morphine.

Administration of oligodeoxynucleotides

AS-ODN or MS-ODN (10 μg, i.c.v.) was administered daily for 3 days before measurements of oedema or pain threshold, and for 4 days after the start of these measurements. When evaluating the analgesic effects of morphine, AS-ODN or MS-ODN (10 μg, i.c.v.) was administered daily for 3 days before the start of the measurement of analgesic effects, and AS-ODN or MS-ODN was also administered on days 1–3 of the repeated morphine treatment.

Statistical analysis

Data are presented as mean ± s.e.m. Statistical significance was assessed using a one-way repeated analysis of variance followed by Dunnett's test. Differences were regarded as significant when the P value was less than 0.05.

Results

Effect of formalin treatment on pain threshold

Formalin provoked intermittent licking or biting 10–15 min after s.c. administration to the dorsal part of the left hind paws of the mice, and swelling and reddening were observed at the application site 3 h after administration.

Compared with the untreated paw, significant swelling was observed at the application site. Swelling peaked 3 h after administration and persisted for at least 10 days (Figure 1A). The nociceptive sensitivity measured by the Randall–Selitto method also peaked 3 h after administration. Hypersensitivity was maintained for 10 days, but nociceptive sensitivity returned to normal 2 weeks after administration (Figure 1B).

Effect of formalin treatment on morphine analgesia

On day 1 of the repeated morphine treatment, a prominent analgesic effect of morphine (10 mg kg^{-1}, s.c) was observed 30 min after administration and lasted for at least 90 min, in both control and formalin-treated mice (Figure 2A). However, in control mice, the analgesic effect gradually

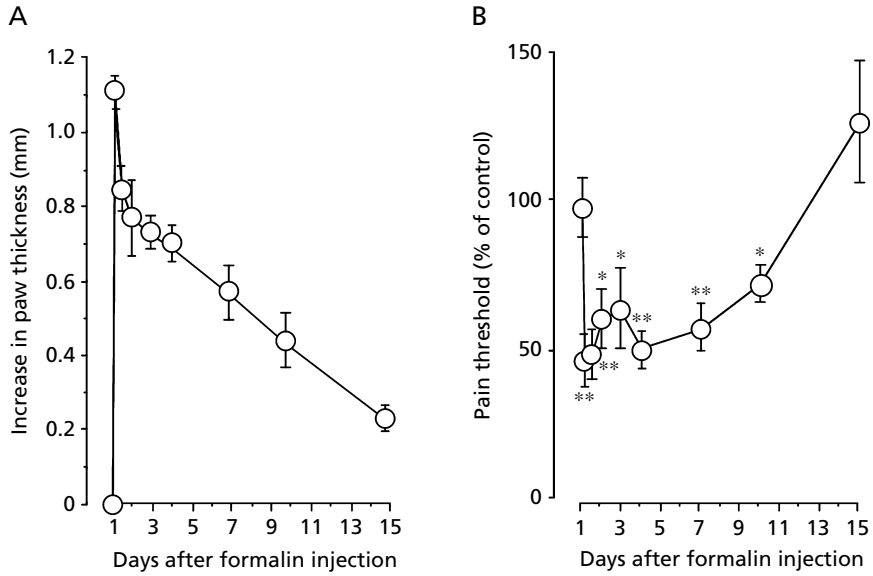

Figure 1 Time course of the changes in paw thickness (A) and pain threshold (measured using a modified Randall–Selitto method (B) after formalin administration (20 μL 2% solution injected s.c. into the dorsal part of the left hind paw). Data are mean ± s.e.m., n = 8 mice. *$P < 0.05$; **$P < 0.01$ compared with the value on day 1.

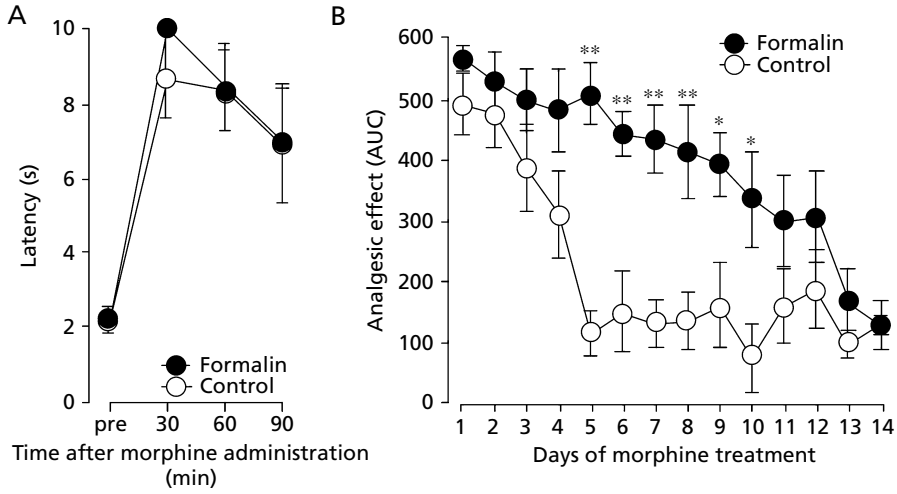

Figure 2 Analgesic effects of morphine and the formalin-induced inhibition of tolerance to morphine analgesia. Formalin (20 μL 2% solution) was injected s.c. into the dorsal part of the left hind paw; control mice were treated with saline. All mice were given morphine (10 mg kg^{-1}) daily for 14 days, starting 24 h after formalin/saline treatment. The analgesic effect after morphine administration was measured using the tail-flick method. A. The analgesic effect of morphine on day 1. B. Change in the analgesic effect of morphine with repeated daily morphine administration (expressed as the area under the curve (AUC) of the increase in response time plotted against time interval). Data are mean ± s.e.m., n = 8 mice. *$P < 0.05$; **$P < 0.01$ vs control.

decreased from day 3 of repeated morphine treatment, and was completely diminished by day 5 (Figure 2B). The decrement in morphine analgesia (i.e. morphine tolerance) was maintained until day 14 of repeated morphine treatment in control mice, whereas in formalin-treated mice morphine tolerance was significantly inhibited on day 5 of repeated morphine treatment and this inhibition lasted until day 10 (Figure 2B).

Effects of nor-binaltorphimine and an antisense oligodeoxynucleotide against kappa opioid receptor

When we administered morphine (10 mg kg^{-1}, s.c.) daily for 5 days, a significant decrement in morphine analgesia (morphine tolerance) was observed on days 3–5 of repeated morphine treatment; formalin treatment significantly inhibited morphine tolerance (Figure 3A). However, when the KOR antagonist nor-BNI (1 mg kg^{-1}) was administered 10 min before morphine administration, formalin-induced inhibition of morphine tolerance was significantly inhibited (Figure 3A). Furthermore, administration of the AS-ODN (10 μg, i.c.v.) completely suppressed the formalin-induced inhibition of morphine tolerance, and morphine tolerance developed on day 4–5 of repeated morphine administration (Figure 3B). MS-ODN had no effect (Figure 3B). AS-ODN completely inhibited the analgesic effect of the KOR agonist U-50488H (20 mg kg^{-1}), whereas MS-ODN had no effect (data not shown).

Effect of naltrindole on formalin-induced inhibition of development of analgesic tolerance to morphine

Administration of the DOR antagonist naltrindole (2 mg kg^{-1}, i.p.) 10 min before daily morphine administration did not affect inhibition of morphine tolerance in formalin-treated mice (Figure 4). Naltrindole had no effect on analgesia or the development of analgesic tolerance to morphine (data not shown).

Effect of U-50488H on the development of analgesic tolerance to morphine

On day 1 of repeated morphine treatment, morphine had a prominent analgesic effect after 30 min that lasted for at least 90 min, in both control and U-50488H-treated mice (Figure 5A). In control mice, this analgesic effect gradually decreased from day 3 of repeated morphine treatment, and was completely diminished by day 5, showing that tolerance to the analgesic effect of morphine had developed (Figure 5B). In U-50488H-treated mice, the initial analgesic effect persisted for 5 days, and the development of morphine tolerance was inhibited (Figure 5B). After day 5, when concomitant U-50488H treatment was ceased, tolerance developed in the same way as in the control group (Figure 5B).

Discussion

Although the development of tolerance to morphine analgesia is thought to limit its prolonged use in the treatment of pain

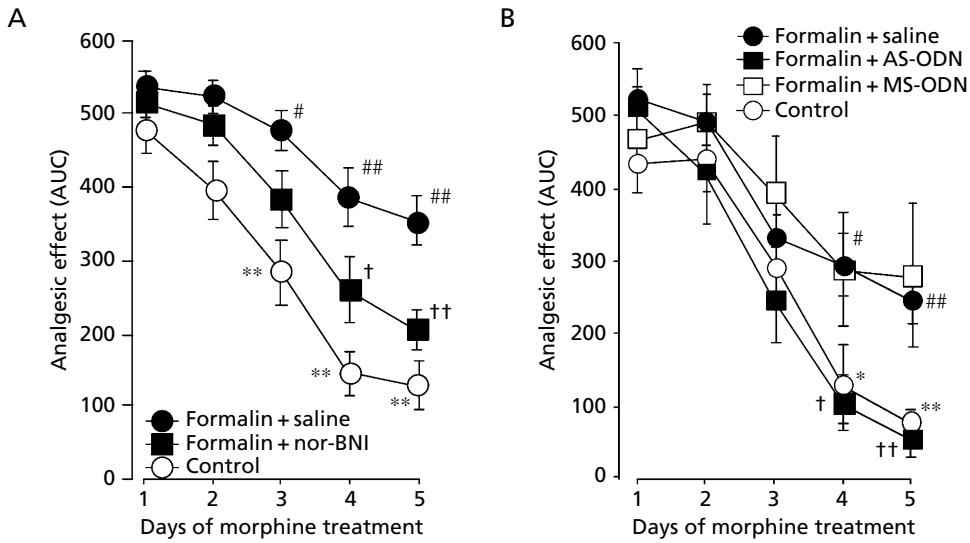

Figure 3 Effects of nor-binaltorphimine (nor-BNI; a kappa opioid receptor (KOR) antagonist) and antisense (AS-ODN) and missense oligodeoxynucleotides (MS-ODN) against KOR on the formalin-induced inhibition of tolerance to morphine analgesia (expressed as the area under the curve (AUC) of the increase in response time plotted against time interval). A. Beginning 24 h after formalin administration, mice were treated daily with nor-BNI (1 mg kg^{-1} s.c.) 10 min before morphine (10 mg kg^{-1} s.c.) for 5 days. The analgesic effect of morphine was measured using the tail-flick method. The control group were treated daily with saline instead of formalin and nor-BNI. Data are mean ± s.e.m., n = 18 mice. B. AS-ODN and MS-ODN (both 10 μg in 10 μL i.c.v.) or saline (10 μL, i.c.v.) were administered for 3 days before and 4 days after injection of formalin. All mice were given morphine daily (10 mg kg^{-1} s.c.) for 5 days, starting 24 h after formalin treatment. Control mice were treated with saline instead of formalin, AS-ODN or MS-ODN. Data are mean ± s.e.m., n = 6 mice. *$P < 0.05$, **$P < 0.01$ vs value on day 1. #$P < 0.05$, ##$P < 0.01$ vs vehicle control; †$P < 0.05$, ††$P < 0.01$ vs formalin + saline treatment.

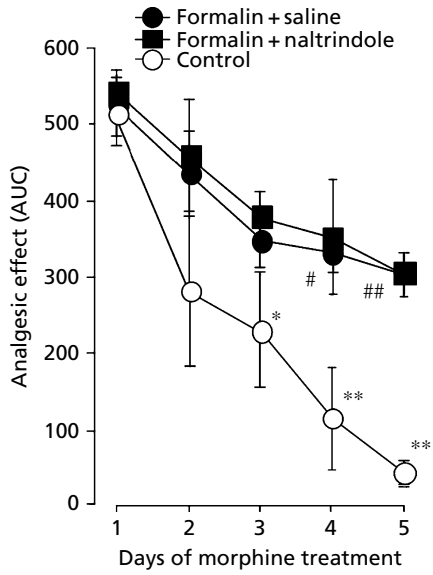

Figure 4 Effect of naltrindole on formalin-induced inhibition of tolerance to morphine analgesia (expressed as the area under the curve (AUC) of the increase in response time plotted against time interval). Formalin (20 μL 2% solution) was injected s.c. into the dorsal part of the left hind paw. After 24 h mice were treated daily with naltrindole (2 mg kg^{-1} i.p.), 10 min before administration of morphine (10 mg kg^{-1}, s.c.) for 5 days. The analgesic effect after morphine administration was measured using the tail-flick method. Control mice were given saline instead of formalin or naltrindole. Data are mean ± s.e.m., n = 5 mice. *$P < 0.05$, **$P < 0.01$ vs value on day 1. #$P < 0.05$, ##$P < 0.01$ vs vehicle control.

(Ellison 1993), it has been suggested that pain conditions may inhibit the development of tolerance to the analgesic effect of opioids (Suzuki 2001). In this study, we confirmed that the decrease in the analgesic effect of morphine with repeated daily administration was completely inhibited by chronic formalin-induced pain (Figure 2). Here, formalin-related mechanical hyperalgesia was shown by assessment of paw-withdrawal thresholds in the Randall–Selitto paw-pressure test. Our observations show an increment in sensitivity to mechanical noxious stimuli after formalin injection.

We assessed the effect of morphine on sensory responses using the tail-flick test, in which the time to movement of the tail from a noxious heat source is measured. This test reflects the activity of a simple spinal reflex arc and provides information on peripheral nerve and spinal function in isolation from higher nociceptive processing and cognitive systems (Ilnytska et al 2006).

Although there are many reports on the mechanism underlying the inhibitory effect of pain on the development of tolerance to the analgesic effect of opioids, this mechanism is not yet fully understood (Rahman et al 1994; Javan et al 2005). We focused on the interactions between opioid receptors, which have been implicated in the development of morphine tolerance and physical dependence (Daniels et al 2005). MOR is an important opioid receptor that mediates analgesic effects. Signalling pathways downstream of KOR and DOR are known to interact with the pathways mediating this effect of MOR. For example, dynorphin and U-50488H, which are KOR agonists, inhibit the development of tolerance to the analgesic effect of morphine induced by repeated morphine administration; this inhibitory effect is blocked by nor-BNI, a

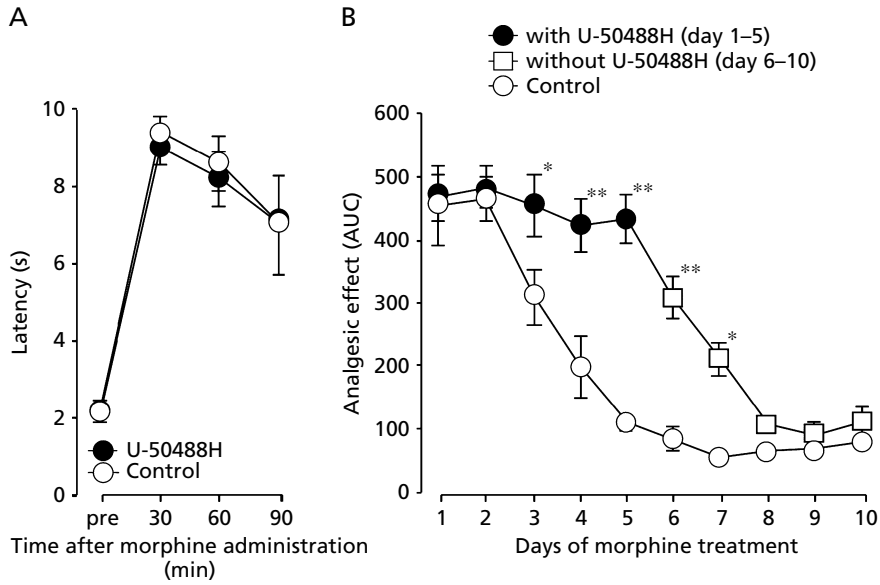

Figure 5 Blockade of tolerance to morphine analgesia by the kappa opioid receptor agonist U-50488H (2 mg kg^{-1} i.p.) administered 5 min after injection of morphine (10 mg kg^{-1} s.c.) daily for 5 days. Mice were given morphine alone from day 6. The analgesic effect of morphine was measured using the tail-flick method. A. Analgesic effect on day 1. B. Analgesic effect is expressed as the area under the curve (AUC) of the increase in response time plotted against time intervals. Control mice were given saline instead of U-50488H. Each point indicates the mean ± s.e.m., n = 8 mice. *$P < 0.05$; **$P < 0.01$ vs controls.

KOR antagonist (Takahashi et al 1991; Takemori et al 1993; Tao et al 2000). We found that formalin-induced inhibition of morphine tolerance was prevented by nor-BNI (Figure 3A), suggesting that KOR may play an important role in the mechanism underlying the inhibition of morphine tolerance in the chronic pain condition induced by formalin. Furthermore, an AS-ODN against KOR significantly blocked the inhibitory effect of formalin (Figure 3B). It has been reported that KOR and MOR exert opposite effects. Systemic or local administration to the ventrotegmental area of morphine or DAMGO (D-Ala(2), NMe-Phe(4), Gly(5) ol]enkephalin), a MOR agonist, has been observed to increase dopamine levels or promote dopamine release in the limbic system of the midbrain. Conversely, dopamine levels in the nucleus accumbens (the location of the dopamine terminals of the ventrotegmental region) decrease following systemic or local administration of KOR agonists such as U-50488H or U-69593 (Spanagel et al 1992; Devine et al 1993). Furthermore, behavioural studies have reported that MOR activation impairs learning and memory, while KOR agonists reverse this impairment (Itoh et al 1994). In-vitro studies have shown opposite effects of these receptors on neurotransmission in the hippocampus, an important region for learning and memory (Simmons & Chavkin 1996; Jamot et al 2003). In addition, it is known that acute administration of MOR agonists causes euphoria in humans, whereas KOR agonists cause anxiety or aversion (Vaupel et al 1993; Vetulani 2001; Narita et al 2002). These opposing actions of KOR and MOR could explain the observed KOR-mediated inhibition of the development of morphine tolerance induced by chronic pain. However, we could not elucidate the detailed mechanism of interaction between KOR and MOR under the chronic pain condition in this study.

The DOR antagonist naltrindole had no effect on the development of analgesic tolerance to morphine (Figure 4). Thus, DOR do not seem to be involved in the inhibition of morphine tolerance induced by chronic pain.

These findings indicate that KOR, but not DOR, play important roles in the mechanism underlying inhibition of morphine tolerance induced by chronic pain. Whether chronic pain causes functional changes in KOR in morphine-treated animals remains to be determined. Nevertheless, morphine has been shown to up-regulate KOR gene expression (Suzuki et al 2001), and we have shown that chronic morphine administration leads to increased expression of KOR protein in the midbrain (unpublished data). In addition, Narita et al (2005) reported that chronic pain facilitates the endogenous kappa opioidergic system, suggesting the possibility of an involvement of KOR activation in chronic-pain-induced suppression of tolerance to morphine analgesia. Furthermore, it has been suggested that psychological stress and social defeat stress, which may suppress tolerance to morphine analgesia, stimulate KOR (Fukunaga et al 1999; McLaughlin et al 2006). Thus, the link between KOR and anxiety, or some other psychological factors, in the chronic-pain-induced inhibition of morphine tolerance is also attracting attention. This link remains to be examined.

Finally, we found that the KOR agonist U-50488H completely suppressed the development of tolerance to the analgesic effects of morphine (Figure 5), suggesting that KOR activation may prevent development of morphine tolerance in animals in a state of chronic pain. This is in line with previous reports showing that the coadministration of a KOR agonist and morphine reduced the development of tolerance to morphine (Tao et al 1994; Lee et al 1997; Jang et al 2006). Furthermore, our observations suggest that KOR stimulation will prevent the apparent loss of opioid analgesia upon repeated opioid administration, and may therefore increase the clinical benefits of opioid analgesics.

Conclusions

Using a chronic pain model in mice, we have reproduced the inhibitory effect of pain on the development of tolerance to morphine analgesia, which is known to occur in patients. KOR have a significant role in the mechanism underlying prevention of morphine tolerance, whereas DOR have little involvement. Although patients with chronic pain do not readily develop morphine tolerance, fairly high doses of morphine may be used when treatment is extended over a long period of time. Our results suggest that stimulation of KOR would avoid the need for such high doses of morphine and the development of morphine tolerance, and may therefore be useful in the clinic.

References

Chien, C. C., Brown, G., Pan, Y. X., Pasternak, G. W. (1994) Blockade of U50488H analgesia by antisense oligodeoxynucleotides to a k opioid receptor. *Eur. J. Pharmacol.* **253**: R7–8

Daniels, D. J., Lenard, N. R., Etienne, C. L., Law, P. Y., Roerig, S. C., Portoghese, P. S. (2005) Opioid induced tolerance and dependence in mice is modulated by the distance between pharmacolphores in a bivalent ligand series, *Proc. Natl. Acad. Sci. USA* **102**: 19208–19213

Devine, D. P., Leone, P., Pocock, D., Wise, R. A. (1993) Differential involvement of ventral tegmental mu, delta and kappa opioid receptors in modulation of basal mesolimbic dopamine release: in-vivo microdialysis studies. *J. Pharmacol. Exp. Ther*. **266**: 1236–1246

Ellison, N. M. (1993) Opioid analgesics for cancer pain: toxicities and their treatment. In: Patt, R. B. (eds) *Cancer pain*. Philadelphia: JB Lippincott, pp 185–194

Fukunaga, H., Takahashi, M., Kaneto, H., Yoshikawa, M. (1999) Effects of Tyr MIF 1 on stress induced analgesia and the blockade of development of morphine tolerance by stress in mice. *Jpn J. Pharmacol*. **79**: 231–235

Gray, A. C., Coupar, I. M., White, P. J. (2006) Comparison of opioid receptor distribution in the rat central nervous system. *Life Sci*. **79**: 674–685

Haley, T. J., McCormick, W. G. (1957) Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmacol. Chemother*. **12**: 12–15

Ilnytska, O., Lyzogubov, V. V., Stevens, M. J., Drel, V. R., Mashtalir, N., Pacher, P., Yorek, M. A., Obrosova, I. G. (2006) Poly(ADP ribose) polymerase inhibition alleviates experimental diabetic sensory neuropathy. *Diabetes* **55**: 1686–1694

Iyengar, S., Webster, A. A., Hemrick Luecke, S. K., Xu, J. Y., Simmons, R. M. (2004) Efficacy of duloxetine, a potent and balanced serotonin norepinephrine reuptake inhibitor, in persistent pain models in rats. *J. Pharmacol. Exp. Ther*. **311**: 576–584

Itoh, J., Ukai, M., Kameyama, T. (1994) Dynorphin A (1–13) potently improves the impairment of spontaneous alternation

performance induced by the mu selective opioid receptor agonist DAMGO in mice. *J. Pharmacol. Exp. Ther*. **269**: 15–21

Jamot, L., Matthes, H.W. D., Simonini, F., Kieffer, B. L., Roder, J. C. (2003) Differential involvement of the Mu and Kappa opioid receptors in spatial learning. *Genes Brain Behav*. **2**: 80–92

Jang, S., Kim, H., Kim, D., Jeong, M. W., Ma, T., Kim, S., Ho, I. K., Oh, S. (2006) Attenuation of morphine tolerance and withdrawal syndrome by coadministration of nalbuphine. *Arch. Pharm. Res*. **29**: 677–684

Javan, M., Ahmadiani, A., Motamedi, F., Kazwmi, B. (2005) Changes in G protein gene expression in rat lumbar spinal cord support the inhibitory effect of chronic pain on the development of tolerance to morphine analgesia. *Neurosci. Res*. **53**: 250–256

Jordan, B. A., Devi, L. A. (1999) G protein coupled heterodimerization modulates receptor function. *Nature* **399**: 697–700

Kieffer, B. L. (1995) Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. *Cell. Mol. Neurobiol*. **15**: 615–635

Kieffer, B. L., Evans, C. J. (2002) Opioid tolerance – in search of the Holy Grail. *Cell* **108**: 587–590

Lee, S. C., Wang, J. J. Ho, S. T., Tao, P. L. (1997) Nalbuphine coadministered with morphine prevents tolerance and dependence. *Anesth Analg* **84**: 810–815

McLaughlin, J. P., Li, S., Valdez, J., Chavkin, T. A., Chavlin, C. (2006) Social defeat stress induced behavioral responses are mediated by the endogeneous kappa opioid system. *Neuropsychopharmacology* **31**: 1241–1248

Mochizucki, D. (2004) Serotonin and noradrenaline reuptake inhibitors in animal models of pain. *Hum. Psychopharmacol.* **19 Suppl 1**: S15–19

Narita, M., Ozaki, S., Suzuki, T. (2002) Endomorphin induced motivational effect: differential mechanism of endomorphin 1 and endomorphin 2. *Jpn J. Pharmacol*. **89**: 224–228

Narita, M., Kishimoto, Y., Ise, Y., Yajima, Y., Misawa, K., Suzuki, T. (2005) Direct evidence for the involvement of the mesolimbic kappa opioid system in the morphine induced rewarding effect under an inflammatory pain like state. *Neuropsychopharmacology* **30**: 111–118

Pan, Z. Z. (1998) mu-Opposing actions of the kappa opioid receptor. *Trends Pharmacol. Sci*. **19**: 94–98

Rahman, A. F., Takahashi, M., Kaneto, H. (1994) Involvement of pain associated anxiety in the development of morphine tolerance in formalin treated mice. *Jpn J. Pharmacol*. **65**: 313–317

Randall, L. O. Selitto, J. J. (1957) A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn. Ther*. **111**: 409–419

Reisine, T., Pasternak, G. (1996) Opioid analgesics and antagonists. In: Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W. (eds) *Goodman and Gilman's the pharmacological basis of therapeutics*, 9th edn. New York: McGraw Hill, pp 521–555

Simmons, M. L., Chavkin, C. (1996) Endogenous opioid regulation of hippocampal function. *Int. Rev. Neurobiol*. **39**: 145–196

Snook, L. A., Milligan, G., Kieffer, B. L., Massotte, D. (2006) Mu delta opioid receptor functional interaction: Insight using receptor G protein fusions. *J. Pharmacol. Exp. Ther*. **318**: 683–690

Snyder, S. H., Pasternak, G. W. (2003) Historical review: Opioid receptors. *Trends Pharmacol. Sci*. **24**: 198–205

Spanagel, R., Herz, A., Shippenberg, T. S. (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. USA* **89**: 2046–2050

Suzuki, S., Chuang, T. K., Chuang, L. F., Doi, R. H., Chuand, R. Y. (2001) Morphine upregulates kappa opioid receptors of human lymphocytes. *Adv. Exp. Med. Biol*. **493**: 81–87

Suzuki, T. (2001) Modification of morphine dependence under chronic pain and its mechanism. *Yakugaku Zasshi* **121**: 909–914

Suzuki, T., Narita, M., Takahashi, T., Misawa, M., Nagase, H. (1992) Effects of nor binaltorphimine on the development of analgesic tolerance to and physical dependence on morphine. *Eur. J. Pharmacol*. **213**: 91–97

Takahashi, M., Senda, T., Kaneto, H. (1991) Role of spinal kappa opioid receptors in the blockade of the development of antinociceptive tolerance to morphine. *Eur. J. Pharmacol*. **200**: 293–297

Takemori, A. E., Loh, H. H., Lee, N. M. (1993) Suppression by dynorphin A and [des Tyr1]dynorphin A peptides of the expression of opiate withdrawal and tolerance in morphine dependent mice. *J. Pharmacol. Exp. Ther*. **266**: 121–124

Tao, P. L., Hwang, C. L., Chen, C. Y. (1994) U 50,488 blocks the development of morphine tolerance and dependence at a very low dose in guinea pigs. *Eur. J. Pharmacol.* **256**: 281–286

Tao, P. L., Wu, S. C., Yang, C. H., Wu, C. C. (2000) Study the mechanisms of U50488H to prevent the development of morphine tolerance in guinea pigs. *Chin. J. Physiol*. **43**: 179–184

Vaupel, D. B., Lange, W. B., London, E. D. (1993) Effects of verapamil on morphine induced euphoria, analgesia and respiratory depression in humans. *J. Pharmacol. Exp. Ther*. **267**: 1386–1394

Vetulani, J. (2001) Drug addction. Part I. Psychoactive substances in the past and presence. *Pol J Pharmacol*. **53**: 201–214