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European Journal of Pharmacology 312 (1996) 349–355

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Pharmacological characterization of KT-90 using cloned μ-, δ- and κ-opioid receptors

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Received 11 June 1996; accepted 14 June 1996

Abstract

We analyzed the pharmacological characteristics of (−)-3-acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine (KT-90) using Chinese hamster ovary (CHO) cells expressing cloned rat μ-, δ- and κ-opioid receptors. KT-90 displaced the specific binding of the following radiolabeled ligands selective to the μ-, δ- and κ-opioid receptors, [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO), [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE), [^{3}H] (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8-yl]benzeneacetamide (U69,593), with K_i values of 3.3 ± 0.7, 22.8 ± 1.5 and 1.9 ± 0.3 nM, respectively. In CHO cells expressing the μ-, δ- and κ-opioid receptors, KT-90 inhibited forskolin (10 μM)-induced cyclic AMP accumulation in a concentration-dependent manner with IC_{50} values of 2337 ± 750, 17.3 ± 4.6 and 2.0 ± 0.1 nM, respectively. The maximal inhibitory effects of KT-90 in the cells expressing μ-, δ- and κ-opioid receptors were significantly lower than those of the type-selective agonists DAMGO, DPDPE and U69,593, respectively. These results indicated that KT-90 acts as a partial agonist on μ-, δ and κ-opioid receptors. KT-90 (10 and 100 nM), when added with morphine, produced a rightward shift of the concentration-response curve of morphine to inhibit the cyclic AMP accumulation in CHO cells expressing μ-, but not δ- or κ-, opioid receptors. This finding is consistent with the findings that lower doses of KT-90 antagonize morphine analgesia in vivo.

Keywords: KT-90 ((−)-3-acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine); Opioid receptor; Binding characteristics; Partial agonist; cAMP

1. Introduction

The compound (−)-3-acetyl-6β-acetylthio-*N*-cyclopropylmethyl-normorphine (KT-90) has acetyl, acetylthio and *N*-cyclopropylmethyl groups instead of hydroxy groups at the 3- and 6-positions and of the *N*-methyl group at the 17-position of morphine, respectively (Fig. 1) (Kanematsu et al., 1990). KT-90 binds to μ-, δ- and κ-opioid receptors with 4-, 19- and 13-fold higher affinity than morphine in the rat brain membrane preparations (Takayanagi et al., 1990b). In the rabbit vas deferens which exclusively contains κ-opioid receptors (Oka et al., 1980), KT-90 inhibits the electrically stimulated twitch response about 6-fold more potently than dynorphin A-(1–13) (Takayanagi et al., 1990b). On the other hand, in the guinea-pig ileum which contains functional μ- and κ-opioid receptors (Hutchinson et al., 1975; Lord et al., 1977; Miller et al., 1986), the inhibitory effect of morphine is inhibited by KT-90 at concentrations which are too low to produce an effect by itself (Takayanagi et al., 1990b). Because morphine preferentially binds to the μ-opioid receptor, these results suggested that KT-90 acts on κ-opioid receptor as an agonist and on the μ-opioid receptor as an antagonist at least in lower doses.

The antinociceptive effects of an s.c. injection of KT-90 are 5–10 times as potent as morphine in rat paw pressure test and in the mouse acetic acid-induced writhing test (Takayanagi et al., 1990b). An i.c.v. injection of KT-90 reportedly produces antinociception in the mouse hot plate test. This antinociceptive effect of KT-90 is antagonized by norbinaltorphimine, a κ-opioid receptor antagonist, but not by β-funaltrexamine, a μ-opioid receptor antagonist

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PII S0014-2999(96)00469-4

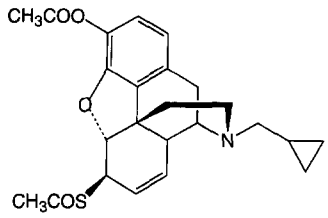

Fig. 1. Structure of KT-90.

(Takayanagi et al., 1990a). On the other hand, lower doses of KT-90 antagonize morphine analgesia (Takayanagi et al., 1990a). In addition to the antinociceptive effects, KT-90 reportedly produces conditioned place aversion in mice (Takayanagi et al., 1990a) as well as κ-selective agonists, such as U50,488H (Mucha and Herz, 1985; Bals-Kubik et al., 1989). These findings support the notion that KT-90 acts on the κ-opioid receptor as an agonist and on the μ-opioid receptor as an antagonist, while its effect on the δ-opioid receptor is unclear.

The pharmacological properties of opioid ligands have been characterized using brain membrane and isolated tissue preparations in vitro. However, tissue preparations purely expressing a single type of opioid receptor are unusual. Furthermore, the distribution of μ-, δ- and κ-opioid receptors varies among tissue preparations. Usually, the circumstances for the signal transduction of opioids in the tissue preparations, such as the species and amounts of GTP-binding proteins, adenylyl cyclases, Ca^{2+} channels and K^+ channels, are heterogeneous. Thus it is difficult to directly compare the agonistic activities of opioids on μ-, δ- and κ-opioid receptors when examined in different tissue preparations. The cDNAs coding the μ-, δ- and κ-opioid receptors have been cloned (for review, see Minami and Satoh, 1995), which has allowed the use of preparations to express a single type of opioid receptor. Furthermore, the agonistic activities of opioids on cloned μ-, δ- and κ-opioid receptors can be examined in a specific cell line. In such preparations, the circumstances for the signal transduction of opioids are homogenous among the cells expressing the μ-, δ- and κ-opioid receptors. In this study, we examined the binding affinity and agonistic activity of KT-90 in CHO cell lines expressing μ-, δ- or κ-opioid receptors.

2. Materials and methods

2.1. Materials

Rat μ- and κ-opioid receptor cDNAs were cloned as described (Minami et al., 1993, 1994). Rat δ-opioid receptor cDNA was a gift from Dr K. Fukuda (Fukuda et al., 1993). KT-90 was synthesized as reported (Kanematsu et al., 1990). Morphine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). [D-Ala^2, MePhe^4,Gly(ol)^5]enkephalin (DAMGO), a μ-opioid selective agonist, and [D-Pen^2,D-Pen^5]enkephalin (DPDPE), a δ-opioid selective agonist, were from Bachem Feinchemikalien AG (Bubendorf, Switzerland). A κ-opioid selective agonist (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8-yl]benzeneacetamide (U-69,593) was a gift from the Upjohn Company (Kalamazoo, USA). [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69,593 were purchased from DuPont-New England Nuclear (Boston, USA). Cyclic AMP assay kits (code No. RPA. 509) were from Amersham (Buckinghamshire, UK).

2.2. Cell culture

The Chinese hamster ovary (CHO) cells stably expressing the rat μ-, δ- and κ-opioid receptors were established as described (Katsumata et al., 1995) and cultured in F-12 medium containing 10% fetal calf serum and 200 μg/ml G418 (GibcoBRL, Gaithersburg, USA) under a 5% CO_2 atmosphere at 37°C. The cells were seeded into 75 cm^2 or 175 cm^2 flasks at a density of 10^4–10^6 cells/cm^2, and grown to subconfluence in 5–7 days. The growth medium was changed every 3 or 4 days. The cells were used between the third and fifteenth passage after cloning.

2.3. Binding assay

Binding was assayed as described (Katsumata et al., 1995). In brief, the CHO cells expressing the μ-, δ- or κ-opioid receptor were homogenized in 50 mM Tris (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM EDTA. The membranes were pelleted by centrifugation at 30 000 × *g* for 20 min, resuspended in the same buffer, then incubated for 1 h at 25°C with 1 nM [^{3}H]DAMGO, 1 nM [^{3}H]DPDPE or 2 nM [^{3}H]U69,593 in the presence of various opioid ligands. Non-specific binding to the μ-, δ- and κ-opioid receptors was determined in the presence of 1 μM DAMGO, 1 μM DPDPE and 2 μM U69,593, respectively. After rapid passage through Whatman GF/C filters soaked with 0.1% polyethyleneimine and washed with the ice-cold buffer, the amount of radioactivity retained on each filter was measured by liquid scintillation spectrometry. Binding assays were performed in duplicate and the results are presented as the means ± S.E.M. of 3–4 separate experiments.

2.4. Cyclic AMP assay

CHO cells expressing μ-, δ- or κ-opioid receptors were seeded into 24-well plates at a density of 10^5 cells/well. After 24 h, the cells were washed, then incubated for 10 min at 37°C each with Hepes-buffered saline containing 1 mM 3-isobutyl-1-methylxanthine followed by a mixture of 10 μM forskolin and 1 mM 3-isobutyl-1-methylxanthine in the presence of various concentrations of opioid ligands. The incubation was stopped with an equal volume of 10%

trichloroacetic acid. The cyclic AMP contents were measured using a radioimmunoassay kit. Cyclic AMP assays were performed in triplicate and the results are presented as the means ± S.E.M. of 3–6 separate experiments.

2.5. Data analysis

The inhibitory constant (K_i) of each opioid ligand in the binding assay was calculated from the IC_{50} value, which is the concentration required to displace 50% of the radiolabeled ligands, according to the equation published by Cheng and Prusoff (1973). In the cyclic AMP assay, the IC_{50} value of each opioid agonist was calculated as the concentration required to reduce the forskolin-induced cyclic AMP accumulation by 50% of the maximal inhibition by each agonist, which was determined by curve fitting. Statistics were analyzed by Student's *t*-test. Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Binding characteristics of KT-90 in the membrane preparations from the CHO cells expressing the μ-, δ- and κ-opioid receptors

As reported (Katsumata et al., 1995), the dissociation constants (K_d) of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69,593 in CHO cells expressing the μ-, δ- and κ-opioid receptors determined from Scatchard analyses were 1.1 ± 0.27 (mean ± S.E.M., $n = 7$), 2.5 ± 0.26 ($n = 6$) and 1.1 ± 0.09 nM ($n = 4$), respectively. The receptor densities (B_{max}) in these cells were 453 ± 69 ($n = 7$), 869 ± 95 ($n = 6$) and 483 ± 20 fmol/mg protein ($n = 4$), respectively.

The binding experiments using the membrane preparations from the CHO cells expressing the μ-opioid receptor revealed that KT-90, morphine and DAMGO displaced the specific binding of 1 nM [^{3}H]DAMGO with IC_{50} values of 6.3 ± 1.4 ($n = 3$), 2.9 ± 0.1 ($n = 3$) and 1.9 ± 0.1 nM ($n = 3$), respectively (Fig. 2A). The calculated K_i values were 3.3 ± 0.7, 1.5 ± 0.04 and 0.99 ± 0.1 nM, respectively.

In the membrane preparations from the CHO cells expressing the δ-opioid receptor, KT-90, morphine and DPDPE displaced the specific binding of 1 nM [^{3}H]DPDPE with IC_{50} values of 31.9 ± 2.1 ($n = 3$), 425 ± 62 ($n = 4$) and 9.1 ± 0.9 nM ($n = 3$), respectively (Fig. 2B). The calculated K_i values were 22.8 ± 1.5, 303 ± 44 and 6.5 ± 0.6 nM, respectively. Thus, KT-90 had about 4-fold lower affinity than DPDPE but about 13-fold higher affinity than morphine for the δ-opioid receptor.

In the membrane preparations from the CHO cells expressing the κ-opioid receptor, KT-90, morphine and U69,593 displaced the specific binding of 2 nM [^{3}H]U69,593 with IC_{50} values of 5.3 ± 0.8 ($n = 3$), 401 ± 8.3 ($n = 3$) and 4.3 ± 1.0 nM ($n = 3$), respectively (Fig. 2C). The calculated K_i values were 1.9 ± 0.3, 145 ± 3.0 and 1.6 ± 0.4 nM, respectively. The affinity of KT-90 was similar to that of U69,593 but much higher than that of

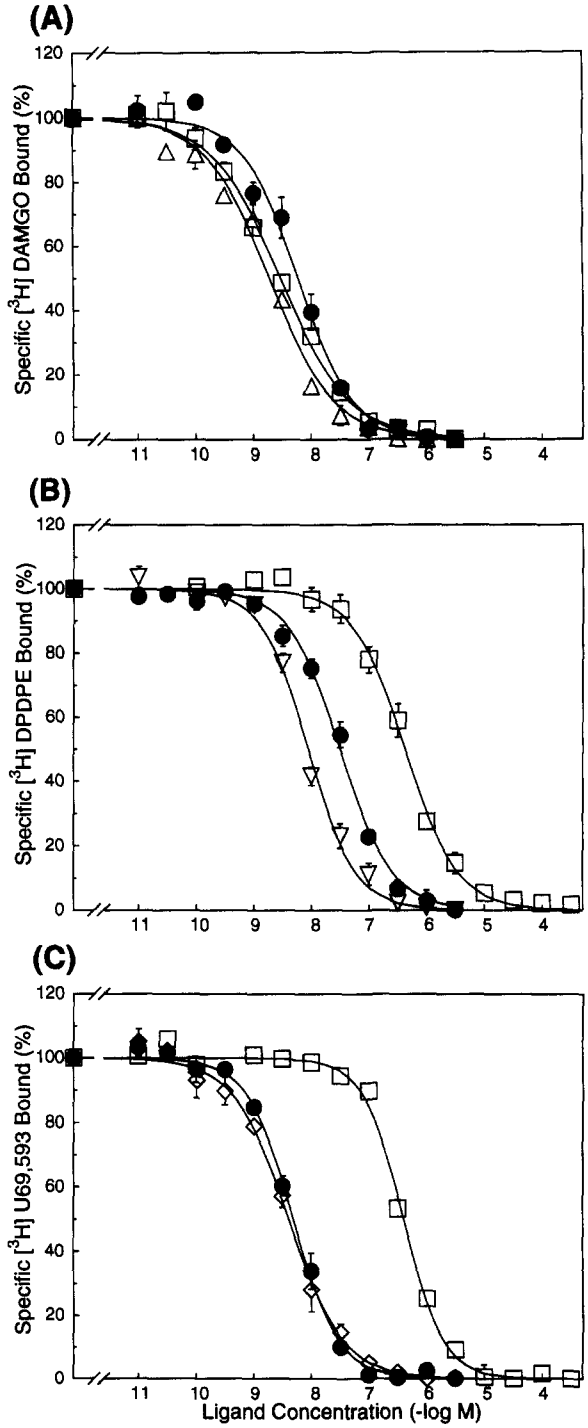

Fig. 2. Competitive inhibition of specific binding of 1 nM [^{3}H]DAMGO (A), 1 nM [^{3}H]DPDPE (B) or 2 nM [^{3}H]U69,593 (C) in membrane preparations from CHO cells expressing μ-, δ- or κ-opioid receptors, respectively. The unlabeled opioid ligands were KT-90 (●), morphine (□), DAMGO (△), DPDPE (▽) and U69,593 (◇). Results are expressed as percentages of the specific binding in the absence of competitive ligands. The data are presented as means ± S.E.M. of 3–4 separate experiments performed in duplicate.

morphine for the κ-opioid receptor. Thus, the calculated K_i values of KT-90 for the μ-, δ- and κ-opioid receptors were 3.3 ± 0.7, 22.8 ± 1.5 and 1.9 ± 0.3 nM, respectively, indicating that KT-90 had high affinities for all three types of opioid receptors with a little preference for the μ- and κ-types.

3.2. Effects of KT-90 on forskolin-induced cyclic AMP accumulation in the CHO cells expressing μ-, δ- and κ-opioid receptors

In CHO cells expressing the μ-opioid receptor, KT-90, morphine and DAMGO inhibited forskolin (10 μM)-induced cyclic AMP accumulation with IC_{50} values of 2337 ± 750 ($n = 6$), 25.9 ± 3.1 ($n = 5$) and 3.1 ± 0.8 nM ($n = 3$), respectively (Fig. 3A). At a concentration of 1 μM, DAMGO and morphine suppressed the cyclic AMP accumulation to 7.4 ± 1.4 and 12.2 ± 1.9% of the control level, respectively and the inhibitory effects almost reached a plateau. In contrast, KT-90 hardly inhibited the cyclic AMP accumulation at a concentration of 100 nM (95.2 ± 2.2% of the control) and suppressed it to 75.1 ± 3.3% of the control level even at a concentration of 1 μM. The values of the maximal inhibition by KT-90, morphine and DAMGO were 38.7 ± 4.6%, 9.9 ± 1.5% and 5.7 ± 0.2% of the control levels, respectively. There were significant differences between the maximal inhibition of KT-90 and those of morphine and DAMGO ($P < 0.01$).

In the CHO cells expressing the δ-opioid receptor, KT-90, morphine and DPDPE inhibited forskolin (10 μM)-induced cyclic AMP accumulation with IC_{50} values of 17.3 ± 4.6 ($n = 3$), 868 ± 245 ($n = 3$) and 1.5 ± 0.1 nM ($n = 3$), respectively (Fig. 3B). Although DPDPE maximally inhibited cyclic AMP accumulation to 11.7 ± 2.7% of the control level, the values for the maximal inhibition by KT-90 and morphine were 29.5 ± 0.5 and 35.9 ± 2.2% of the control, respectively, which were significantly different from that by DPDPE ($P < 0.01$). There was also a significant difference between those by KT-90 and morphine ($P < 0.05$).

In the CHO cells expressing the κ-opioid receptor, KT-90, morphine and U69,593 inhibited forskolin (10 μM)-induced cyclic AMP accumulation with IC_{50} values of 2.0 ± 0.1 ($n = 3$), 1095 ± 183 ($n = 4$) and 17.3 ± 4.5 nM ($n = 3$), respectively (Fig. 3C). The extent of the maximal inhibition by U69,593 was 17.3 ± 1.0% of the control, whereas by KT-90 and morphine were 31.4 ± 2.5 and 44.1 ± 2.2% of the control, respectively, which were significantly different ($P < 0.01$). There was a significant difference also between the maximal inhibition by KT-90 and by morphine ($P < 0.05$).

3.3. Antagonistic property of KT-90 against morphine in CHO cells expressing the μ-opioid receptor

KT-90 at 10 and 100 nM did not inhibit forskolin (10 μM)-induced cyclic AMP accumulation in CHO cells expressing the μ-opioid receptor (Fig. 3A), while it reduced the specific binding of 1 nM [^{3}H]DAMGO to 39.6 ± 5.6% and 3.2 ± 2.2% of the control, respectively. Thus, KT-90 at these concentrations seemed to antagonize

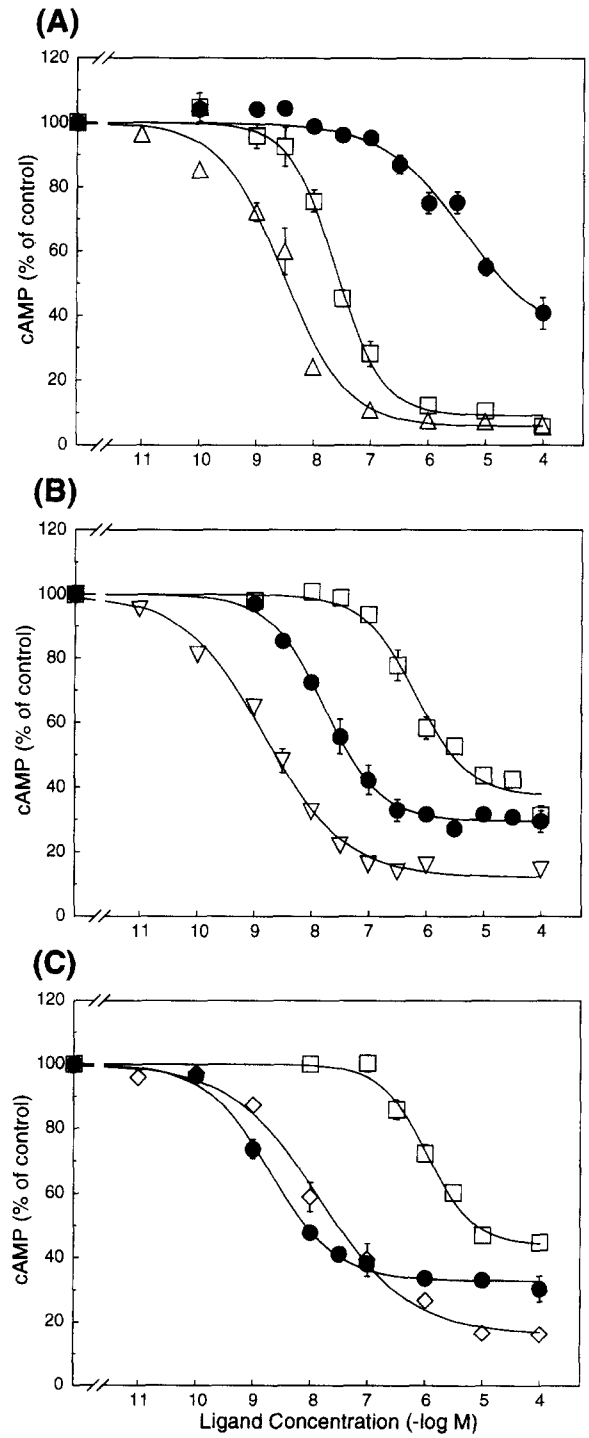

Fig. 3. Effects of opioids on forskolin (10 μM)-induced cyclic AMP accumulation in CHO cells expressing μ- (A), δ- (B) or κ- (C) opioid receptors. Intracellular cyclic AMP levels of the cells incubated with 10 μM forskolin alone served as controls (100%). Opioid ligands used were KT-90 (●), morphine (□), DAMGO (△), DPDPE (▽) and U69,593 (◇). Results are presented as means ± S.E.M. of 3–6 separate experiments performed in triplicate.

the effect of other opioid agonists, such as morphine and DAMGO, in the μ-opioid receptor. In fact, the co-application of KT-90 (10 and 100 nM) with morphine produced rightward shifts of the concentration-response curves of morphine in CHO cells expressing the μ-opioid receptor (Fig. 4A). The IC_{50} value of morphine in the absence of KT-90 was 25.9 ± 3.1 nM ($n = 5$). The IC_{50} values were significantly ($P < 0.01$) changed to 109.9 ± 23 ($n = 3$) and 2939 ± 557 nM ($n = 3$) in the presence of 10 and 100 nM KT-90, respectively. In contrast, co-application of KT-90 (100 nM) with morphine produced a downward shift of the concentration-response curve of morphine in CHO cells expressing the δ- and κ-opioid receptors (Fig. 4B,C).

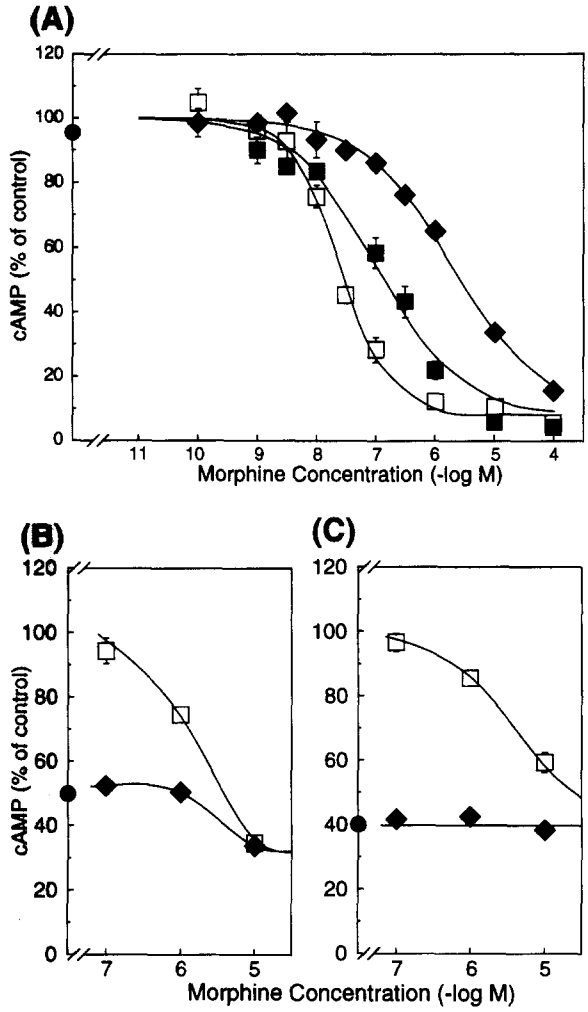

Fig. 4. Effects of KT-90 applied with morphine on the concentration-response curve of morphine to inhibit forskolin (10 μM)-induced cyclic AMP accumulation in CHO cells expressing μ-, δ- and κ-opioid receptors. KT-90 produced rightward shifts of the concentration-response curve of morphine in CHO cells expressing the μ-opioid receptor (A). KT-90 produced downward shifts in the δ- (B) and κ-opioid receptors (C). Intracellular cyclic AMP levels of the cells incubated with 10 μM forskolin alone served as controls (100%). KT-90 (100 nM) alone (●), morphine alone (□), morphine + 10 nM KT-90 (■) and morphine + 100 nM KT-90 (◆). Results are presented as means ± S.E.M. of 3–5 separate experiments performed in triplicate.

4. Discussion

KT-90 showed high affinities for the μ-, δ- and κ-opioid receptors with K_i values of 3.3 ± 0.7, 22.8 ± 1.5 and 1.9 ± 0.3 nM, respectively. KT-90 had 7 and 12 times higher affinities for the μ- and κ-opioid receptors, respectively, than for the δ-opioid receptors. KT-90 bound to the μ-, δ- and κ-opioid receptors with 2-fold lower but 13- and 76-fold higher affinity than morphine, respectively. Previously, Takayanagi et al. (1990b) have reported that KT-90 bound to these receptors with 4-, 19- and 13-fold higher affinity than morphine, respectively, in rat brain membrane preparations. The differences in the affinities of KT-90 between their study and ours may be due to the radiolabeled ligands used for labeling the opioid receptors. They used [^{3}H]naloxone, [^{3}H]DADLE and [^{3}H]ethylketocyclazocine to label the μ-, δ- and κ-opioid receptors, respectively, whereas we used [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69,593. Because naloxone, DADLE and ethylketocyclazocine are not highly selective to the μ-, δ- and κ-opioid receptors, respectively (Raynor et al., 1994), these radiolabeled ligands cannot specifically bind to a single type of opioid receptor in brain membrane preparations that contain heterogeneous types of opioid receptors. Thus, their results obtained by using these radiolabeled ligands in the brain membrane preparations may not necessarily reveal the correct K_i values of KT-90 for the μ-, δ- and κ-opioid receptors.

In CHO cells expressing the μ-opioid receptor, KT-90, morphine and DAMGO inhibited forskolin-induced cyclic AMP accumulation with IC_{50} values of 2337 ± 750, 25.9 ± 3.1 and 3.1 ± 0.8 nM, respectively. KT-90 was about 90- and 750-fold less potent in inhibiting the cyclic AMP accumulation than morphine and DAMGO, respectively, whereas it had only 2–3-fold lower affinity than morphine and DAMGO. At a concentration of 100 nM, KT-90 as well as DAMGO and morphine almost fully displaced the specific binding of [^{3}H]DAMGO to the μ-opioid receptor. On the other hand, at this concentration, KT-90 inhibited the cyclic AMP accumulation only to 95.2 ± 2.2% of the control, whereas DAMGO and morphine inhibited it to 10.9 ± 1.5 and 28.3 ± 3.9% of the control, respectively. Furthermore, KT-90 maximally inhibited the cyclic AMP accumulation only to 38.7 ± 4.6% of the control, whereas DAMGO and morphine maximally inhibited it to 5.7 ± 0.2% and 9.9 ± 1.5% of the control. The significant differences between the extent of the maximal inhibition by KT-90 and those by DAMGO and morphine ($P < 0.01$) indicated that KT-90 is a partial agonist in the μ-opioid receptor.

Because KT-90 considerably displaced the specific binding of [^{3}H]DAMGO at these concentrations of 10 and 100 nM without producing agonistic effects, KT-90 at these concentrations was thought to antagonize the effect of morphine in the μ-opioid receptor. As shown in Fig. 4A, KT-90 produced rightward shifts of the con-

centration-response curve of morphine at concentrations of 10 and 100 nM. In contrast, co-application of KT-90 at a concentration of 100 nM with morphine, produced downward shifts of the concentration-response curve of morphine for the δ- and κ-opioid receptors. These findings indicated that KT-90 can antagonize the effect of morphine upon the μ-, but not δ- or κ-, opioid receptor. These results are consistent with other studies in which KT-90 antagonized the inhibitory effect of morphine on the electrically stimulated twitch response in the guinea-pig ileum (Takayanagi et al., 1990b) and the analgesic effect of morphine in mice (Takayanagi et al., 1990a).

In CHO cells expressing the δ-opioid receptor, KT-90, morphine and DPDPE inhibited forskolin-induced cyclic AMP accumulation with IC_{50} values of 17.3 ± 4.6, 868 ± 245 and 1.5 ± 0.1 nM, respectively. The potency of KT-90 was about 50-fold higher than that of morphine. KT-90 and morphine maximally inhibited cyclic AMP accumulation to 29.5 ± 0.5 and $35.9 \pm 2.2\%$ of the control, respectively, while DPDPE inhibited it to $11.7 \pm 2.7\%$. There was a significant difference between the extent of maximal inhibition by KT-90 or morphine and that by DPDPE ($P < 0.01$). This indicated that KT-90 and morphine act on the δ-opioid receptor as partial agonists, while the efficacy of KT-90 is significantly ($P < 0.05$) higher than that of morphine. The property of morphine as a partial agonist has also been identified in the δ-opioid receptor expressed on NG108-15 cells (Law et al., 1982) and in the cloned mouse δ-opioid receptor expressed on CHO cells (Law et al., 1994).

In CHO cells expressing the κ-opioid receptor, KT-90 inhibited forskolin-induced cyclic AMP accumulation with an IC_{50} value of 2.0 ± 0.1 nM, which was about 8- and 500-fold lower than those of U69,593 and morphine. On the other hand, KT-90 and morphine maximally inhibited the cyclic AMP accumulation to 31.4 ± 2.5 and $44.1 \pm 2.2\%$ of the control, respectively, while U69,593 maximally inhibited it to $17.3 \pm 1.0\%$. There was a significant difference between the extent of the maximal inhibition by KT-90 or morphine and that by U69,593 ($P < 0.01$). These findings indicated that KT-90 and morphine act on the κ-opioid receptor as partial agonists, while the efficacy of KT-90 is significantly ($P < 0.05$) higher than that of morphine. The agonistic property of KT-90 in the κ-opioid receptor is consistent with the findings that KT-90 inhibits the electrically stimulated twitch response in the rabbit vas deferens (Takayanagi et al., 1990b), which exclusively contains κ-opioid receptors (Oka et al., 1980). In addition, KT-90 reportedly produces conditioned place aversion in mice (Takayanagi et al., 1990a), as well as typical κ-opioid receptor agonists, such as U50,488H (Mucha and Herz, 1985; Bals-Kubik et al., 1989).

In this study, we examined the pharmacological properties of KT-90 using cloned rat μ-, δ- and κ-opioid receptors expressed on the CHO cells. This has allowed the use of preparations that express a single type of opioid receptor and provide homogeneous conditions for the signal transduction systems for opioids, such as GTP-binding proteins and adenylyl cyclases. KT-90 showed high affinity for all three types of opioid receptors. Furthermore, we revealed that KT-90 acted as a partial agonist in the μ-, δ- and κ-opioid receptors. However, because of the extremely low efficacy in the μ-opioid receptor, KT-90 antagonized the effect of morphine in the μ-, but not δ- or κ-, opioid receptors at concentrations of 10 and 100 nM. Reportedly KT-90 exhibits analgesic effects at 5–10-fold lower doses than morphine (Takayanagi et al., 1990b). Because KT-90 shows little agonistic effect on the μ-opioid receptor at lower doses, the potent analgesic effects of KT-90 are not considered attributable to its action on the μ-opioid receptor. KT-90 binds to the δ- and κ-opioid receptors with 13–76-fold higher affinity than morphine and the efficacies of KT-90 to inhibit forskolin-induced cyclic AMP accumulation in these receptors were significantly higher than those of morphine. The δ- and κ-opioid receptor agonists elicit antinociception (Heyman et al., 1988; Millan, 1990). Moreover, the simultaneous administration of DPDPE and U50,488H produces analgesic synergy (Miaskowski et al., 1990). These findings suggest that KT-90 produces potent analgesic effects in vivo through acting on both the δ- and κ-opioid receptors.

In this study, we examined the effects of opioids only on the adenylyl cyclase activity, whereas the opioid receptors can also couple to other signal transduction systems, such as Ca^{2+} and K^+ channels. Nevertheless, the present results suggest mechanisms for the analgesic effects of KT-90 in vivo. Because the effects of opioids on the adenylyl cyclase are thought to reflect their ability to activate heterotrimeric GTP-binding proteins, the adenylyl cyclase inhibition probably parallels other activities, such as Ca^{2+} channel inhibition and K^+ channel activation, which are also mediated by GTP-binding proteins. Indeed, in several studies using the expression systems of the cloned opioid receptors, the opioid agonists inhibiting adenylyl cyclase activity can also inhibit Ca^{2+} channels and/or activate K^+ channels (Kaneko et al., 1994; Tallent et al., 1994; Kovoor et al., 1995). However, the effects on the ion channels are thought to be important for the opioid agonists to produce analgesic effects in vivo. Thus, further studies upon the effects of KT-90 on the ion channels in cells expressing cloned opioid receptors are necessary to fully elucidate the mechanisms involved in the analgesic effects of KT-90.

Acknowledgements

This study was supported by a Grant from the Japanese Society for the Promotion of Science, a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan and the grants from the Japan Health Sciences Foundation and the Uehara Memorial Foundation.

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