

Effects of Morphine and *Panax ginseng* on the Opioid Receptor-G protein Interactions

Young-Ran KIM, Ae-Young KIM and Kyeong-Man KIM*

Pharmacology Laboratory, College of Pharmacy, Chonnam National University,
Kwang-Ju, 500-757, Korea

(Received February 20, 1999; accepted March 22, 1999)

Abstracts – Effects of *Panax ginseng* on the morphine toxicity were studied in relation to its effects on the opioid receptor-G protein interactions. Morphine treatments (3 days) reduced the body weight increment rate and the weight of the thymus and spleen. These changes were usually recovered by the concomitant administration of ginseng total saponin (GTS) but occasionally further deteriorated. This discrepancy was studied in relation to the opioid receptor coupling to G protein, that is, the effects of morphine and GTS on the opioid receptors were studied using the antagonist-agonist competitive binding studies. When GTS recovered the morphine toxicity, morphine shifted the striatal δ receptors to slightly higher affinity state, and this was partly recovered by the GTS treatment. However, morphine did not have any effect on the affinity state of δ receptor from NG108-15 cells, suggesting that additional factors were needed for the modulation of the affinity states of δ receptor. Effects of morphine and GTS on μ receptor were complicate and variable, and we could not reach a clear conclusion. The morphine toxicity might accompany complicate biological involvements, and the modulation of the affinity states of the opioid receptors might explain a part of the effects of GTS on the morphine toxicity.

Keywords □ morphine, opioid receptor, G protein, Ginseng

Morphine is an excellent analgesic in that it greatly reduces the distress associated with pain by working on the affective component of the nociception. However, along with the mental and physical dependence, morphine when used for a long period, results in serious immune suppressions (Bryant *et al.*, 1987; Bidlack and Hemmick, 1990).

Morphine seems to have more than one pathways for the immune suppressions (Shavit *et al.*, 1986; Pruett *et al.*, 1992). The activation of hypothalamic-hypophyseal-adrenal (HPA) axis, leading to an increased release of adrenal corticosterone, seems to be the most plausible pathway involved in the morphine-mediated immune suppressions (Bryant *et al.*, 1991; Hernandez *et al.*, 1993). However, it is still unclear how morphine increases the plasma corticosterone level.

One of the typical pharmacological actions of ginseng is considered to be an immunomodulation (Kim *et al.*, 1990; Lee *et al.*, 1997). In support of these findings, our previous studies have shown that oral administration of

ginseng total saponin (GTS) exerts excellent protective effects against morphine-induced depression of the B cell and T cell functions (Lee *et al.*, 1995). We also have shown that the oral administration of GTS generally blocked the morphine-induced increase in the plasma corticosterone, and apoptic cell death of thymocytes (Kim *et al.*, 1999). However, the molecular mechanism of *Panax ginseng* on the morphine-induced immunosuppression has not been reported yet.

The ternary complex model (De Lean *et al.*, 1980) predicts that the alterations in the receptor-G protein coupling change the shape of the competition curve. There have been reports showing that morphine in chronic administration changed the opioid receptor-G protein coupling (Puttfarcken *et al.*, 1988; Nestler *et al.*, 1989; Selly *et al.*, 1997). Interestingly, ginseng is known to possess variety of pharmacological actions to antagonize the morphine-induced alternations in brain functions (Ramarao and Bhargava, 1990; Suh *et al.*, 1997; Kim *et al.*, 1998).

Based on these results, in this study, we conducted a series of experiments to study the molecular mechanisms of morphine and GTS, focusing on the relationship

* To whom correspondence should be addressed.

between the physiological status of the experimental animals and the opioid receptor-G protein interaction.

MATERIALS AND METHODS

Ginseng saponins

The ginseng total saponin extracted from Korean red ginseng was provided by Korea Ginseng & Tobacco Research Institute (Taejeon, Korea).

Cell culturing

NG108-15 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% heat-inactivated fetal calf serum (FBS, Gibco) and HAT (10 mM hypoxanthine, 10 mM aminopterin, 16.1 mM thymidine).

Animal treatments

Balb/c mice were obtained from Korea Institute of Chemical Technology Animal Center (Taejeon, Korea), and were maintained on a 12-hr light/dark cycles. They were freely accessible to food and water. Four to five week old male balb/c mice were used. GTS (100 mg/kg, oral) was administered once a day for 9 days, and morphine was subcutaneously injected from 6th day to 9th day (last 4 days). The route, dose, and period of ginseng administration were determined based on the traditional folk medicine and other studies (Ramarao and Bhargava, 1990; Kim *et al.*, 1990). The mice were decapitated at the 9th day, 2 hrs after drug treatments, and the blood was collected from each mouse. For each step, any sustained pain or noxious stimuli were not applied to the animals.

Competitive binding studies of opioid receptors

Membrane proteins were prepared from the control, morphine-treated, and morphine/GTS-treated animals. Striatum or hypothalamus was removed and homogenized with polytron homogenizer in lysis buffer (50 mM Tris

HCl, pH 7.6, containing 1 mM EDTA and 1 mM PMSF and other protease inhibitors), and was centrifuged at $48,000\times g$ for 10 min at 4°C. The same procedure was repeated. Pellet was resuspended in 50 mM Tris-HCl, pH 8.0, containing 5 mM $MgCl_2$.

For competition binding studies of μ -opioid receptors, 150 μg of membrane protein was incubated with [3H] naloxone (Du Pont, 5 nM final) either in the absence or in the presence of 50 μM GppNHp and 0.1 M NaCl with increasing concentration (0.1-100 nM) of a specific μ receptor agonist, DAGO. For δ -opioid receptors, the same procedures were followed except that it was conducted both for the brain tissues and NG108-15 cells using a δ receptor-specific ligand and agonist. Membrane proteins were incubated with [3H]naltrindole (Du Pont, Kd: 0.18 nM, 0.36 nM final) with increasing concentration of (0.1-1,000 nM) of DSLET. Nonspecific binding was defined as the radioactivity remaining in the presence of 10 nM naloxone. After 1 hr incubation at 37°C, the reaction was stopped by adding ice cold binding buffer (100 mM NaCl, 50 mM Tris, and 5 mM $MgCl_2$). After vacuum filtration through GF/B filter, the radioactivity was counted with liquid scintillation counter.

RESULTS AND DISCUSSION

Effects of GTS on the morphine-induced changes in physiological parameters

Morphine treatments almost invariably induced physiological changes such as the decrease in the body weight increment rate, thymus and spleen weight, the increase in serum corticosterone level, and the apoptosis in thymocytes. The concomitant treatment with GTS usually recovered those changes (Lee *et al.*, 1995; Kim *et al.*, 1999; Table 1) but sometimes it failed to recover the morphine toxicity (Table 2). In this study, we tried to ex-

Table I. Effects of morphine and GTS on body weight increment rate and immune organ weight (recovered by GTS)

| Experimental Group | Dose (mg/kg) | Change of Body Weight (%) | % per Body Weight | |
|--------------------|--------------|---------------------------|---------------------|----------------------|
| | | | Thymus | Spleen |
| Control | — | 2.75 ± 2.26 | 0.14 ± 0.01 | 0.49 ± 0.03 |
| GTS | 100 | 2.81 ± 2.32 | 0.13 ± 0.01 | 0.47 ± 0.03 |
| Morphine | 20 | $-3.55\pm 2.21^{**}$ | $0.10\pm 0.01^{**}$ | $0.33\pm 0.02^{***}$ |
| Mor/GTS | 20/100 | $2.25\pm 3.60^*$ | 0.12 ± 0.01 | $0.38\pm 0.02^*$ |

Mice were treated with GTS (100 mg/kg, oral) for 5 days, followed by morphine/GTS treatment for subsequent 4 days. Control mice were treated with saline instead of morphine and GTS. Each value represents the mean \pm S.E. of 5-7 mice. Experiments were repeated three times. ++: $p<0.01$, significance of difference compared with control group. +++: $p<0.01$, significance of difference compared with control group. *: $p<0.1$, significance of difference compared with morphine-treated group.

Table II. Effects of morphine and GTS on the body weight increment rate and immune organ weight (deteriorated by GTS)

| Experimental Group | Dose (mg/kg) | Change of Body Weight (%) | % per Body Weight | |
|--------------------|--------------|---------------------------|-------------------------|-------------------------|
| | | | Thymus | Spleen |
| Control | — | 6.34±1.18 | 0.23±0.02 | 0.44±0.08 |
| GTS | 100 | 3.91±2.74 | 0.25±0.03 | 0.37±0.06 |
| Morphine | 20 | 0.56±3.38 ⁺⁺ | 0.18±0.02 ⁺⁺ | 0.37±0.04 |
| Mor/GTS | 20/100 | -2.34±3.61 ⁺⁺ | 0.15±0.04 | 0.31±0.02 ⁺⁺ |

Mice were treated as in table 1. ++: $p < 0.01$, significance of difference compared with control group.

plain this discrepancy in terms of the effects of morphine and GTS on the affinity states of opioid receptors.

Effects of GTS on the affinity states of δ -opioid receptors when GTS recovered the morphine toxicity

Opioid receptors exist either in a high affinity state (G protein-coupled) or low affinity state (uncoupled state). According to the ternary complex model, agonists distinguish between the high and low affinity states, but antagonists do not. Agonists have higher affinity for the receptor-G protein complex, and they displace antagonists from the G protein-coupled receptors at lower concentration. A nonhydrolyzable GTP analog, GppNHp was used as a decoupling agent. The binding of agonists to the opioid receptor is highly sensitive to nonhydrolyzable GTP analogs which (in the presence of Na^+) cause a marked decrease in the affinity to the receptor without changing the B_{max} , and promote the dissociation of receptor-G protein-agonist complex (Childers, 1991).

Table 1 is one of the cases where GTS significantly recovered the morphine-induced changes in physiological parameters. Membrane proteins were prepared from the same animals and the competition studies were conducted for the δ -opioid receptors. The membrane proteins were labelled with [^3H]naltrindole, a specific antagonist for δ receptor (K_d : 0.18 nM, Yasuda *et al.*, 1993). As the concentration of DSLET, a specific agonist for δ receptor (K_d : 21 nM, Yasuda *et al.*, 1993), was increased, [^3H]naloxone was competitively displaced from the membrane protein (control group). When this curve was compared with that of GppNHp-treated group (GppNHp completely uncouples receptor-G protein), the control curve was, shallower suggesting that the δ -opioid receptor was G protein-coupled (Fig. 1). Morphine increased the agonist-induced displacement of the high affinity sites suggesting that the δ receptor was shifted to the high affinity state by the morphine treatments. GTS partly recovered it. The same pattern was observed from the hypothalamus (data not shown).

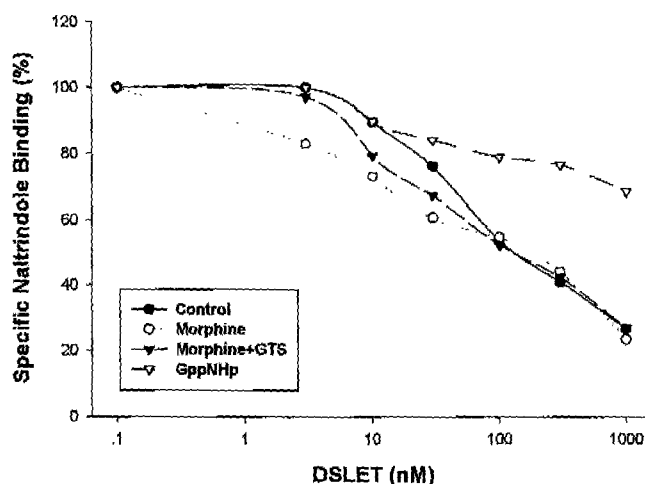


Fig. 1. Effects of morphine and GTS on the δ -opioid receptor-G protein interaction in the mice striatum when GTS recovered the morphine actions. Mice were treated as in table 1. Membrane proteins were prepared from the striatum and competition bindings were conducted using [^3H]naltrindole (0.36 nM) and DSLET (3-1,000 nM). Each point represents the average of three distinct measurements.

Effects of morphine on the affinity states of δ receptor in NG108-15 cells

To study the effects of morphine on the affinity states of δ receptor without the interference through the interaction with μ receptor, NG108-15 cells were chosen. NG108-15 cell is a hybrid of mouse neuroblastoma and rat glioma, and it expresses mouse δ receptors. In contrast to the results obtained from the striatal membrane proteins as shown in Fig. 1, the morphine treatment (100 μM , 48 hrs) did not affect the shape of competition curve suggesting that morphine did not affect the δ receptor-G protein interaction (Fig. 2).

The roles of the δ -opioid receptor in the development of morphine tolerance and dependence have been well established (Abdelhamind *et al.*, 1991; Miyamoto *et al.*, 1993), nevertheless, the effects of morphine on δ -opioid receptor were not consistent. Some reported that the treatments of NG108-15 cells with δ agonist down-regulated

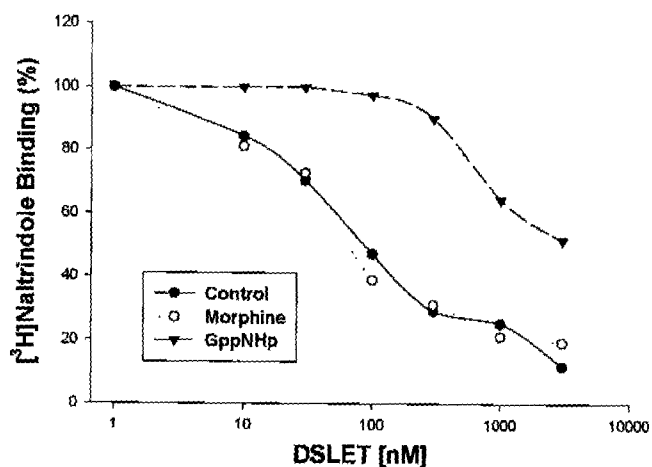


Fig. 2. Effects of morphine on the δ -receptor-G protein interaction in NG108-15 cells. NG108-15 cells were treated with 100 μ M morphine for 48 hrs. Competition bindings were conducted as in fig. 1. Each point represents the average of three distinct measurements.

the δ -opioid receptor (Kim *et al.*, 1995), but others showed that the morphine-induced down-regulation of δ -opioid receptor was observed only in the presence of functional μ receptors in the same cell (Baumhaker *et al.*, 1993; Jiang *et al.*, 1990; Palazzi *et al.*, 1996). Our study supports the latter, that is, the modulation of δ -receptor by morphine might require the interaction between δ and μ receptors.

Effects of GTS on the affinity states of μ -opioid receptors when GTS recovered the morphine toxicity

Effects of morphine and ginseng on the μ receptor-G protein coupling were technically difficult to study. Since we did not have a specific radio-labeled antagonist for the μ receptor other than [3 H]naloxone, DAGO (Kd, 0.57 nM, Martin and Eades, 1966), a specific μ receptor agonist displaced only a small proportion of the [3 H] naloxone bound from brain tissue, at most 40 to 50%. In addition, morphine treatments have been known to bring in complicate effects on μ receptor making it more difficult to interpret, furthermore, these effects seemed to vary depending on the brain regions studied (Sim *et al.*, 1996; Petruzzi *et al.*, 1997).

As shown in Fig. 3, in the striatum prepared from experimental animals treated with the morphine, DAGO, a specific μ agonist, more readily displaced [3 H]naloxone binding compared with the control group, suggesting that morphine increased the affinity of μ receptor to the agonist. Surprisingly the concomitant administration of GTS and morphine consistently shifted the competition

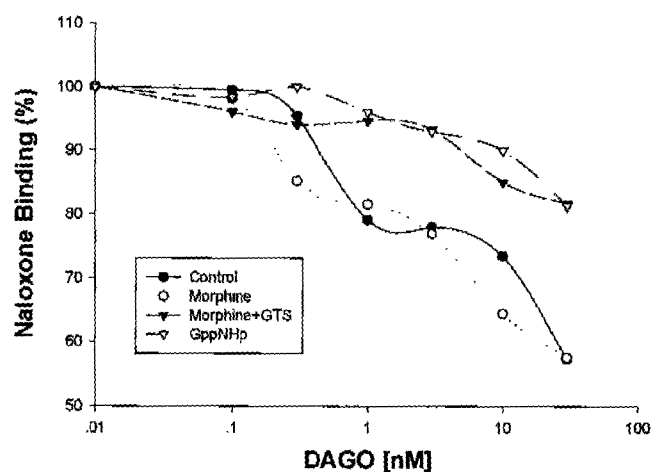


Fig. 3. Effects of morphine and GTS on the μ -opioid receptor-G protein interaction in mice striatum when GTS recovered the morphine actions. Mice were treated as in table 1. Competition bindings were conducted using [3 H]naloxone (5 nM) and DAGO (0.1-30 nM). Each point represents the average of three distinct measurements.

curve far to the right suggesting that the μ receptor was uncoupled from G protein. This would result in inefficient signaling through μ receptor.

Effects of GTS on the affinity states of μ -opioid receptors when GTS failed to recover the morphine toxicity

Occasionaly, as shown in Table 2, GTS failed to recover the morphine toxicity, rather it deteriorated the physiological parameters. Competitive binding studies were conducted from the same experimental animals using the

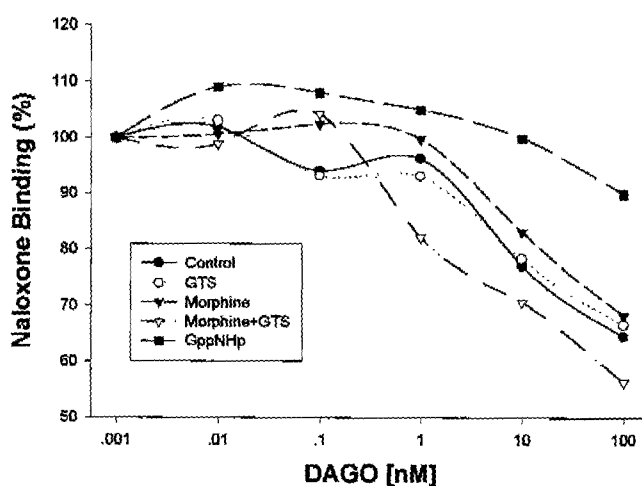


Fig. 4. Effects of morphine and GTS on the μ -opioid receptor-G protein interaction in mice striatum when GTS failed to recover the morphine actions. Mice were treated as in table 1. Competition bindings were conducted using [3 H]naloxone (5 nM) and DAGO (0.01-100 nM). Each point represents the average of three distinct measurements.

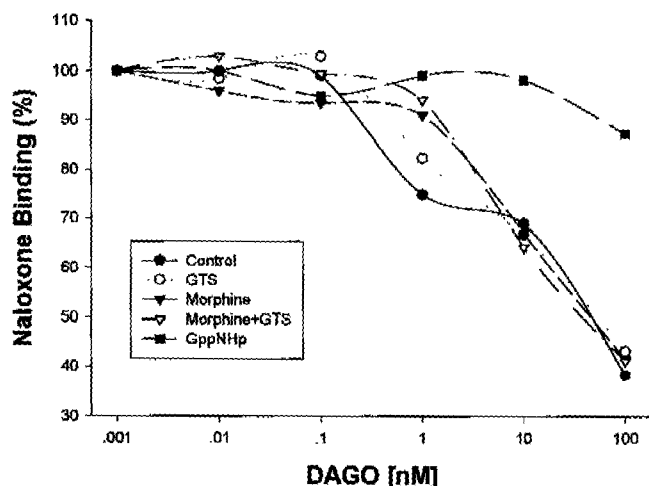


Fig. 5. Effects of morphine and GTS on the μ -opioid receptor-G protein interaction in mice hypothalamus when GTS failed to recover the morphine actions. Mice were treated as in table 1. Competition bindings were conducted using [3 H]naloxone (5 nM) and DAGO (0.01-100 nM). Each point represents the average of three distinct measurements.

membrane proteins prepared from the striatum and hypothalamus.

Fig. 4 shows the competitive binding curves from the striatum. Compared with the control group of the Fig. 3 (where GTS recovered the morphine toxicity), the competition binding curve did not show a good shallow-shape suggesting that the coupling between the μ receptor and G protein is different from Fig. 3. GTS itself did not cause any noticeable change in the shape of competition curve but morphine shifted the competition curve to the far right side with a steeper slope (G protein is uncoupled). Interestingly the concomitant treatment with GTS recovered the shape of morphine-induced competition curve close to that of control group in Fig. 3.

Fig. 5 shows the effects of morphine and GTS on the competition curve in the hypothalamus for the μ receptor. The membrane proteins were prepared from the same experimental animals in the Table 2 (Fig. 4). The effects of morphine on μ receptor from the hypothalamus were similar to those from striatum (Fig. 4) but GTS exerted totally different effects (Fig.4 vs Fig.5). The control group showed a good G protein-coupled curve and GTS itself did not exert any significant effect on the competition curve. Morphine shifted the competition curve to the right as in the striatum (uncoupling, Fig. 4), however, concomitant treatment with the morphine and GTS only slightly enhanced morphine-induced the uncoupling of μ

receptor. We do not have any clear explanation for this discrepancy between the striatum and hypothalamus from the same experimental animals. The biological environments might be different in two brain regions.

When we compared the Fig. 3 (Table 1, the toxicity was recovered by GTS) and the Fig. 4 (Table 2, the toxicity was worsened by GTS), the affinity states of μ receptors in two control groups were different. Because a number of experimental variables are involved with the differences between two studies, it is hard to discuss them separately, however, at the least, the differences in the states of μ receptor-coupling with G protein might be the starting point for the discussion.

Even though morphine exerted deteriorative effects on the physiological parameters (morphine toxicity) both in Fig. 3 and Fig. 4, the effects of morphine on the affinity states of μ receptor were totally different. In Fig. 3, morphine increased the affinity of μ receptor to the agonist (G protein-coupled), but in Fig. 4, it significantly decreased it (G protein-uncoupled). This discrepancy might reflect the different functional states of experimental animals, especially in connection with opioid receptors. We speculate that the morphine administration might put the animals either in the tolerant or in the abstinent state depending on the affinity states of μ receptors at the resting condition. It has been reported that analgesic and the cellular immune functions could be differently regulated depending on the status of the experimental animals, whether they are in physical tolerance or abstinence condition (Albrecht *et al.*, 1997). The effects of morphine and GTS might just follow the path set by the affinity states of μ receptor at the resting states.

ACKNOWLEDGEMENT

This paper was supported by 1997 Research Fund, Chonnam National University.

REFERENCES

- Abdelhamid, E. E., Sultana, M., Portoghese, P. S. and Take-mori, A. E. (1991). Selective blockade of delta opioid receptor prevents the development of morphine tolerance and dependence in mice. *J. Pharmacol. & Exp. Ther.* **258**, 299-303.
- Albrecht, E., Heinrich, N., Lorenz, D., Baeger, I., Samovilova, N., Fechner, K. and Berger, H. (1997). Influence of continuous levels of fentanyl in rats on the mu-opioid receptor in the central nervous system. *Pharmacol. Biochem. Behav.*

- 58, 89-94.
- Baumhaker, Y., Gafni, M., Keren, O. and Sarne, Y. (1993). Selective and interactive down-regulation of mu- and delta-opioid receptors in human neuroblastoma SK-N-SH cells. *Mol. Pharmacol.* **44**, 461-467.
- Bhargava, H. N. and Ramarao, P. (1991). The effects of Panax ginseng on the development of tolerance to the pharmacological actions of morphine in the rat. *Gen. Pharmacol.* **2**, 521-525.
- Bidlack, J. M. and Hemmick, L. M. (1990). Morphine-induced immune alterations *in vivo*. *Cellular Immunol.* **126**, 343-353.
- Bryant, H. U., Bernton, E. W., Kenner, J. R. and Holaday, J. W. (1991). Role of adrenal cortical activation in the immunosuppressive effects of chronic morphine treatment. *Endocrinol.* **128**, 3253-3258.
- Childers, S. R. (1991). Opioid receptor-coupled second messenger systems. *Life Sci.* **48**, 1991-2003.
- De Lean, A., Stadal, J. M., Lefkowitz, R. J. (1980). A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β -adrenergic receptor. *J. Biol. Chem.* **155**, 7108-7117.
- Hernandez, M. C., Flores, L. R. and Bayer, B. M. (1993). Immunosuppression by morphine is mediated by central pathways. *J. Pharmacol. & Exp. Ther.* **267**, 1336-1341.
- Jiang, Q., Mosberg, H. I. and Porreca, F. (1990). Selective modulation of morphine antinociception, but not development of tolerance, by δ receptor agonists. *Eur. J. Pharmacol.* **186**, 137-141.
- Kim, D. S., Chin, H. and Klee, W. A. (1995). Agonist regulation of the expression of the δ opioid receptor in NG 108-15 cells. *FEBS Lett.* **376**, 11-14.
- Kim, H. S., Jang, C. G., Oh, K. W., Oh, S., Rhee, H. M., Rhee, G. S., Seong Y. H. and Park, W. K. (1998). Effects of ginseng total saponin on morphine-induced hyperactivity and conditioned place preference in mice. *J. Ethnopharmacol.* **60**, 33-42.
- Kim, J. Y., Germolec, D. R. and Luster, M. I. (1990). Panax ginseng as potential immunomodulator: studies in mice. *Immunopharmacol. and Immunotoxicol.* **12**, 257-276.
- Kim, Y. R., Lee, S. Y., Shin, B. A. and Kim, K. M. (1999). *Panax ginseng* blocks morphine-induced thymic apoptosis by lowering plasma corticosterone level. *Gen Pharmacol.* **In press**.
- Lee, S. Y., Kim, A. Y., Kim, Y. R. and Kim, K. M. (1995). Effect of *Panax ginseng* on morphine-induced immune suppression. *Kor. J. Appl. Pharmacol.* **3**, 177-181.
- Lee, Y. S., Chung, I. S., Lee, I. R., Kim, K. H., Hong, W. S. and Yun, Y. S. (1997). Activation of multiple effector pathways of immune system by the antineoplastic immunostimulatory acidic polysaccharide ginsan isolated from *Panax ginseng*. *Cancer Res.* **17**, 323-331.
- Martin, W. R. and Eades, M. L. (1966). Demonstration of tolerance and physical dependence in the dog following a short-term infusion of morphine. *J. Pharmacol. Exp. Ther.* **133**, 262-267.
- Miyamoto, Y., Portoghese, P. S. and Takemori, A. E. (1993). Involvement of δ_2 opioid receptors in the development of morphine dependence in mice. *J. Pharmacol. Exp. Ther.* **264**, 1141-1145.
- Nestler, E. J., Erdos, J. J., Terwiliger, R., Duman, R. S. and Tallman, J. F. (1989). Regulation of G proteins by chronic morphine in the locus coeruleus. *Brain Res.* **476**, 230-239.
- Palazzi, E., Ceppi, E., Guglielmetti, F., Catozzi, L., Amoroso, D. and Groppetti, A. (1996). Biochemical evidence of functional interaction between μ - and δ -opioid receptors in SK-N-BE neuroblastoma cell line. *J. Neurochem.* **67**, 138-144.
- Petrucci, R., Ferraro, T. N., Kurschner, V. C., Golden, G. T. and Berrettini, W. H. (1997). The effects of repeated morphine exposure on μ opioid receptor number and affinity in C57BL/6J and DBA/2J mice. *Life Sci.* **61**, 2057-2064.
- Pruett, S. B., Han, Y. C. and Fuchs, B. A. (1992). Morphine suppresses primary humoral immune responses by a predominantly indirect mechanism. *J. Pharmacol. & Exp. Ther.* **262**, 923-928.
- Puttfarcken, P. S., Werling, L. L. and Cox, B. M. (1988). Effects of chronic morphine exposure on opioid inhibition of adenylyl cyclase in 7315c cell membranes: a useful model for the study of tolerance at μ opioid receptors. *Mol. Pharmacol.* **33**, 520-527.
- Ramarao, P. and Bhargava, H. N. (1990). Antagonism of the acute pharmacological actions of morphine by *Panax ginseng* extract. *Gen. Pharmacol.* **21**, 877-880.
- Selly, D. E., Nestler, E. J., Breivogel, C. S., Childers, S. R. (1997). Opioid receptor-coupled G-proteins in rat locus coeruleus membranes: decrease in activity after chronic morphine treatment. *Brain Res.* **746**, 10-18.
- Shavit, Y., Depaulis, A., Martin, F. C., Terman, G. W., Perchnick, R. N., Zane, C. J., Gale, R. P. and Liebeskind, J. C. (1986). Involvement of brain opiate receptors in the immune-suppressive effect of morphine. *Proc. Natl. Acad. Sci.* **83**, 7114-7117.
- Sim, L. J., Selly, D. E., Dworkin, S. I. and Childers, S. R. (1996). Effects of chronic morphine administration on μ opioid receptor-stimulated [³⁵S]GTP γ S autoradiography in rat brain. *J. Neurosci.* **16**, 2684-2692.
- Suh, H. W., Song, D. K. and Kim, Y. H. (1997). Effects of ginsenosides injected intrathecally or intracerebroventricularly on antinociception induced by morphine administered intracerebroventricularly in the mouse. *Gen. Pharmacol.* **29**, 873-877.
- Yasuda, K., Raynor, K., Kong, H., Breder, C. D., Takeda, J., Reisine, T. and Bell, G. I. (1993). Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. *Proc. Natl. Acad. Sci. USA* **90**, 6736-6740.