# $G_z$ coupling to the rat κ-opioid receptor

Helen W.L. Lai[a], Masabumi Minami[b], Masamichi Satoh[c], Yung H. Wong[a,*]

[a]*Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong*
[b]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-01, Japan*
[c]*Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-01, Japan*

Received 9 January 1995

**Abstract We have expressed the cloned rat κ-opioid receptor in human embryonic kidney 293 cells and studied the ability of κ-selective ligands to inhibit adenylyl cyclase. In transfected 293 cells, activation of the κ-opioid receptor by U50,488 and the dynorphins resulted in the inhibition of cAMP accumulation. The inhibitory response was sensitive to pertussis toxin and highly selective for κ-agonists; neither μ- nor δ-opioids were able to activate the κ-opioid receptor. Upon co-transfection with the α subunit of $G_z$, inhibition of cAMP accumulation by κ-agonist became refractory to pertussis toxin, indicating that the κ-opioid receptor can couple to both $G_i$ and $G_z$ proteins.**

*Key words:* κ-Opioid receptor; Adenylyl cyclase; G protein; signal transduction

## 1. Introduction

The opioid peptides are known to regulate diverse physiological functions ranging from analgesia to the regulation of gastrointestinal motility. The actions of opioid peptides are manifested through a number of specific opioid receptors. Amongst the different classes of opioid receptors (μ, δ, and κ), the κ-opioid receptors are activated by dynorphin-related peptides. Based on pharmacological prolfiles, the κ-opioid receptors can be further classified into $\kappa_1$ and $\kappa_2$ subtypes [1].

Intense cloning efforts have resulted in the isolation of cDNAs encoding the mouse [2], rat [3,4], and guinea pig [5] $\kappa_1$-opioid receptors. Like the cloned μ- [6] and δ-opioid [7] receptors, these $\kappa_1$-receptors belong to the superfamily of G protein-coupled serpentine receptors. Indeed, κ-opioid receptors are known to regulate voltage dependent calcium [8,9] and potassium [10] channels via G proteins. The evidence for the inhibition of adenylyl cyclase by κ-opioids is somewhat more controversial. Several recent reports suggest that κ-opioids can inhibit adenylyl cyclase [11,12] whereas others failed to demonstrate similar responses [13]. However, expression of the cloned κ-opioid receptors in COS cells has revealed that this receptor can in fact inhibit adenylyl cyclase [2,4,5].

The specificity of interactions between the κ-opioid receptor and G proteins are poorly defined. It is generally believed that the κ-opioid receptors are coupled to $G_i$-like proteins which serve as substrates for pertussis toxin (PTX) catalyzed ADP-ribosylations. This concept is especially attractive since $G_i$ proteins have been shown to inhibit adenylyl cyclase and stimulate potassium channels [14]. However, an increasing number of $G_i$-coupled receptors appeared to have the capacity to interact with PTX-insensitive G proteins. For example, the dopamine-$D_2$ and adenosine $A_1$ receptors can interact with $G_z$ [15] while the C5a receptor can stimulate phospholipase C via $G_{16}$ [16]. Thus far, there is no indication of the possible coupling of PTX-insensitive G proteins to κ-opioid receptors. In this study, we report that the rat $\kappa_1$-opioid receptor is capapble of inhibiting adenylyl cyclase via the pertussis toxin-insenitive G protein, $G_z$.

## 2. Materials and methods

### 2.1. Materials

The $\kappa_1$-opioid receptor (in the pcDNA3 vector) was isolated from a rat cDNA library [3]. The cDNAs of $\alpha_z$ in pcDNAI and the rat luteinizing hormone receptor (LHR) were constructed or obtained as previously described [15]. PTX was purchased from List Biological Laboratories (Campbell, CA). Human choriogonadotropin (hCG) was a generous gift from the National Pituitary Agency (Bethesda, MD). 293 cells were obtained from the American Type Culture Collection (ATCC CRL-1573). [2-$^3$H]Adenine was purchased from Amersham Corp. Plasmid purification columns were obtained from Qiagen Inc. Cell culture reagents were obtained from Gibco and all other chemicals were purchased from Sigma.

### 2.2. Transient transfection of 293 cells

Human embryonic kidney 293 cells were maintained at 5% $CO_2$, 37°C in Earle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 50 units/ml penicillin and 50 μg/ml streptomycin. Cells were seeded in 12-well plates at $2 \times 10^5$ cells per well the day before transfection. The various cDNAs were purified via Qiagen columns and transfected using the DEAE-dextran method as previously described [17].

### 2.3. cAMP accumulation

Cells were labelled 16–20 h post-transfection with 1 μCi/ml of [2-$^3$H]adenine with or wthout 100 ng/ml of PTX. 16–24 hours later they were washed once with HEPES buffered MEM (pH 7.4) and incubated for 30 min at 37°C in the presence of 1 mM 1-methyl-3-isobutylxanthine, with or without 5 ng/ml hCG and opioid ligands as specified. The reaction was stopped by adding 5% TCA containing 1 mM ATP. The [$^3$H]cAMP fraction was isolated via the Dowex columns as described in [17]. Absolute values for cAMP accumulation varied between experiments, but variability within a given experiment was less than 10% in general.

## 3. Results and discussion

We have previously established that the cloned rat $\kappa_1$-opioid receptor can activate $Ca^{2+}$-dependent $Cl^-$ channels when expressed in *Xenopus* oocytes [3]. In this report we used a mammalian expression system to study the receptor's ability to inhibit adenylyl cyclase through different G proteins. The human embryonic kidney 293 cells were co-transfected with cDNAs

*Corresponding author. Fax: (852) 358 1559.

*Abbreviations:* DPDPE, [D-Pen$^2$,D-Pen$^5$]enkephalin; DADLE, [D-Ala$^2$,-D-Leu$^5$]enkephalin; DAMGO, [D-Ala$^2$,*N*-Me-Phe$^4$,Gly$^5$-ol]enkephalin; G protein, guanine nucleotide-binding regulatory protein; $\alpha_z$, α subunit of the G protein $G_z$; PTX, pertussis toxin; hCG, human choriogonadotropin; LHR, luteinizing hormone receptor.


*SSDI* 0014-5793(95)00088-7

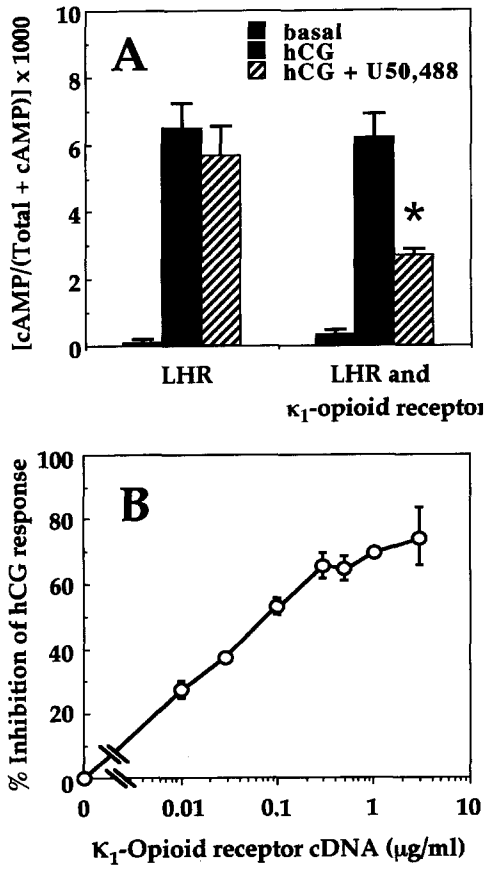


Fig. 1. U50,488-mediated inhibition of cAMP accumulation in transfected 293 cells. 293 Cells were transiently transfected, labelled with [2-$^3$H]adenine and then assayed for cAMP accumulation as described in section 2. (A) Cells were transfected with cDNAs encoding the LHR (0.15 μg/ml) with or without $\kappa_1$-opioid receptor (0.25 μg/ml). Transfected cells were assayed for responses to hCG (5 ng/ml) with or without U50,488 (100 nM). *U50,488 significantly reduced the hCG-stimulated activity; paired $t$-test, $P < 0.05$. (B) Cells were transfected with cDNAs encoding the LHR (0.15 μg/ml) and varying concentrations of the $\kappa_1$-opioid receptor (up to 3 μg/ml). Results are expressed as% inhibition of the hCG-stimulated activity as compared with that measured in the absence of the κ-agonist. U50,488 (100 nM) significantly inhibited the hCG-stimulated activity at all cDNA concentrations tested; paired $t$-test, $P < 0.05$. Data shown represent the mean ± S.D. of triplicate determinations in a single experiment; two additional experiments yielded similar results.

encoding the rat luteinizing hormone receptor (LHR in the pCIS vector) and the rat $\kappa_1$-opioid receptor. Co-expression of LHR allows us to selectively study the sub-population of cells that have taken up the cDNAs, and activation of LHR by human chorionic gonadotropin (hCG) is used to raise the intracellular cAMP levels. As indicated in Fig. 1A, addition of hCG to 293 cells transiently transfected with LHR-pCIS boosted intracellular cAMP levels by 19-fold above basal. In the presence of 100 nM of U50,488, a κ-selective ligand, the hCG-stimulated cAMP accumulation was reduced by 60–70%. Inhibition of cAMP accumulation by U50,488 was dependent on the amount of $\kappa_1$-opioid receptor cDNA used in the transfections (Fig. 1B) and was not seen in cells transfected with LHR-pCIS alone (Fig. 1A).

We then verified the pharmacological profile of the $\kappa_1$-opioid receptors that were expressed in 293 cells by examining the ability of different opioid ligands to activate $\kappa_1$-receptors. In 293 cells co-transfected with cDNAs encoding LHR (0.15 μg/ml) and $\kappa_1$-receptors (0.25 μg/ml), U50,488 dose-dependently inhibited the hCG response (Fig. 2) with an $EC_{50}$ of 1.4 ± 0.4 nM (mean ± S.E.M.; $n = 3$). Dyn 1–8, a non specific opioid agonist also inhibited the hCG response in a dose dependent manner, yielding an $EC_{50}$ of 0.32 ± 0.2 nM ($n = 3$) but with a slightly lower average maximal inhibition of 55% (Fig. 2). DAMGO and DPDPE (μ- and δ-selective agonists, respectively) had little or no effect on the hCG response. Their $EC_{50}$ values were greater than 1 μM (data not shown). At a single saturating dose of 100 nM, other μ- and/or δ-selective ligands were also unable to elicit κ-opioid receptor mediated inhibition of the hCG response. For example, the hCG responses in the presence of morphiceptin or DADLE were 107 ± 16% and 107 ± 7%, respectively, of those determined in the absence of opioid ligands. Nor-binaltorphimine, a specific κ antagonist, significantly ($P < 0.05$) abolished the ability of U50,488 to inhibit cAMP accumulation (Fig. 2). These results are consistent with the known pharmacological profile of the cloned $\kappa_1$-opioid receptor [18].

Both μ- and δ-opioid receptors are coupled to the PTX-sensitive $G_i/G_o$ proteins [19], and $G_i$-like proteins have also been implicated in the coupling of κ-opioid receptors to their effectors [20]. We therefore examined the possible involvement of $G_i$ proteins in $\kappa_1$-opioid receptor mediated inhibition of cAMP accumulation. In 293 cells co-expressing the LHR and $\kappa_1$-receptor, activation of the latter by 100 nM of U50,488 significantly inhibited the hCG response (Figs. 2 and 3) and this effect was PTX-sensitive (Fig. 3), indicating the involvement of $G_i$ proteins. For many $G_i$-coupled receptors, the PTX-insensitive $G_z$ protein [21] can functionally replace $G_i$ in the negative regulation of adenylyl cyclase [15]. Indeed, we have recently demonstrated that the cloned δ-opioid receptor can inhibit adenylyl cyclase via $G_z$ in co-transfected 293 cells [22]. Thus we sought to examine if similar coupling between $G_z$ and the $\kappa_1$- opioid receptor may exist. When the cDNA encoding the a subunit of $G_z$ was included in the transfection medium,

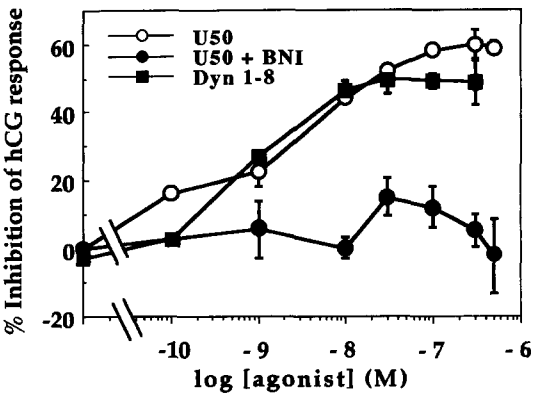


Fig. 2. Dose-dependent effects of κ-selective ligands. 293 cells were co-transfected with cDNAs encoding the LHR (0.15 μg/ml) with or without $\kappa_1$-opioid receptor (0.25 μg/ml), and assayed for cAMP accumulation after exposure to varying concentrations of κ-selective agonists (U50,488 and dynorphin 1–8) in the absence or presence of 1 μM of the κ-antagonist, nor-binaltorphimine (BNI). Data shown represent the mean ± S.E.M. of triplicate determinations from three independent experiments.

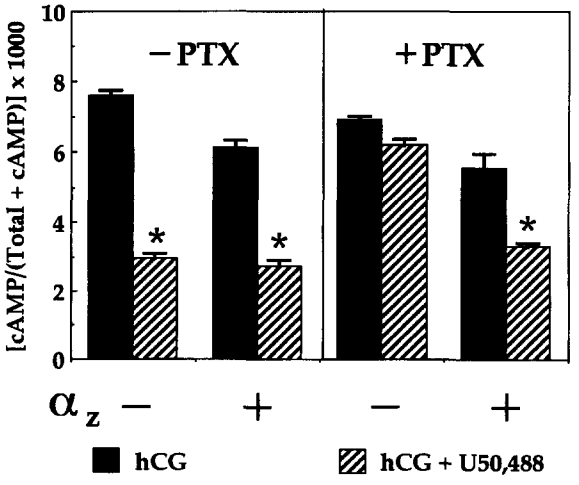


Fig. 3. κ-Opioid receptor coupling to the PTX-insensitive $\alpha_z$. 293 cells were co-transfected with cDNAs encoding the LHR (0.15 μg/ml) and the κ-opioid receptor (0.25 μg/ml) with or without $\alpha_z$ (0.125 μg/ml). Transfected cells were treated in the absence or presence of PTX (100 ng/ml) and assayed for cAMP accumulation in the presence of hCG (5 ng/ml) with or without 100 nM U50,488. The data represent triplicate determinations in a single experiment; two independent experiments yielded similar results. *U50,488 significantly reduced the hCG-stimulated activity; paired Bonferroni $t$-test, $P < 0.05$.

U50,488-mediated inhibition of the hCG response became refractory to PTX treatment (Fig. 3). The retainment of the U50,488 response following the inactivation of endogenous $G_i$ proteins by PTX indicated that the $\kappa_1$-opioid receptor can functionally interact with $G_z$. The significance of this finding is presently unclear. In terms of distribution, $G_z$ is most highly expressed in the brain and retina with lesser concentrations in the adrenal gland, kidney and pancreatic islet cells [21]. It is noteworthy that $G_z$ might be involved in the $\mu/\delta$ mediated supraspinal antinociceptive pathways [23].

In this study, we report for the first time that the cloned rat $\kappa_1$-opioid receptor can couple to a PTX-insensitive G protein, $G_z$, to inhibit adenylyl cyclase. It remains to be determined whether the $\kappa_1$-opioid receptor can utilize additional PTX-insensitive G proteins (e.g. $G_q$ and $G_{12}$) to perform other signaling functions such as inhibition of phospholipase C [24].

*Acknowledgements:* We are grateful to Yoshito Kaziro and Deborah Segaloff for cDNAs encoding the rat $\alpha_z$ and LHR, respectively. This work was supported by a grant from the Research Grants Council (RGC) of Hong Kong (HKUST 169/93M) to YHW.

## References

[1] Zukin, R.S., Eghbali, M., Olive, D., Unterwald, E.M. and Tempel, A. (1988) Proc. Natl. Acad. Sci. USA 85, 4061–4065.
[2] Yasuda, K., Raynor, K., Kong, H., Breder, C.D., Takeda, J., Reisine, T. and Bell, G.I. (1993) Proc. Natl. Acad. Sci. USA 90, 6736–6740.
[3] Minami, M., Toya, T., Yoshikazu, K., Maekawa, K., Nakamura, S., Onogi, T., Kaneko, S. and Satoh, M. (1993) FEBS Lett. 329, 291–295.
[4] Chen, Y., Mestek, A., Liu, J. and Yu, L. (1993) Biochem. J. 295, 625–628.
[5] Xie, G.X., Meng, F., Mansour, A., Thompson, R.C., Hoversten, M.T., Golstein, A., Watson, S.J. and Akil, H. (1994) Proc. Natl. Acad. Sci. USA 91, 3779–3783.
[6] Chen Y., Mestek A., Liu J., Hurley J. A. and Yu L. (1993) Mol. Pharmacol. 44, 8–12.
[7] Evans C. J., Keith D. E., Morrison H., Magendzo K. and Edwards R. H. (1992) Science 258, 1952–1955.
[8] Attali, B., Saya, D., Nah, S. Y. and Vogel, Z. (1989) J. Biol. Chem. 264, 347–353.
[9] Eriksson, P. S., Nilsson, M., Wogberg, M., Hansson, E. and Ronnback, L. (1993) Neuroscience. 54, 401–407.
[10] Grudt, T.J. and Williams, J. T. (1993) Proc. Natl. Acad. Sci. USA 90, 11429–11432.
[11] Attali, B., Saya, D. and Vogel, Z. (1989) J. Neurochem. 52, 360–369.
[12] Konkoy, C.S. and Childers, S.R. (1989) Mol. Pharmacol. 36, 627–633.
[13] Polastron, J., Boyer, M.J., Quertermont, Y., Thouvenot, J.P., Meunier, J.C. and Jauzac, P. (1990) J. Neurochem. 54, 562–570.
[14] Bourne H. R., Sanders D. A. and McCormick F. (1990) Nature 348, 125–132.
[15] Wong Y. H., Conklin B. R. and Bourne H. R. (1992) Science 255, 339–342.
[16] Amatruda, T.T., Gerard, N.P., Gerard, C. and Simon M.I. (1993) J. Biol. Chem. 268, 10139–10144.
[17] Wong Y. H. (1994) Methods Enzymol. 238, 81–94.
[18] Raynor K., Kong H., Chen Y., Yasuda K., Yu L., Bell G. I. and Reisine T. (1994) Mol. Pharmacol. 45, 330–334.
[19] Wong Y. H., Demoliou-Mason C. D. and Barnard E. A. (1988) J. Neurochem. 51, 114–121.
[20] Childers, S.R., Fleming, L., Konkoy, C., Marckel, D., Pacheco, M., Sexton, T. and Ward, S. ( 1992 ) Ann. N.Y. Acad. Sci. 654, 33–51.
[21] Matsuoka, M., Itoh, H., Kozas, T. and Kaziro, Y. (1988) Proc. Natl. Acad. Sci. USA 85, 5384–5388.
[22] Tsu, R.C., Chan, J.S.C. and Wong, Y.H. (1995) J. Neurochem., in press.
[23] Sanshez-Blazquez P., Juarros J. L., Martinez-Pena Y., Castro, M.A. and Garzon J. (1993) Life Sci. 53, 381–386.
[24] Misawa, H., Ueda, H. and Satoh, M. (1990) Neurosci. Lett. 112, 324–327.