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Effects of *Panax Ginseng* on the Development of Morphine Tolerance and Dependence
—on antagonisms of morphine analgesia by ginsenosides—

Hack-Seang Kim, Sung-Hee Shin, Kang-Ju Choi* and Suk-Chang Kim*
*College of Pharmacy, Chungbuk National University, Cheongju 310, and *Korea Ginseng and Tobacco Research Institute, Taejon 300-31, Korea*
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인삼이 몰핀내성 및 의존성 형성에 미치는 영향
— 수종의 ginsenoside에 의한 몰핀진통력 길항작용에 관하여 —

김학성·신성희·최강주*·김석창*
충북대학교 약학대학, 한국인삼연초연구소*
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Abstract

Antagonisms of the analgesic effect of morphine in mice by ginsenoside Rb_1, Rb_2, Rg_1 and Re were investigated in these experiments. These ginsenosides antagonized the analgesic effect induced by morphine in mice and the administration of 2,4-dihydroxyphenylalanine or 5-hydroxytryptophan reduced the antagonisms of morphine analgesia by the ginsenosides. Possible mechanisms involved in the antagonistic actions of the ginsenosides on morphine analgesia were described.

Introduction

Morphine is employed primarily as an analgesic, but it has many other pharmacological effects as well. The development of tolerance and physical dependence with repeated use is a characteristic feature of morphine, and the possibility of developing psychological dependence on the effect of morphine is one of the major limitations of its clinical use.

The history of opium and its alkaloids and the problems of addiction are described by Terry and Pellens[1] and by Musto.[2] The problem of addiction to opioids stimulated a search for potent analgesics that would be free of the potential to produce addiction. Similarly, long acting and orally effective narcotic antagonists with minimum secondary effects have been sought to treat narcotic addicts. A folk medicinal preparation

prescribed by seven herbal drugs including *Panax ginseng* has been used as an antidote in the treatment of morphine tolerant-dependent patients and its effective component is known to be keratin of *Manis squama.*[3] However, they did not discuss any effects of *Panax ginseng* on morphine tolerant-dependent patients. Nabata *et al.* reported the analgesic and hypothermic effects in ginseng extract and its saponins.[4] We reported the inhibition of development of morphine induced tolerance and dependence in ginseng saponins, protopanaxadiol saponins and protopanaxatriol saponins,[5,6] and that ginseng saponins antagonized morphine analgesia in mice. These actions may be attributed to decreases in catecholamines and serotonin levels.[7]

The present study was undertaken to determine whether ginsenoside Rb_1, Rb_2, Rg_1 and Re treated in acute antagonize the analgesic effect of morphine and are related to the changes of catecholamines and serotonin levels.

Materials and Methods

White ICR male mice weighing 18-22g in each group of 10-15 mice were used in all experiments. L-DOPA hydrochloride (Sigma), 5-HTP hydrochloride (Sigma) and ginsenoside Re in 0.5% CMC suspension, ginsenoside Rb_1, Rb_2 and Rg_1 dissolved in distilled water were administered intraperitoneally but morphine hydrochloride (Dae-Won Pharm. Co.,) was injected subcutaneously.

Methods

The analgesic action of morphine 5 mg/kg(s.c.) was tested by the fail flick method described by the D'Amour and Smith.[8] The measurement was made every 30 min during 90 min. by the fail flick method. The tail flick latencies to thermal stimulation were determined in seconds prior to morphine injection and at 0, 30, 60 and 90 min after its injection. A value of 10 sec was used as the cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula:

$$\text{Percent Analgesia} = \frac{\text{Tt-To}}{\text{Tc-To}} \times 100$$

where To is base line or pre-morphine tail flick reaction time; Tt is the reaction time at t min after morphine injection, and Tc is cut-off time. The base lines of tail flick latencies in different groups were around 2 ± 0.2 seconds. The effect was calculated as area under a curve (A.U.C.) that was obtained by plotting the analgesic percent on the ordinate and the time intervals (min) on the abscissa, and was expressed as percent of the effect obtained in morphine alone treated control animals.[9] To test the antagonized degrees of morphine analgesia by ginsenosides, both doses of ginsenosides 100 and 200 mg/kg were administered to mice 4 hours prior to the morphine injection.

The influence of L-DOPA or 5-HTP 100 mg/kg on the antagonisms of morphine analgesia by ginsenosides was tested in the groups pretreated with ginsenosides 200 mg/kg because significant antagonisms were not observed in the Re group pretreated with ginsenosides 100 mg/kg. L-DOPA or 5-HTP 100 mg/kg was injected intraperitoneally 30 min prior to morphine administration.

Statistics

The differences in the means for different responses in different groups were analyzed by student's t-test.

Results

The analgesic effects of ginsenosides 100 and 200 mg/kg were studied in a preliminary experiment. When compared with morphine 5 mg/kg alone activity, the analgesic effects of ginsenosides 100 mg/kg showed 36.4% in the Rb_1 group and showed 40.7% in the Rb_2 group. Ginsenosides 200 mg/kg showed 112.5% in the Rb_1 group and 85.95% in the Rb_2 group. But Rg_1 and Re groups produced less than 10% of analgesic activity in both doses. (Fig. 1)

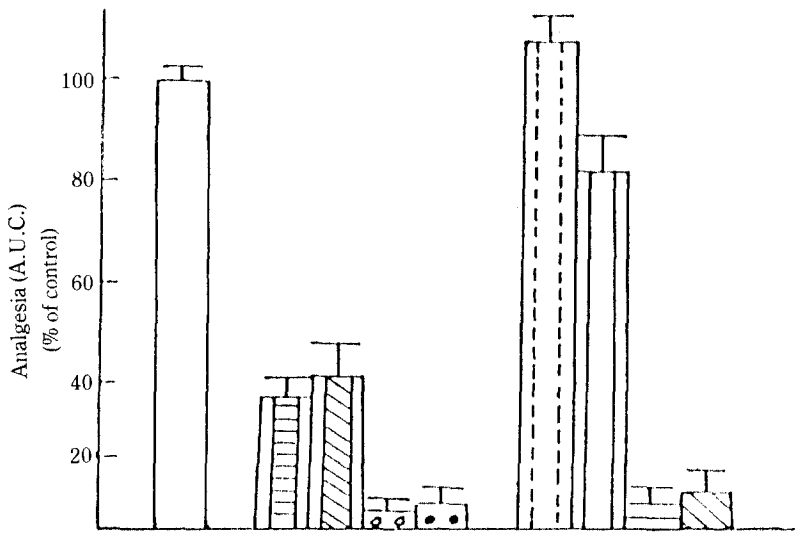

Fig. 1. Analgesic activity of ginsenosides 100 or 200 mg/kg when compared with morphine 5 mg/kg analgesia.

Mor 5 mg/kg
Rg_1 100mg/kg
Rb_2 200 mg/kg
Rb_1 100 mg/kg
Re 100 mg/kg
Rg_1 200 mg/kg
Rb_2 100 mg/kg
Rb_1 200mg/kg
Re 200 mg/kg

The significant antagonisms of morphine analgesia showed 36.22% in the Rb_1 group, 57.16% in the Rb_2 group and 36.62% in the Rg_1 group when pretreated with the respective ginsenoside 100 mg/kg, but the Re group did not show the significant antagonism. Meanwhile the groups treated with the respective ginsenoside 200 mg/kg showed significant antagonisms of morphine analgesia of 84.48% in the Rb_1 group, 98.01% in the Rb_2 group, 86.33% in the Rg_1 group and 91.08% in the Re group. (Fig. 2) A single injection of L-DOPA or 5-HTP 100 mg/kg had no significant effect on the morphine analgesia. (Fig. 3)

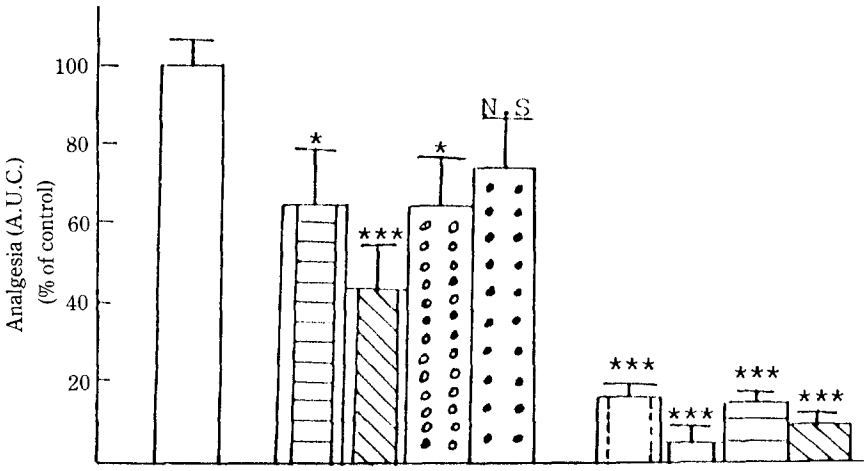

Fig. 2. Analgesic activity of morphine 5 mg/kg when pretreated with each ginsenoside 100 or 200 mg in mice.
Experimental details are described in Material and Methods. * $P<0.05$ *** $P<0.001$

Mor 5 mg/kg
Rg_1 100 mg/kg + Mor
Rb_2 200 mg/kg + Mor
Rb_1 100 mg/kg + Mor
Re 100 mg/kg + Mor
Rg_1 200 mg/kg + Mor
Rb_2 100 mg/kg + Mor
Rb_1 200 mg/kg + Mor
Re 200 mg/kg + Mor

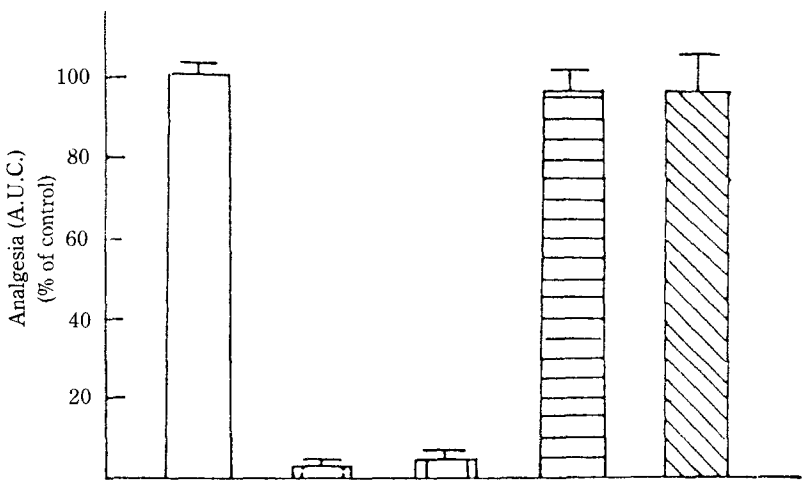

Fig. 3. Analgesic activity of L-DOPA or 5-HTP 100 mg/kg when compared with morphine 5 mg/kg analgesia, and morphine analgesia when pretreated with L-DOPA or 5-HTP 100 mg/kg

Mor 5mg/kg
5-HTP 100 mg/kg
5-HTP + Mor
L-DOPA 100 mg/kg
L-DOPA + Mor

As compared with morphine 5 mg/kg alone activity, administration of L-DOPA 100 mg/kg in the groups pretreated with ginsenosides 200 mg/kg restored the reduced analgesic activity of morphine to 56.57% in the Rb_1 group, 94.02% in the Rg_1 group, 86.72% in the Re group but to over 100% in the Rb_2 group. (Fig. 4) As com-

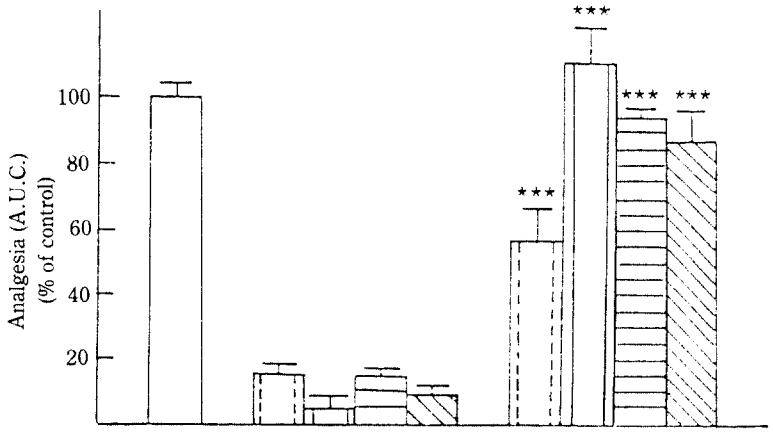

Fig. 4. Analgesic activity of morphine when pretreated with ginsenosides 200mg/kg, and recovery of the morphine analgesia when injected L-DOPA 100 mg/kg.

Mor 5mg/kg | Rb_2 200mg/kg | Re 200mg/kg | Rb_1 200mg/kg | Rg_1 200 mg/kg

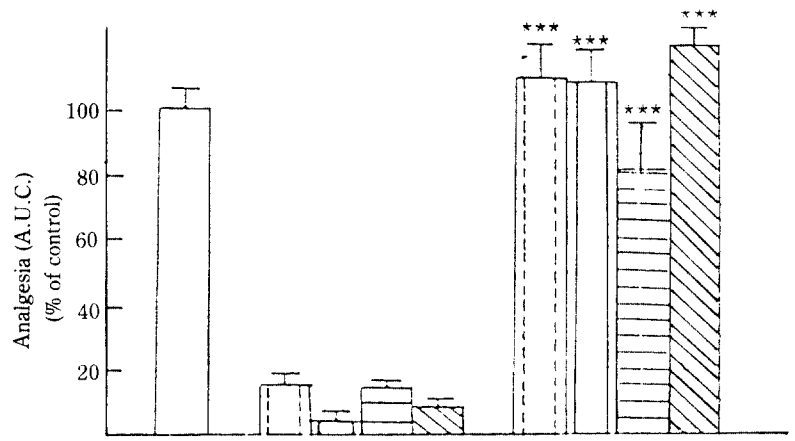

Fig. 5. Analgesic activity of morphine when pretreated with ginsenosides 200mg/kg, and recovery of the morphine analgesia when injected 5-HTP 100mg/kg

Mor 5 mg/kg | Rb_2 200 mg/kg | Re 200 mg/kg | Rb_1 200 mg/kg | Rg_1 200 mg/kg

pared with morphine 5 mg/kg alone activity, administration of 5-HTP 100 mg/kg in the groups pretreated with ginsenosides 200 mg/kg restored the reduced morphine analgesia to 80.26% in the Rg_1 group but to over the degrees of morphine alone activity in other groups. (Fig. 5)

Discussion

Morphine-induced analgesis is due to actions at several sites within the CNS and involves several systems of neurotransmitters. Morphine like drugs are thought to de-

crease the release of neurotransmitters, such as substance P, that mediate transmission of pain impulses. Other neurotransmitters, such as 5-HT and norepinephrine, are also involved. There is a remarkable capacity to modulate the perception of pain in the CNS. Electrical stimulations of the periaqueductal gray matter produce analgesia by mechanisms that involve both opioid like peptides and biogenic amines (*e.g.*, 5-HT and norepinephrine). There also appears to be an opioid system in the CNS that enhances susceptivility to painful stimuli. Non-opioid pathways also participate in the modulation of nociception.

The analgesic effects of stimulation at supraspinal sites are reduced by intrathecal administration of adrenergic and tryptaminergic antagonists.[10] The antagonistic effect of reserpine on the analgesic effect of morphine has been widely accepted, and it is suggested that brain catecholamines play a role in the mechanism.[11] It has been described that tetrabenazine antagonized the analgesia induced by morphine in mice and the effect of tetrabenazine was attributed to reduction of brain dopamine and norepinephrine.[12]

Kim *et al.* reported that G.S, PD and PT antagonized morphine analgesia. Their maximal antagonized effects on the time course appeared at 4 hours prior to the injection of morphine and the antagonisms of morphine analgesia by ginseng saponins were reduced by the administration of L-DOPA or 5-HTP.[6,7] Therefore ginsenosides were injected in mice at 4 hours prior to the injection of morphine to test their antagonized effects in this experiment. It was reported that ginseng saponins might have reserpine or tetrabenazine like action and deplete catecholamines and serotonin levels.[13,14] Also the newly equilibrated state of neurogic functions on the antagonisms of morphine analgesia by ginseng saponins could be thought as shown in the groups of daily treatment with small dose of reserpine.[15]

The present results showed that ginsenosides antagonized the analgesic effect of morphine. These findings suggested that the antagonisms of morphine analgesia produced by ginsenosides might be primarily due to their central or peripheral action of analgesic pathways of morphine. In order to investigate the possibility that ginsenosides induced decreases in CNS or PNS levels of noradrenaline or serotonin are responsible for their antagonistic actions, the administration of each amine precursor was determined. The marked suppressive effects of L-DOPA and 5-HTP were observed upon ginsenosides antagonisms of morphine analgesia. When both precursors, L-DOPA and 5-HTP in subeffective dose were injected into ginsenosides treated mice, morphine showed nearly the same analgesic effect as or over that of morphine alone.

Considering these findings, ginsenosides antagonisms of morphine analgesia may be attributed to several factors: a decrease of noradrenaline and serotonin content, a change in the concentration ratio of both amines in CNS or PNS, and the newly equilibrated state of neurogic functions.

Conclusion

In the study of the antagonisms of morphine analgesia by ginsenoside Rb_1, Rb_2, Rg_1 and Re, these ginsenosides antagonized the morphine analgesia and the administration of 2,4-dihydroxyphenylalanine or 5-hydroxytryptophan reduced the antagonisms of the morphine analgesia.

Acknowledgement

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