ELSEVIER

European Journal of Pharmacology 396 (2000) 23–28

ejp

www.elsevier.nl/locate/ejphar

Different effects of NMDA/group I metabotropic glutamate receptor agents in δ- and μ-opioid receptor agonist-induced supraspinal antinociception

Tsutomu Suzuki [a,*], Takeshi Aoki [a], Orie Ohnishi [a], Hiroshi Nagase [b], Minoru Narita [a]

[a] *Department of Toxicology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *Pharmaceutical Research Laboratories, Toray Industries, Inc., Kamakura 248-0036, Japan*

Received 2 December 1999; received in revised form 21 February 2000; accepted 29 February 2000

Abstract

The *N*-methyl-D-aspartate (NMDA) and metabotropic glutamate (mGlu) receptors are involved in nociceptive transmission in the central nervous system. The present study was designed to study the effects of NMDA and group I mGlu receptor agents on δ- and μ-opioid receptor agonist-induced antinociception in the mouse brain. Intracerebroventricular (i.c.v.) treatment with the non-competitive NMDA receptor antagonist dizocilpine and the group I mGlu receptor antagonist (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) significantly attenuated the antinociception induced by the δ-opioid receptor agonists [D-Pen^2, Pen^5]enkephalin (DPDPE), (−)-TAN 67 and [D-Ala^2]deltorphin II. On the contrary, i.c.v. administration of dizocilpine and (*S*)-4CPG slightly but significantly enhanced the antinociception induced by the μ-opioid receptor agonist [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO). Under these conditions, i.c.v. administration of NMDA and the group I mGlu receptor agonist 3,5-dihydrophenylglycine (DHPG) significantly enhanced the antinociception induced by δ-opioid receptor agonists, whereas both reduced DAMGO-induced antinociception. These findings suggest that the supraspinal antinociceptive actions of μ- and δ-opioid receptor agonists appear to be modulated differently by NMDA and group I mGlu receptors in the mouse.

Keywords: Antinociception; Opioid; Dizocilpine (MK-801); NMDA receptor; (*S*)-4CPG ((*S*)-4-carboxyphenylglycine); Metabotropic glutamate receptor

1. Introduction

Opioid drugs produce their actions by interacting with at least three types of opioid receptors; μ, κ and δ. In recent years, several studies have been undertaken to determine the potential role of excitatory amino acids in the antinociceptive responses to opioid drugs in mice and rats. Clinically, to obtain complete analgesia, a combination of the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine and an opioid agonist is a very powerful approach when the pain cannot be controlled by morphine alone (Oshima et al., 1990; Yang et al., 1996; Rabben et al., 1999).

Glutamate is a major transmitter in central pain pathways. The excitatory effect of glutamate is mediated by ionotropic (NMDA and α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) types) and metabotropic receptors. NMDA receptors consist of multiple subunits that regulate transmembrane ion flux, whereas metabotropic glutamate (mGlu) receptors contain seven transmembrane domain proteins that couple to intracellular second messenger systems through G-proteins (Sugiyama et al., 1987; Nakanishi, 1992; Hayashi et al., 1994; Hollmann and Heinemann, 1994). Recently, molecular cloning and pharmacological studies have revealed the existence of at least eight mGlu receptor subtypes ($mGlu_1$ receptor–$mGlu_8$ receptor). The $mGlu_1$ receptor and $mGlu_5$ receptor, which are classified as group I, are positively coupled to the phosphatidylinositol-phospholipase C system, whereas the others are negatively coupled to adenylate cyclase. The $mGlu_2$ receptor and $mGlu_3$ receptor are classified as

* Corresponding author. Tel.: +81-3-5498-5831; fax: +81-3-5498-5831.
E-mail address: suzuki@hoshi.ac.jp (T. Suzuki).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00183-7

group II, while $mGlu_4$ receptor, $mGlu_6$ receptor, $mGlu_7$ receptor and $mGlu_8$ receptor are classified as group III.

NMDA and group I mGlu receptors are both located in postsynaptic elements (Martin et al., 1992; Shigemoto et al., 1993). Opioid receptors and glutamate (NMDA, mGlu) receptors have been shown to be similarly distributed in the brain (Masu et al., 1994; Mansour et al., 1995), suggesting that some of these receptors may control nociceptive neurotransmission through a physiological balance in the same plasma membrane site. Thus, it is possible that opioid receptors and group I mGlu receptors share common pools of intracellular second messengers. However, little is known about the effects of direct interaction, if any, between μ- or δ-opioid and glutamate receptors on antinociceptive responses.

The present study was designed to investigate the effects of the pharmacological interaction of NMDA or group I mGlu receptor agents on the antinociception induced by the i.c.v. administration of a δ- or μ-opioid receptor agonist in mice.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

2.1. Animals

Male ddY mice (17–19 g) were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed at a room temperature of 22 ± 1°C with a 12-h light–dark cycle (light on 8:30 a.m. to 8:30 p.m.), and were allowed to adapt to this environment for a period of 1 week before the experiments. Food and water were available ad libitum.

2.2. Antinociceptive assay

The antinociceptive response was evaluated by recording the latency to paw licking, tapping or realing in the warm-plate test, where the metal plate was thermostatically controlled at 51 ± 0.5°C. To prevent tissue damage, we established a 30-s cut-off time. The test was repeated every 10 min for 60 min after drug or saline treatment. Each animal served as its own control, and the latency to responses was measured both before and after drug administration. All drugs were co-administered. Antinociception was calculated as a percentage of the maximum possible effect (percentage antinociception) according to the following formula: percentage antinociception = (test latency − predrug latency)/(cut-off time − predrug latency) × 100. Predrug latency, measured at 30-min intervals, was the mean of two values for each animal. Each group consisted of 8–10 mice per drug or saline treatment, and each animal was used for only one treatment.

2.3. Intracerebroventricular (i.c.v.) injection

Intracerebroventricular administration was performed as described previously (Haley and McCormick, 1957; Suzuki et al., 1996). The injection was made with a 2-mm double-needle (Natsume Seisakusho, Tokyo) attached to a 25-μl Hamilton microsyringe. Solution was injected in a volume of 4 μl per mouse.

2.4. Drugs

The drugs used in the present study were [D-Pen^2, Pen^5]enkephalin (DPDPE) and NMDA, which were obtained from Research Biochemicals (Natick, MA, USA). [D-Ala^2]deltorphin II and [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) were purchased from Sigma (St. Louis, MO, USA). (−)-TAN 67 (2-methyl-4*a*α-(3-hydroxyphenyl)-1,2,3,4,4*a*,5,12,12*a*α-octahydroxyquinolino[2,3,3-*g*]isoquinoline), a non-peptidic selective δ_1-opioid receptor agonist (Tseng et al., 1997), was synthesized by Dr. Nagase. Dizocilpine maleate ((+)-5-methyl-10, 11 dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5,10-imine maleate) was obtained from Merck/Banyu (Tokyo, Japan). The group I mGlu receptor agonist 3,5-dihydrophenylglycine (DHPG) and antagonist (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) were purchased from Tocris Cookson (Bristol, UK). All drugs were dissolved in saline.

2.5. Statistical analysis

Antinociceptive effects were calculated using the general equation and were expressed as the mean with S.E.M. One-way repeated analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for the statistical evaluation.

3. Results

Both selective δ- and μ-opioid receptor agonists produced maximal antinociceptive responses at 10 min after these drugs were given i.c.v. (data not shown). The glutamate receptor agents, dizocilpine (0.03–3.0 nmol/mouse), NMDA (10 and 30 pmol/mouse), (*S*)-4CPG (10–56 nmol/mouse) and DHPG (3.0–10 nmol/mouse), were co-administered with δ-opioid receptor agonists. When administered i.c.v. at the doses used in the present study, none of glutamate receptor agents produced any change of the warm-plate latency or other behavioral effects.

Under these conditions, the non-competitive NMDA receptor antagonist dizocilpine significantly attenuated

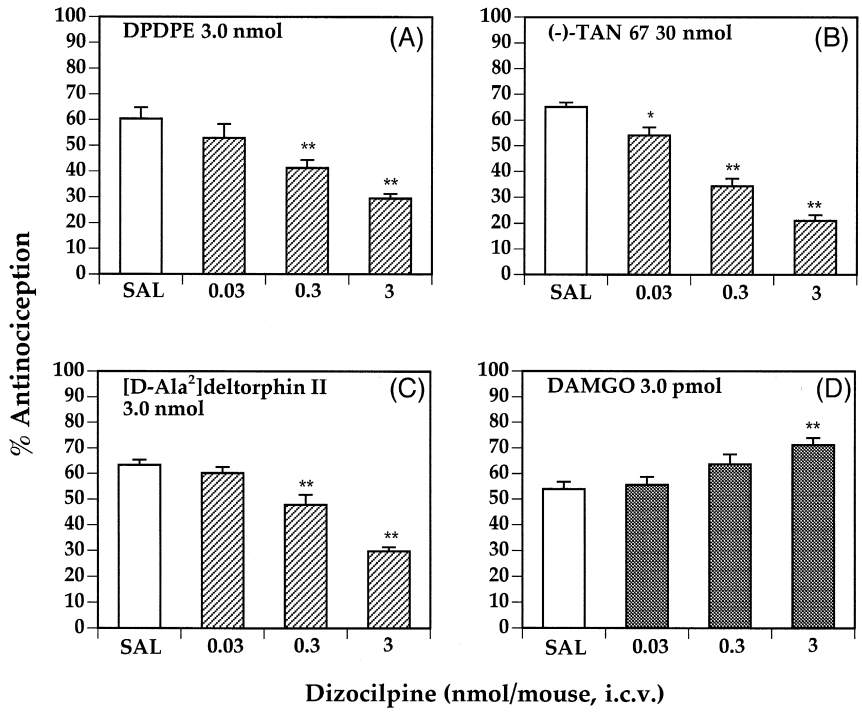

Fig. 1. Effects of the non-competitive NMDA receptor antagonist dizocilpine (0.03–3.0 nmol/mouse, i.c.v.) on DPDPE (A; 3.0 nmol)-, (−)-TAN 67 (B; 30 nmol)-, [D-Ala^2]deltorphin II (C; 3.0 nmol)- and DAMGO (D; 3.0 pmol)-induced antinociception at 10 min after the administration of opioid agonists using 51 ± 0.5°C warm-plate test. Each column represents the mean with S.E.M. of 8–10 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group.

DPDPE-, (−)-TAN 67- and [D-Ala^2]deltorphin II-induced antinociception in a dose-dependent manner (Fig. 1A; $F(3, 31) = 4.48$, $P < 0.01$, 1B; $F(3, 31) = 4.48$, $P < 0.01$, 1C; $F(3, 30) = 4.51$, $P < 0.01$). In contrast, the antinociception

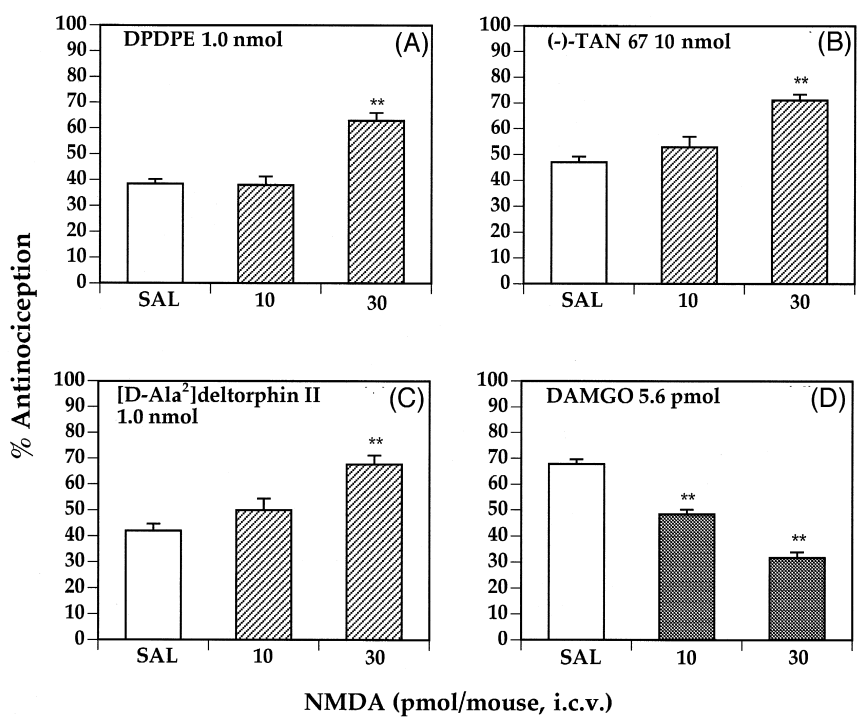

Fig. 2. Effects of NMDA (10 and 30 pmol/mouse, i.c.v.) on DPDPE (A; 1.0 nmol)-, (−)-TAN 67 (B; 10 nmol)-, [D-Ala^2]deltorphin II (C; 1.0 nmol)- and DAMGO (D; 5.6 pmol)-induced antinociception. Each column represents the mean with S.E.M. of eight or nine mice. $^{**}P < 0.01$ vs. control group.

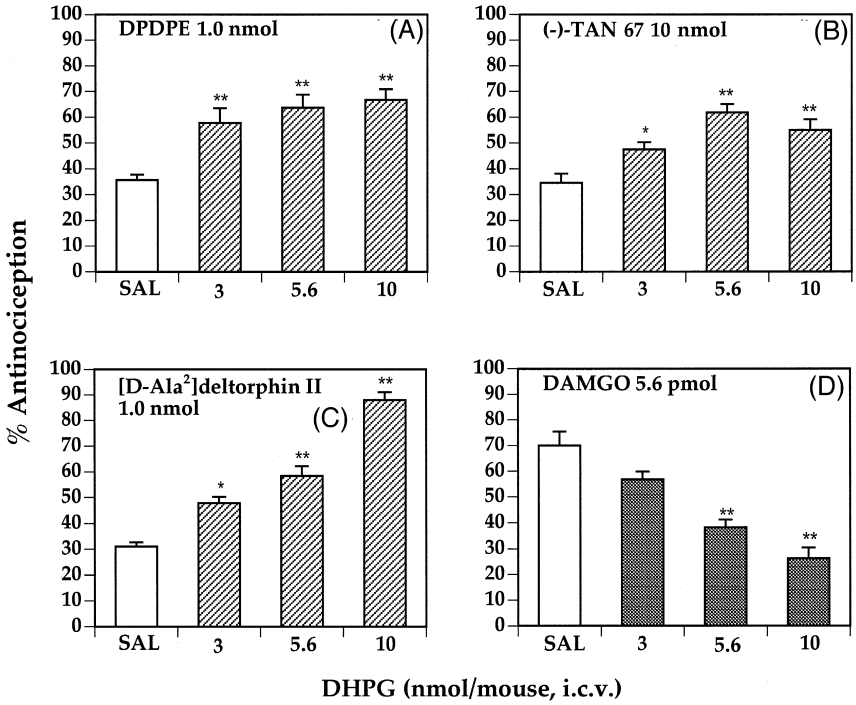

Fig. 3. Effects of the group I metabotropic glutamate (mGlu) receptor antagonist (*S*)-4-carboxyphenylglycine ((*S*)-4CPG; 10–56 nmol/mouse, i.c.v.) on DPDPE (A; 3.0 nmol)-, (−)-TAN 67 (B; 30 nmol)-, [D-Ala^2]deltorphin II (C; 3.0 nmol)- and DAMGO (D; 3.0 pmol)-induced antinociception. Each column represents the mean with S.E.M. of 8–10 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group.

induced by the μ-opioid receptor agonist DAMGO was significantly enhanced by the i.c.v. co-administration of dizocilpine (Fig. 1D; $F(3, 32) = 4.46$, $P < 0.01$). As shown in Fig. 2, the specific NMDA receptor agonist NMDA

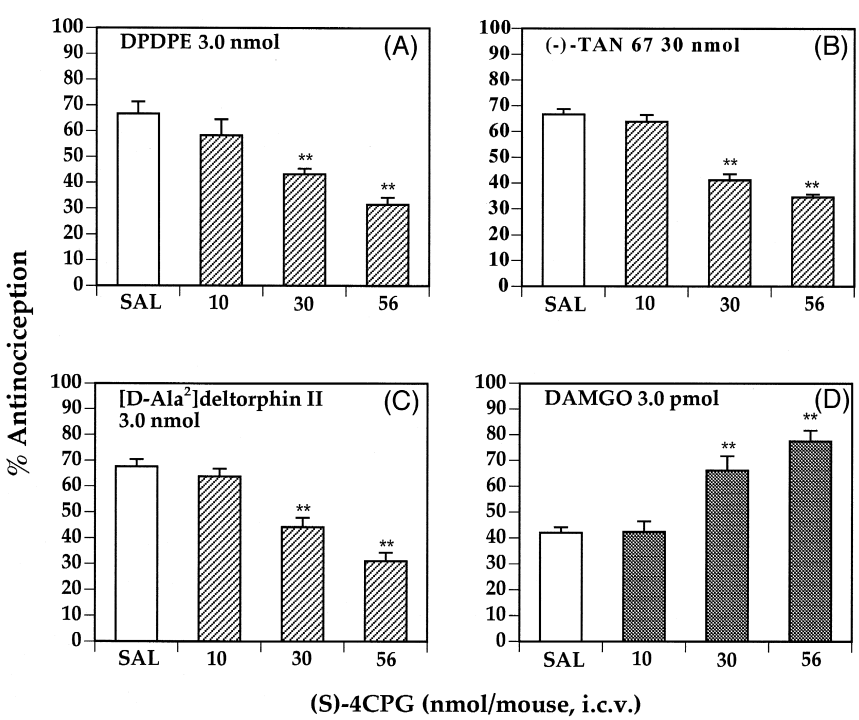

Fig. 4. Effects of the group I metabotropic glutamate (mGlu) receptor agonist 3,5-dihydrophenylglycine (DHPG; 3.0–10 nmol/mouse, i.c.v.) on DPDPE (A; 1.0 nmol)-, (−)-TAN 67 (B; 10 nmol)-, [D-Ala^2]deltorphin II (C; 1.0 nmol)- and DAMGO (D; 5.6 pmol)-induced antinociception. Each column represents the mean with S.E.M. of 9 or 10 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group.

(30 pmol/mouse) significantly enhanced DPDPE-, (−)-TAN 67- and [D-Ala^2]deltorphin II-induced antinociception (Fig. 2A; 63.4%, 2B; 51.0%, 2C; 61.0% vs. each control group), whereas it reduced the antinociception produced by DAMGO (Fig. 2D; 53.2% vs. control group).

The group I mGlu receptor antagonist (*S*)-4CPG attenuated DPDPE-, (−)-TAN 67- and [D-Ala^2]deltorphin II-induced antinociception in a dose-dependent manner (Fig. 3A; $F(3, 31) = 4.48$, $P < 0.01$, 3B; $F(3, 31) = 4.48$, $P < 0.01$, 3C; $F(3, 27) = 4.60$, $P < 0.01$). In contrast, DAMGO-induced antinociception was enhanced by the co-administration of (*S*)-4CPG (Fig. 3D; $F(3, 31) = 4.48$, $P < 0.01$). The group I mGlu receptor agonist DHPG enhanced DPDPE-, (−)-TAN 67- and [D-Ala^2]deltorphin II-induced antinociception, whereas it reduced the antinociception induced by DAMGO (Fig. 4).

4. Discussion

The present results show that the NMDA receptor antagonist dizocilpine given i.c.v. reduces the supraspinal antinociception induced by δ-opioid agonists such as DPDPE, (−)-TAN 67 and [D-Ala^2]deltorphin II in the mouse warm-plate test. These findings are consistent with the finding that intraperitoneal (i.p.) pretreatment with competitive ([3*S*-(3α,4aα,6β,8aα)]-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid; LY 235959) and non-competitive antagonists (dizocilpine; MK-801) of NMDA receptors suppressed DPDPE- and [D-Ala^2]deltorphin II-induced antinociception using the mouse tail-flick test (Bhargava and Zhao, 1996). Unlike the antinociception induced by δ-opioid agonists, the supraspinal antinociception induced by the selective μ-opioid receptor agonist DAMGO was significantly enhanced by dizocilpine at the maximal dose (3.0 nmol) used in the present study. Dizocilpine alone did not induce antinociception, sedation, motor deficits or any other abnormal behaviors. In contrast to the findings with dizocilpine, i.c.v. treatment with NMDA, which did not produce behavioral toxicity such as wild-running at these doses, enhanced δ-opioid agonist-induced antinociception but suppressed DAMGO-induced antinociception.

In terms of interactions between the mGlu receptor and opioid receptor, we found here for the first time that the group I mGlu receptor antagonist (*S*)-4CPG suppressed δ-opioid agonist-induced antinociception. This suppression by (*S*)-4CPG was observed at doses which, when given by itself, did not induce either antinociception or behavioral toxicity. On the contrary, the group I mGlu receptor agonist DHPG enhanced δ-opioid agonist-induced antinociception, whereas it blocked DAMGO-induced antinociception. These findings suggest that the functional activation of both the NMDA receptor and group I mGlu receptor may positively and negatively modulate δ- and μ-opioid agonist-induced antinociception, respectively, in the mouse brain.

Among the different second messenger systems modulated by opioids, the inhibition of adenylate cyclase activity has been considered to be a primary signal transduction mechanism in the action of opioid receptors (Loh and Smith, 1990). Several studies have demonstrated that opioid agonists elicit a decrease in cyclic AMP formation through pertussis toxin-sensitive G-proteins in both brain and cultured neuronal cells (Burns et al., 1983; Loh and Smith, 1990). However, it has been reported that a cloned δ-opioid receptor expressed in *Xenopus* oocytes can mediate agonist activation of phospholipase C (Miyamae et al., 1993). Recently, it has also been reported that δ-opioid receptor-mediated increases in intracellular Ca^{2+} result from inositol-1,4,5-triphosphate-induced Ca^{2+} release from intracellular stores (Smart and Lambert, 1996). Furthermore, it has been reported that the activation of group I mGlu receptors stimulates inositol-1,4,5-triphosphate production and increases intracellular Ca^{2+} levels (Schoepp and Conn, 1993; Pin and Duvorsin, 1995). These findings suggest the possibility that opioid receptors and mGlu receptors may share common pools of intracellular second messengers and activate receptors that modulate the activity of other receptors via common second messengers on the same cells.

There is considerable evidence of a close relationship between opioid-induced antinociception and Ca^{2+} levels within the central nervous system. Agents that increase cytosolic Ca^{2+} in neurons and synaptosomes block μ-opioid receptor agonist-induced antinociception when treated by i.c.v. administration (Hano et al., 1964), whereas Ca^{2+} chelators (i.e., EGTA) or Ca^{2+} channel antagonists potentiate μ-opioid agonist-induced antinociception (Hoffmeister and Tetterburn, 1986). On the contrary, the i.c.v. administration of Ca^{2+} has been shown to enhance the δ-opioid receptor agonist (−)-TAN 67-induced antinociception, which is reduced by i.c.v. treatment with EGTA (Osawa et al., 1998). Taken together, the present findings indicate that the differential modulation of μ- and δ-opioid receptor agonist-induced antinociception by intracellular calcium may be due to the difference in the interaction of NMDA/mGlu receptors and μ- or δ-opioid receptors. In conclusion, we have demonstrated that the stimulation of NMDA and group I mGlu receptors results in enhanced δ-opioid receptor agonist-induced antinociception.

Acknowledgements

This work was supported in part by grants from the Ministry of Health and Welfare, and the Ministry of Education, Science, Sports and Culture of Japan to T. Suzuki. We wish to thank Ms. Aya Myoga for her expert technical assistance.

References

Bhargava, H.N., Zhao, G.-M., 1996. Effects of competitive and noncompetitive antagonists of *N*-methyl-D-aspartate receptor on the analgesic action of δ_1- and δ_2-opioid agonists in mice. Br. J. Pharmacol. 119, 1586–1590.

Burns, D.L., Hewlett, E.L., Moss, J., Vaughan, M., 1983. Pertussis toxin inhibits enkephalin stimulation of GTPase of NG108-15 cell. J. Biol. Chem. 258, 1435–1438.

Haley, M.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Hano, K., Kaneto, H., Kakunaga, T., 1964. Significance of Ca^{2+} ion in the morphine analgesia. Jpn. J. Pharmacol. 14, 227–229.

Hayashi, Y., Sekikawa, N., Jane, S., Sunter, D.E., Birse, D.C., Udvarhelyi, E.F., Watkins, P.M., 1994. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J. Neurosci. 14, 3370–3377.

Hoffmeister, F., Tettenborn, D., 1986. Ca^{2+} agonists and antagonists of the dihydropyridine type: antinociceptive effects, interference with opiate μ-receptor agonists and neuropharmacological actions in rodents. Psychopharmacology 90, 299–307.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Loh, H.H., Smith, A.P., 1990. Molecular characterization of opioid receptors. Annu. Rev. Pharmacol. Toxicol. 30, 123–147.

Mansour, A., Fox, C.A., Akil, H., Watson, S.J., 1995. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci. 18, 22–29.

Martin, L.J., Blackstone, C.D., Huganir, R.L., Price, D.L., 1992. Cellular localization of a metabotropic glutamate receptor in the rat brain. Neuron 9, 259–270.

Masu, M., Nakajima, Y., Moriyoshi, K., Ishii, T., Akazawa, C., Nakanishi, S., 1994. Molecular characterization of NMDA and metabotropic glutamate receptors. Ann. N. Y. Acad. Sci. U. S. A. 707, 153–164.

Miyamae, T., Fukushima, N., Misu, Y., Ueda, H., 1993. δ-Opioid receptor mediates phospholipase C activation via Gi in *Xenopus* oocytes. FEBS Lett. 333, 311–314.

Nakanishi, S., 1992. Molecular diversity of glutamate receptors and implications for brain function. Science 258, 597–603.

Osawa, M., Nagase, H., Kamei, J., 1998. Role of intracellular calcium in modification of mu and delta opioid receptor-mediated antinociception by diabetes in mice. J. Pharmacol. Exp. Ther. 286, 780–787.

Oshima, E., Tei, K., Kayazawa, H., Urabe, N., 1990. Continuous subcutaneous injection of ketamine for cancer pain. Can. J. Anaesth. 37, 385–386.

Pin, J.-P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.

Rabben, T., Skjelbred, P., Øye, I., 1999. Prolonged analgesic effect of ketamine, an *N*-methyl-D-aspartate receptor inhibitor, in patients with chronic pain. J. Pharmacol. Exp. Ther. 289, 1060–1066.

Schoepp, D.D., Conn, P.J., 1993. Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol. Sci. 14, 13–20.

Shigemoto, R., Nomura, S., Ohishi, H., Sugihara, H., Nakanishi, S., Mizuno, N., 1993. Immunohistochemical localization of a metabotropic glutamate receptor, mGluR 5, in the rat brain. Neurosci. Lett. 163, 53–57.

Smart, D., Lambert, D.G., 1996. δ Opioids stimulate inositol 1,4,5-triphosphate formation, and so mobilize Ca^{2+} from intracellular stores, in undifferentiated NG108-15 cells. J. Neurochem. 66, 1462–1467.

Sugiyama, H., Ito, I., Hirano, C., 1987. A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature 325, 531–533.

Suzuki, T., Tsuji, M., Mori, T., Misawa, M., Nagase, H., 1996. The effects of dopamine D_1 and D_2 receptor antagonists on the rewarding effects of δ_1 and δ_2 opioid receptor agonists in mice. Psychopharmacology 124, 211–218.

Tseng, L.F., Narita, M., Mizoguchi, H., Kawai, K., Mizusuna, A., Kamei, J., Suzuki, T., Nagase, H., 1997. Delta-1 opioid receptor-mediated antinociceptive properties of a nonpeptidic delta opioid receptor agonist, (−)TAN-67, in the mouse spinal cord. J. Pharmacol. Exp. Ther. 280, 600–605.

Yang, C.-Y., Wong, C.-S., Chang, J.-Y., Ho, S.-T., 1996. Intrathecal ketamine reduces morphine requirements in patients with terminal cancer pain. Can. J. Anaesth. 43, 379–383.