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Effect of Total Ginseng Saponin on the Opioid Receptor Binding in Mouse Brain

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Abstract□The modulatory effects of total ginseng saponin (TGS) on the μ, δ, and opioid receptor binding in morphine tolerance and dependence were examined in this study. The specific [^{3}H]DAGO ([D-Ala^2, N-Mephe^4, Glycol^5] enkephalin) binding was significantly increased in chronic morphine (10 mg/kg, i.p.) treated mouse striatum. The specific [^{3}H]DPDPE ([D-Pen^2, D-Pen^5] enkephalin) binding was significantly increased following morphine treatment in the mouse striatum and cortex. Also, an apparent decrease in the affinity of [^{3}H]DPN (diprenorphine) was observed after chronic morphine treatment in mouse striatum and cortex. TGS produced a slight increase of specific [^{3}H]DAGO, [^{3}H]DPDPE binding and a significant increase of specific [^{3}H]DPN binding in the mouse brain striatum. In cortex, TGS produced an inhibition of specific [^{3}H]DAGO and [^{3}H]DPDPE binding and increase of the specific [^{3}H]DPN binding. The prolonged administration of TGS (25, 50, 100, and 150 mg/kg, i.p., 3 wks) produced an inhibition of increased [^{3}H]DAGO specific binding following morphine without significant changes in the agonist binding to and receptors in mouse striatum and cortex. These contracted alterations in μ, δ, and κ opiate receptor binding were dependent in TGS doses and brain sites.

Key words□Total ginseng saponin, morphine, opioid receptor binding.

Introduction

Panax ginseng has been used in medicine not only in Korea and China but also in Japan, the Soviet Union and the United States of America.[1] According to the literature, ginseng has been known in chinese ethnopharmacology for more than 3000 years.[1] The studies on the pharmacology and clinical application of ginseng on decrease in blood pressure,[2,3] a suppression of conditioned avoidance response,[4,5] inhibition of gastric ulceration,[6] facilitation of sexual behavior[7] of ginseng were undertaken by many researchers.

Ginseng saponin, such as Rb, has been shown to possess CNS depressant, anticonvulsant, antipsychotics, improvement of learning and memory, analgesic and anti-fatigue action.[8-10] Moreover, Ramarao and Bhargava[11] have demonstrated ginseng extract in high doses produced a mild analgesic activity in the rat, but it was not antagonized by naltrexone. Kim *et al.*[12-14] have reported ginseng inhibits the development of tolerance and physical dependence on morphine in mice, which is not associated with the reduction of the brain biogenic amines.[15] But, cellular and biochemical mechanisms underlying dependence upon opioids are still less understood. Opioids have many pharmacological effects, that could be mediated through various intracellular mechanisms and different opioids can induce the same pharmacological effect via different transduction pathway.[16,17] It is known that following chronic administration of selective or opioid agonist, distinct abstinence signs are manifest.[18] Also, various CNS areas, including the locus coeruleus,[19] the nucleus accumbens,[20] the hippocampus,[21] the ventral midbrain,[22] the periaqueductal grey,[23] the amygdala,[24]

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the caudate nucleus[25] and the spinal cord[26] have been shown to play a role in opioid development or tolerance. Many putative neurotransmitters such as dopamine, noradrenaline, serotonin, acetylcholine, GABA, and excitatory amino acid have been reported to be involved in the appearance and expression of various signs of opioid tolerance and dependence.[27,28]

The purpose of the present study was to investigate whether TGS has the preventive effect on the morphine tolerance in the mice. In order to investigate the influence of TGS on the development of morphine tolerance, we assessed the opioid receptor binding assay on the μ, δ, and κ-specific receptors in chronic TGS and morphine administrated mice.

Materials and Methods

1. Experimental animals

Six male ICR mice weighing 20~30 g were used in each group. The animals were housed in a controlled environment of 23± 1℃ temperature and regular food throughout the experimental period.

2. Treatment

TGS (saponins mixture containing 26 ginsenosides such as Rb_1 18.26%, Rb_2 9.7%, Rc 9.65%, Rd 8.24%, Re 9.28%, Rf 3.48%, Rgi 6.42%, Rg_2 3.62%, Rg_3 4.70%) powder was purified by Ando *et al*'s method[29] and kindly presented from the Korea Ginseng & Tobacco Research Institute. It was suspended in distilled water and administrated intraperitoneally in doses of 25, 50, 100, and 150 mg/kg/day for 3 wks. The control animals were received an equivalent volume (10 ml/kg) of saline for 3 wks. Morphine (10 mg/kg, i.p.) was co-administrated with TGS from second weeks of TGS treatment. The experimental groups were as follows. Control group: saline injection, TGS groups : TGS (25, 50, 100, and 150 mg/kg) injection for 3wks, Morphine group : morphine (10 mg/kg) injection for 1 wk, TGS + morphine group : combined injection of morphine (10 mg/kg) and TGS (25, 50, 100, and 150 mg/kg) for 3 wks.

3. Opiate receptor binding assays

The P_2 membrane preparation was carried out as previously described.[30] Aliquots (100 μl, 1 mg/ml of protein) of freshly prepared homogenate from striatal tissue were incubated with [^{3}H][D-Ala^2, N-Mephe^4, Glycol^5] enkephalin : DAGO (for μ-specific opioid receptor assays), [^{3}H][D-Pen^2, D-Pen^5] enkephalin : DPDPE (for δ-specific opioid receptor assays), [^{3}H] diprenorphine : DPN (for κ-specific opioid receptor assays). Nonspecific binding was determined by the addition of 5 μM DAGO, DPDPE, and dynorphine A. The 2-hour incubation was terminated by collecting the Whatman GF filter, and excess radioactivity was removed by washing the filters three times with 5 ml of HEPES (25 mM, pH 7.4) at 4℃. After incubating at 20℃ overnight in 10 ml of Liquiscint (National diagnostics, Somerville, N.J., USA), its radioactivity on the filters was determined by liquid scintillation counter (Packard, Model TRI-CARB 4530, USA).

4. Protein determinations

The concentrations of protein in membrane preparation were measured by the method of Lowey *et al.*[31] Bovine serum albumin was used as the protein standard.

5. Materials

[^{3}H]DAGO (59 Ci/mmol), [^{3}H]DPDPE (46 Ci/mmol) and [^{3}H]DPN (31 Ci/mmol) were obtained from Amersham Int (Buckinghamshire, England). DAGO, DPDPE, dynorphine A and haloperidol were purchased from Sigma Chemical Company (St. Louis, M.O., USA). Morphine hydrochloride was obtained from Sam-Sung Pharm. Co. (Seoul, Korea).

6. Statistical analysis

Data are presented as mean± S.E. for the 6 mice. Students t-test was used for statistical analysis.

Results

1. Mouse striatum

In the P_2 membrane homogenates of the mouse striatum, the agonist [^{3}H]DAGO and [^{3}H]DPDPE binding by morphine (10 mg/kg, i.p.) was increased following morphine (Fig. 1 and 2). The [^{3}H]DPN specific binding was decreased (Fig. 3).

The specific [^{3}H]DAGO binding to the brain striatal membrane was decreased in TGS (25 mg/kg, i.p.) treated group, but was similar with control

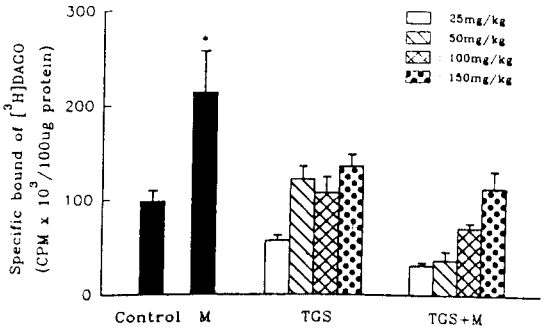

Fig. 1. Effect of TGS on the [^{3}]DAGO binding in mouse brain striatum. M : Morphine (10 mg/kg), TGS : Total ginseng saponin, TGS+M : Total ginseng saponin+Morphine. *p<0.05.

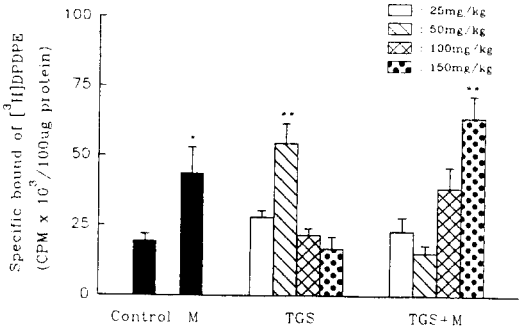

Fig. 2. Effect of TGS on the [^{3}]DPDPE binding in mouse brain striatum. *p<0.05, **p<0.01.

group in TGS (50, 100 and 150 mg/kg) treated groups (Fig. 1). The degree of the specific [^{3}H]DPDPE binding did not present significant difference in each group of TGS treatment (Fig. 2). The specific [^{3}H]DPN binding was significantly increased following 50 and 100 mg/kg TGS (Fig. 3).

The increased specific binding of [^{3}H]DAGO following morphine was inhibited by TGS (25, 50, 100, and 150 mg/kg)(Fig. 1). We could not find a significance of [^{3}H]DPDPE specific binding in each group (Fig. 2). The decreased specific binding of [^{3}H]DPN following morphine was increased by TGS (50, 100, and 150 mg/kg)(Fig. 3).

2. Mouse cortex

In P_2 membrane homogenates of the mouse cortex, morphine had no effect on the [^{3}H]DAGO specific binding (Fig. 4). The agonist [^{3}H]DPDPE specific binding following morphine (10 mg/kg, i.p.) was

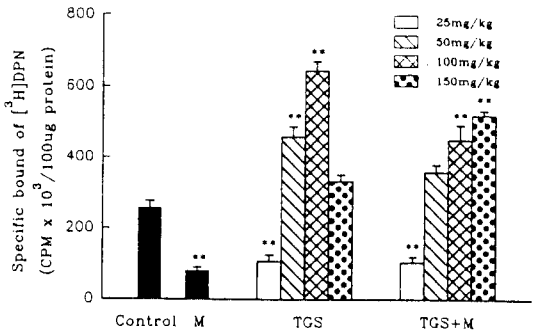

Fig. 3. Effect of TGS on the [^{3}]DPN binding in mouse brain striatum. **p<0.01.

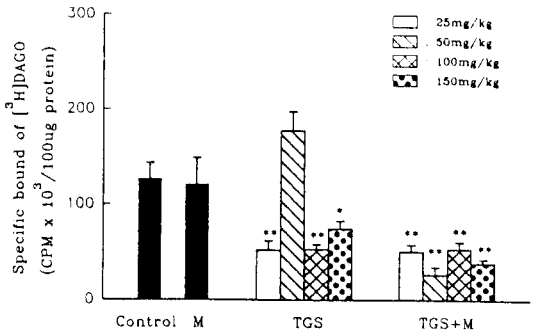

Fig. 4. Effect of TGS on the [^{3}]DAGO binding in mouse brain cortex. *p<0.05, **p<0.01.

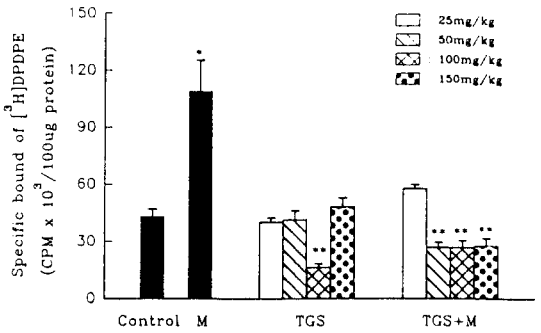

Fig. 5. Effect of TGS on the [^{3}]DPDPE binding in mouse brain cortex. *p<0.05, **p<0.01.

significantly increased (Fig. 5). The [^{3}H]DPN specific binding was significantly decreased (Fig. 6).

The specific [^{3}H]DAGO binding to the brain cortical membrane was decreased in TGS group (25, 100, or 150 mg/kg)(Fig. 4). But the specific [^{3}H]DPDPE binding to the brain cortex did not show sig-

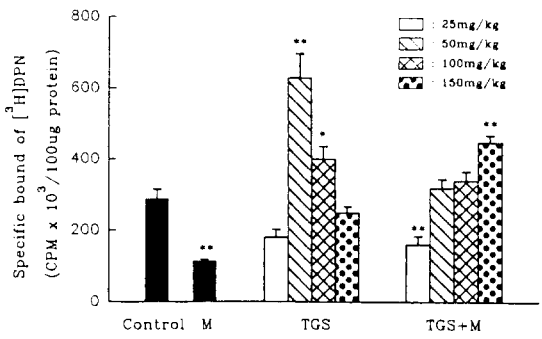

Fig. 6. Effect of TGS on the [^{3}]DPN binding in mouse brain cortex. *p<0.05, **p<0.01.

nificant changes by TGS (Fig. 5). The [^{3}H]DPN specific binding was increased by TGS (50 or 100 mg/kg)(Fig. 6).

The specific binding of [^{3}H]DAGO by morphine was inhibited by TGS treatment (Fig. 4). The specific [^{3}H]DPDPE binding to the brain cortical membrane by morphine was decreased by TGS treatment (Fig. 5). The decrease in specific binding of [^{3}H] DPN following morphine was increased by TGS (50, 100 and 150 mg/kg) (Fig. 6).

Discussion

Long term administration of opioids induces analgesic tolerance.[32] However, the cellular and molecular mechanisms of opioid tolerance are still not clearly understood. Many models of drug addiction include the development of dependence with chronic drug use and the emergence of withdrawal symptoms on abstinence.[33] In case of opioid dependence of animal model, measurements of opiate withdrawal symptom include not only well-characterized somatic signs but also behavioral alterations. The results of a number of studies[34,35] suggest that the neural mechanism contribute to the expression of behavioral and somatic symptoms of opiate withdrawal, which are anatomically distinct. The opioid-sensitive neurons, which are widely distributed in the nervous system, are evidently subject to many other neurons, and exert themselves influences on numerous other neurons.[16] Several types of opioid receptors are present in whole animal tissues and the occurrence of receptor-selective tolerance supports that receptor-specific mechanisms may be critical in the tolerance process.[16] But, attempts to demonstrate any alteration in the opioid receptor density or receptor-ligand interaction as a possible mechanism for opiate tolerance have been inconclusive. Ginseng produces various neuropharmacological effects such as changes in brain biogenic amines, improvement of learning and memory retention, promotion of recovery from fatigue, and an increase of intellectural performance.[8-10] Recently, Kim *et al.*[12-14] reported the inhibitory effects of ginseng on the development of morphine tolerance and dependence, in addition to the antagonism of morphine analgesia. This study was to determine whether TGS has an inhibitory effect on tolerance to morphine through opioid specific receptor binding assay. The present results indicate that chronic administration of TGS results in the decrease of [^{3}H] DAGO binding by morphine treatment and no changes of [^{3}H] DPDPE and in increase of [^{3}H] DPN binding significantly. Morphine can produce a down-regulation of brain μ receptor in neonatal rats and in spinal cord and cerebral cortex in adult rats.[36,37] Chronic infusion of morphine in guinea pigs decreases the number of μ receptors with high affinity for agonists, resulting in a small reduction in overall μ receptor number, without changes in the agonist binding to μ and κ receptors.[38] In the clonal cell line NG108-15 cells, chronic opiate agonist treatment produced a down-regulation of the δ opiate receptor.[39,40] This was due to an internalization of the cell surface receptors into the lysosomal compartment.[40] The observed inhibition of opioid binding sites in the effector system result in an apparent decrease in agonist affinity for the receptor. Otherwise, chronic morphine treatment produced an enhancement of opioid receptor binding[16,41,42] and chronic naloxone treatment produced also an increase in opioid receptor binding.[42,43] Therefore, the cause of the failure of chronic morphine treatment and the mechanism of opiate tolerance were not due to only an alteration of the receptor density. Thus, opiate tolerance might not be due to the loss in opioid receptor binding. Ramarao and Bhargava[11] reported that ginseng-induced analgesia was not reversed by naloxone. There-

fore, they suggested that the analgesia of ginseng did not mediate via opiate receptor system. These data showed agreement on the report[16] that chronic morphine administration resulted in the up-regulation of the μ and δ binding sites but not of κ binding sites complex in rats. Rothman *et al.*[44 47] observed that chronic morphine administration resulted in the upregulation of the μ and δ binding sites, but not of κ binding sites of the complex in rats. These authors provided some evidences that this change could be a biochemical marker related to the development of tolerance and dependence.

However, in this results, we could not explain absolute effect of TGS on the morphine. Further studies on the other molecular action of TGS on morphine tolerance are now needed to assess the binding of TGS to the other endogenous opioid ligand sites in the CNS in order to investigate the possible its pharmacological importance.

In summary, the μ, δ, and κ opiate receptor binding were dependent in TGS doses and brain sites. In addition to the apparent increased action on [^{3}H]DPN binding following chronic morphine treatment, the observed significant decreases of specific [^{3}H]DAGO and [^{3}H]DPDPE binding of the morphine by TGS in mouse striatum or cortex provided a possibility that TGS could have some inhibitory action on the the development of tolerance to morphine. But we could not exclude any mechanisms via another different neural involvement such as putative neurotransmitters in brain.

요 약

이 연구에서는 morphine의 내성에 대한 인삼(total ginseng saponin : TGS)의 영향을 보고자 하였다. 그리하여 μ, δ 및 κ specific receptor에 대하여 [^{3}H]DAGO, [^{3}H]DPDPE 및 [^{3}H]DPN binding assay를 mouse striatum과 cortex의 P_2 membrane분획을 추출하여 시행하였다.

TGS를 25, 50, 100 및 150 mg/kg씩 3주간 복강내로 투여한 결과, [^{3}H]DAGO의 binding은 striatum에서는 약간의 증가를 보였으며, cortex에서는 감소를 나타내었다. [^{3}H]DPDPE binding은 striatum에서는 다소 증가를 보였고 cortex에서는 다소 감소를 나타내었다. [^{3}H]DPN binding은 striatum과 cortex에서 모두 증가를 나타내었다. TGS는 morphine에 의한 [^{3}H]DAGO binding의 증가를 striatum과 cortex에서 모두 억제하였으며, [^{3}H]DPDPE binding은 cortex에서 억제되었으나, [^{3}H]DPN binding에는 별 영향을 미치지 못하였다.

이상의 결과로 보아, TGS에 의한 morphine 장기 투여된 mouse 뇌에서 opioid수용체 binding 정도는 TGS 용량별, 뇌부위별로 다르게 영향을 나타냈으며, μ와 δ opioid 수용체 binding에 대한 TGS의 억제적인 결과는 다른 neural mechanism의 관련성을 배제할 수는 없으나, 수용체 결합정도로 보아 morphine 내성발현에 다소 영향을 줄 수 있으리라고 생각된다.

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