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# FUNCTIONAL COUPLINGS OF THE δ- AND THE κ-OPIOID RECEPTORS WITH THE G-PROTEIN-ACTIVATED K+ CHANNEL

Kazutaka Ikeda*, Toru Kobayashi, Tomio Ichikawa,
Hiroshi Usui and Toshiro Kumanishi

Department of Neuropathology, Brain Research Institute,
Niigata University, Asahimachi 1, Niigata 951, Japan

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**Summary:** The δ- and the κ-opioid receptors were individually co-expressed with the G-protein-activated $K^+$ (GIRK1) channel in *Xenopus* oocytes. Stimulation of these opioid receptors with selective agonists induced currents which were blocked by the opioid receptor antagonist naloxone. The current responses showed inward rectification and were blocked by 300 μM $Ba^{2+}$, indicating that the effect of activated opioid receptors was mainly mediated by the GIRK1 channel. The $EC_{50}$ value obtained from these responses was 45 nM for the agonist DPDPE in the oocytes injected with the δ-opioid receptor mRNA and the GIRK1 mRNA and 15 nM for the agonist U50488H in the oocytes injected with the κ-opioid receptor mRNA and the GIRK1 mRNA. The Hill coefficient was 0.92 in the former and 0.93 in the latter. These results suggest that each of the δ- and the κ-opioid receptors functionally couples with the GIRK1 channel. 

The opioid receptor distributes in various regions in the central and peripheral nervous systems [1], and is considered to play an important role in brain functions involved in for example, emotion, euphoria, analgesia and morphine tolerance and dependence [2-4]. Activation of the opioid receptor alters membrane conductances for $K^+$ and $Ca^{2+}$ and levels of second messengers such as cAMP and inositol 1,4,5-triphosphate, each through G-protein activation, and ultimately results in the inhibition of neural firing and of neurotransmitter

*To whom correspondence should be addressed. FAX: 81-25-229-2959.

**Abbreviations:** DPDPE, [D-Pen$^{2,5}$]-Enkephalin; U50488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide; PCR, polymerase chain reaction.

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release [5]. Previous pharmacological and molecular biological studies have defined at least three major types of opioid receptor, μ, δ and κ [1, 6-15]. Activation of μ- and δ-opioid receptors has been shown to increase an inward rectifying potassium conductance *in vivo* [16-18] and *in vitro* [19, 20]. However, the pharmacological characteristics of the signal transductions mediated by these opioid receptors via $K^+$ channels have been largely unknown. Regarding the κ-opioid receptor, whether or not its signal transduction mechanism involves the $K^+$ channel has also remained unknown. In the present investigation, we demonstrated functional couplings of the δ- and the κ-opioid receptors with the GIRK1 channel (G-protein-activated $K^+$ channel) in a *Xenopus*-oocyte expression system and analyzed their pharmacological characteristics in the signal transduction.

## Materials and Methods

*Cloning of the δ- and the κ-opioid receptor cDNAs*

Based on the cDNA sequences for the mouse δ- and κ-opioid receptors [6-8], pairs of oligonucleotide primers corresponding to the regions containing either a translational initiation codon or a stop codon were synthesized. Primers for the δ-opioid receptor were 5'-GCGCCATGGAGCTG GTGCCCTCTG-3' and 5'-GCTCTAGAGTCAGGCGGCAGCGCCACCGCCCG -3' and those for the κ-opioid receptor were 5'-TCACCATGGAGTCCCCCATTCAGA-3' and 5'-CGACTAGTCATACTGGCTTATTCA-3'. PCR was performed for 35 cycles (94 °C, 45 s; 60 °C, 25 s; 74 °C, 3 min) in 50 μl of a reaction mixture containing 1 x *Pfu* Buffer, 125 μM of each dNTP, 250 nM of each primers, ~100 ng of the adult mouse whole-brain cDNA as a template, 1.25 units of *Pfu* DNA polymerase (Stratagene) and 10 % (vol/vol) dimethyl sulfoxide. PCR products containing the entire coding sequences for the δ- and the κ-opioid receptors were inserted between the *Nco*I and *Xba*I sites of the plasmid pSP35T [21] to yield the plasmids pSPORδ and pSPORκ, respectively. The nucleotide sequences of the inserted regions of the plasmids were confirmed by the dideoxy nucleotide chain-termination method.

*Expression in* Xenopus *oocytes*

The δ- and the κ-opioid-receptor-specific mRNAs were synthesized *in vitro* from linearized pSPORδ and pSPORκ, using 5 mM cap dinucleotide $^7$mGpppG and the Mega-script system (Ambion). The GIRK1-specific mRNA was synthesized by the same method from pSPGIRK1 (manuscript in preparation) which included the entire coding sequence for the mouse brain GIRK1. The deduced amino acid sequence of the mouse brain GIRK1 was the same as that of the rat atrial GIRK1 [22, 23]. *Xenopus laevis* oocytes were injected with either opioid-receptor-specific mRNA (~10 ng/oocyte) together

with the GIRK1-specific mRNA (~12 ng/oocyte) and incubated at 19 °C for two days. Whole-cell currents were recorded with a conventional two-micropipette voltage clamp [24] from the oocytes which were superfused with a high-potassium solution (96 mM KCl, 2 mM NaCl, 1 mM $MgCl_2$ and 1.5 mM $CaCl_2$). A δ-opioid-receptor agonist, DPDPE, a κ-opioid-receptor agonist, U50488H, and an opioid-receptor antagonist, naloxone, were purchased from Sigma. Student's *t*-test was used to determine the statistical significance between different groups.

## Results and Discussion

To determine whether the δ- and the κ-opioid receptors couple with the GIRK1 channel, we performed expression assays on their mRNAs synthesized *in vitro* using *Xenopus* oocytes. The oocytes injected with both the δ-opioid-receptor mRNA and the GIRK1 mRNA responded to the δ-opioid-receptor selective agonist DPDPE (53 ± 12 nA, n=7; mean ± S.E.M.), and the response was blocked by the opioid-receptor antagonist naloxone (Fig. 1A, C). No response to the agonist DPDPE was detected in the oocytes injected with the δ-opioid-receptor mRNA alone or with the GIRK1 mRNA alone (Fig. 1C). Similarly, the oocytes injected with both the κ-opioid-receptor mRNA and the GIRK1 mRNA responded to the κ-opioid-receptor selective agonist U50488H (133 ± 16 nA, n=7), and the response was blocked by naloxone (Fig. 1B, C). The oocytes injected with the κ-opioid receptor mRNA alone responded only weakly to the agonist U50488H (9 ± 2 nA, n=7), and the oocytes injected with the GIRK1 mRNA alone showed no response to U50488H (Fig. 1C). In both cases of co-injections, the current-voltage relationship of the response showed inward rectification, which is characteristic of the GIRK channel (Fig. 2). Furthermore, the responses were blocked by 300 μM $Ba^{2+}$ and recovered very soon after its removal (Fig. 1A, B). These results strongly suggest that the current responses observed in the co-injected oocytes were mainly mediated by the GIRK1 channel. The minor response observed in the oocytes injected with the κ-opioid-receptor mRNA alone might be a result of the opening of $Ca^{2+}$-activated $Cl^-$ channels via inositol triphosphate as suggested by results of a previous study [9].

We examined the relationship between the agonist concentration and the response. The agonist concentration required to produce a half-maximal effect

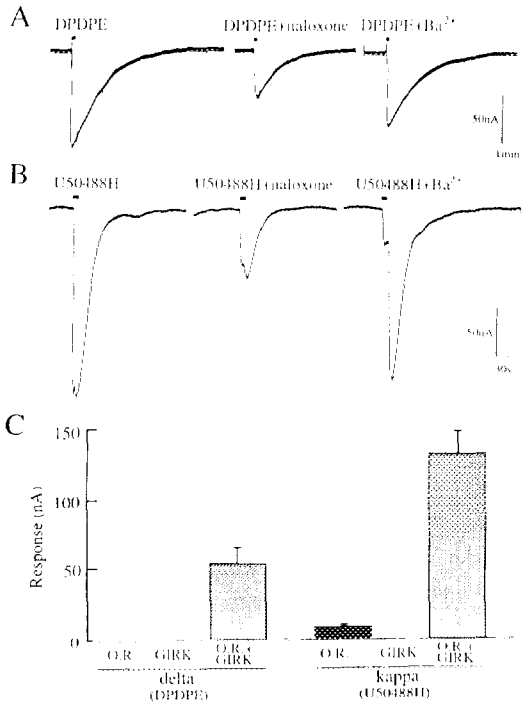


**Fig. 1.** Current responses in *Xenopus* oocytes co-injected with opioid receptor mRNA and GIRK1 channel mRNA. Current responses were measured at -70 mV membrane potential in a high-potassium solution. (A) Current responses in the oocytes injected with the δ-opioid-receptor mRNA and the GIRK1 mRNA to 100 nM DPDPE (left), 100 nM DPDPE and 1 μM naloxone (middle) and 100 nM DPDPE and 300 μM $Ba^{2+}$ (right). Bars above the traces show the duration of application. (B) Current responses in the oocytes injected with the κ-opioid-receptor mRNA and the GIRK1 mRNA to 100 nM U50488H (left), 100 nM U50488H and 1 μM naloxone (middle) and 100 nM U50488H and 300 μM $Ba^{2+}$ (right). Bars above the traces represent the same as in (A). (C) Average current responses of the oocytes injected with opioid-receptor mRNA alone (O.R.), the GIRK1 mRNA alone (GIRK), and both the opioid-receptor mRNA and the GIRK1 mRNA (O.R. + GIRK). Types of O.R. and agonists (100 nM) are indicated. Data are presented as mean ± S.E.M. of measurements on 7 oocytes.

($EC_{50}$) was 45 nM for DPDPE in the oocytes co-injected with the δ-opioid-receptor mRNA and the GIRK1 mRNA (Fig 3A), while it was 15 nM for U50488H in the oocytes co-injected with the κ-opioid-receptor mRNA and the GIRK1 mRNA (Fig. 3B). The Hill coefficient was 0.92 in the former and 0.93 in the latter. These results suggest that each of the δ- and the κ-opioid receptors involved in these response systems is activated by a low concentration of the selective agonist, probably in a one-to-one manner regarding the number of receptor and agonist molecules. The extremely high resolution of ligand effects

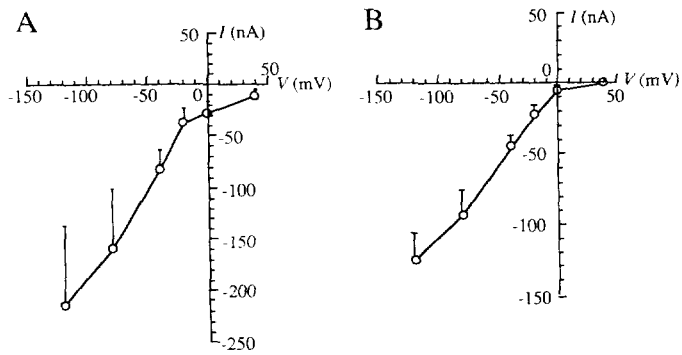


**Fig. 2.** Average current-voltage relationships of opioid-induced currents in *Xenopus* oocytes co-injected with opioid-receptor mRNA and GIRK1 mRNA. (A) Current response to 1 μM DPDPE in the oocytes injected with the δ-opioid-receptor mRNA and the GIRK1 mRNA. (B) Current response to 1 μM U50488H in the oocytes injected with the κ-opioid-receptor mRNA and the GIRK1 mRNA. Each circle and error bar represents the mean and S.E.M. of measurements on 5 oocytes. Note that the current shows a prominent inward rectification in both (A) and (B).

observed in our oocyte expression system might also provide an advantage in further detailed analyses of known and novel opioid ligands.

We demonstrated the functional couplings of the δ- and the κ-opioid receptors with the GIRK1 channel in this study. These couplings might actually occur *in vivo*, since we have found both opioid-receptor and GIRK1 mRNAs in

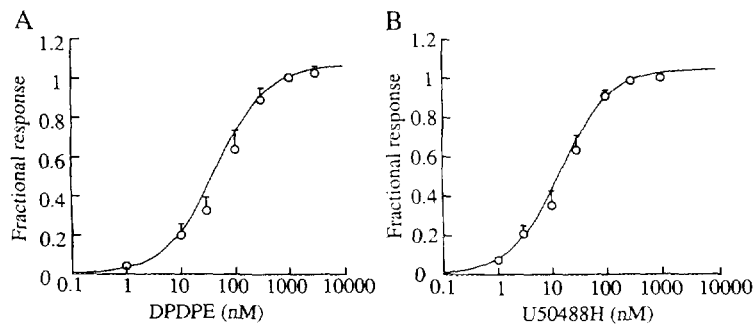


**Fig. 3.** *Dose-response relationships for the specific agonists.* (A) The oocytes injected with the δ-opioid-receptor mRNA and the GIRK1 mRNA. (B) The oocytes injected with the κ-opioid-receptor mRNA and the GIRK1 mRNA. Each circle and error bar represents the mean of fractional responses and S.E.M. obtained from 7 oocytes. The calculated curves have been drawn according to the equation $I = I_{max} / [1 + (EC_{50} / A)^n]$, where $I$ represents the current response, $I_{max}$ the maximum current response, $A$ the concentration of agonist, and $n$ the Hill coefficient. The $EC_{50}$ and Hill coefficient values obtained from the curves are,respectively, 45 nM and 0.92 (A), and 15 nM and 0.93 (B).

the same neurons in various brain regions by *in situ* hybridization analysis (manuscript in preparation). Considering the present findings together with recent works [19, 20] on couplings of different combinations of opioid receptors and GIRK channels, it is suggested that the GIRK channels mediate a major signal transduction pathway for opioid effects in general.

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## References

1. Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. and Watson S. J. (1988) Trends Neurosci. 11, 308-314.
2. Pasternak, G. W., Childers, S. R. and Snyder, S. H. (1980) Science 208, 514-516.
3. Koob, G. F., Maldonado, R. and Stinus, L. (1992) Trends Neurosci. 15, 186-191.
4. Nestler, E. J., Hope, B. T. and Widnell, K. L. (1993) Neuron 11, 995-1006.
5. Loh, H. H. and Smith, A. P. (1990) Annu. Rev. Pharmacol. Toxicol. 30, 123-147.
6. Kieffer, B. L., Befort, K., Gaveriaux-Ruff, C. and Hirth C. G. (1992) Proc. Natl. Acad. Sci. USA 89, 12048-12052.
7. Evans, C. J., Keith Jr., D. E., Morrison, H., Magendzo, K. and Edwards, R. H. (1992) Science 258, 1952-1955.
8. 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.
9. Minami, M., Toya, T., Katao, Y., Maekawa, K., Nakamura, S., Onogi, T., Kaneko, S. and Satoh, M. (1993) FEBS Lett. 329, 291-295.
10. Meng, F., Xie, G., Thompson, R. C., Mansour, A., Goldstein, A., Watson, S. J. and Akil, H. (1993) Proc. Natl. Acad. Sci. USA 90, 9954-9958.
11. Nishi, M., Takeshima, H., Fukuda, K., Kato, S. and Mori, K. (1993) FEBS Lett. 330, 77-80.
12. Chen, Y., Mestek, A., Liu, J., Hurley, J. A. and Yu, L. (1993) Mol. Pharmacol. 44, 8-12.
13. Fukuda, K., Kato, S., Mori, K., Nishi, M., Takeshima, H. (1993) FEBS Lett. 327, 311-314.
14. Thompson, R. C., Mansour, A., Akil, H. and Watson, S. J. (1993) Neuron 11, 903-913.
15. Wang, J. B., Imai, Y., Eppler, C. M., Gregor, P., Spivak, C. E. and Uhl, G. R. (1993) Proc. Natl. Acad. Sci. USA 90, 10230-10234.

16. Williams, J. T., North, R. A. and Tokimasa, T. (1988) J. Neurosci. 8, 4299-4306.
17. Loose, M. D. and Kelly, M. J. (1990) Brain Res. 513, 15-23.
18. Wimpey, T. L. and Chavkin, C. (1991) Neuron 6, 281-289.
19. Chen, Y. and Yu, L. (1994) J. Biol. Chem. 269, 7839-7842.
20. Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M. and Hugnot, J. P. (1994) FEBS Lett. 353, 37-42.
21. Amaya, E., Musci, T. J. and Kirschner, M. W. (1991) Cell 66, 257-270.
22. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N. and Jan, L. Y. (1993) Nature 364, 802-806.
23. Dascal, N., Schreibmayer, W., Lim, N. F., Wang, W., Chavkin, C., DiMagno, L., Labarca, C., Kieffer, B. L., Gaveriaux-Ruff, C., Trollinger, D., Lester, H. A. and Davidson, N. (1993) Proc. Natl. Acad. Sci. USA 90, 10235-10239.
24. Sakimura, K., Morita, T., Kushiya, E. and Mishina, M. (1992) Neuron 8, 267-274.