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Effect of Ginseng Saponin on the Analgesic Effect and Tolerance Development of Clonidine

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Abstract—The antagonism against clonidine-induced analgesia by ginseng saponin (GS) and the inhibitory effect of GS on the development of clonidine-induced tolerance were evaluated in mice. GS, when administered systemically, intracerebrally and intrathecally, antagonized significantly the analgesic effect of clonidine. GS, when injected intraperitoneally not only inhibited the development of clonidine-induced analgesic tolerance, but also enhanced the analgesic effect of clonidine on the 2nd and 5th day. Naloxone did not antagonize the analgesic effect of clonidine and had no influence on the deveolpment of tolerance of both acute and delayed types. These results indicate that the antagonism against clonidine-induced analgesia and the inhibition of the deveolpment of clonidine-induced tolerance by GS are not mediated by the opioid mechanism.

Keywords—Ginseng saponin • clonidine • analgesic effect • tolerance • naloxone

Clonidine is a potent alpha adrenoceptor stimulating agent, acting on both adrenergic alpha-1 and alpha-2 receptors. As an alpha-2 adrenoceptor agonist in low doses, clonidine decreased noradrenergic neurotransmission, whereas the high concentration of clonidine increased postsynaptic alpha-1 adrenoceptor responses.[1~3]

The analgesic effect of clonidine was dose-dependent up to 5 mg/kg, and about 10 times more potent than morphine. Five mg/kg of clonidine elicited nearly equipotent analgesia with 50 mg/kg of morphine.[4]

Recently, Paalzow and others suggested that the analgesic effects of clonidine and morphine on brain noradrenergic mechanism might be similar.[5~7] Chance and Kaneto reported that the treatment with clonidine produced the development of analgesic tolerance.[4,8] But there was no report that discussed the effects of ginseng saponin(GS) on the analgesia and tolerance development of clonidine.

We studied the effects of GS on the analgesic effect and tolerance of clonidine.

Materials and Methods

Animals and test drugs

ICR male mice weighing 20~23 g in a group of 10~15, were used in all experiments. GS (gift from Korea Giseng and Tobacco Research Institute), clonidine hydrochloride (gift from Boehringer Ingelheim Pharm. Co.), naloxone hydrochloride(Samjin Pharm. Co.) and reserpine (Aju Pharm. Co.) were dissolved in saline just before the tests and administered to mice intraperitoneally (i.p.), intrathecally (i.t.) and/or

intracerebrally (i.c.) as indicated.

Measurement of analgesic effect and tolerance development of clonidine

On the 1st day, mice were treated i.p. with 1, 2.5, 5 and 10 mg/kg of clonidine. After the injection of clonidine, the analgesic effect was measured at 30 min intervals for 3 hr by the tail pinch method[9]. A limit of 6 sec to each tail pinch test was used as a cut-off time to avoid damage to the tail.

The analgesic response for each mouse was calculated by the following formula;

$$\text{Percent of analgesia}(\%) = \frac{Tt - To}{Tc - To} \times 100$$

where To is the baseline or pre-clonidine tail pinch reaction time, Tt is the reaction time at t sec after the injection of clonidine and Tc is the cut-off time. The baseline of tail pinch latencies was around 1 ± 0.1 sec. The effect was calculated as an area uder the curve (AUC) that was obtained by plotting the analgesic percents on the ordinate and the timé intervals (min) on the abscissa, and expressed as percent of the effect obtained in control animals treated only with 5 mg/kg of clonidine. The degree of tolerance was assessed by measuring the analgesic effect of the test dose of clonidine, 1 mg/kg (i.p.) on the 2nd, 5th and 10th day.

Measurement of the effects of naloxone on the clonidine alalgesia and tolerance development

On the 1st day, to test the antagonism, mice were treated i.p. with 10 mg/kg of naloxone, 30 min before the injection of clonidine (5 mg/kg, i.p.). To evaluate the degree of the development of clonidine-induced tolerance, 1 mg/kg of clonidine was injected i.p. in the mice pretreated the combination of naloxone(10 mg/kg) and clonidine(5 mg/kg) on the 2nd and 5th day, respectively.

Measurement of the effects of pretreatment time intervals and doses of GS

To decide the most effective pretreatment time of GS prior to the test of clonidine antagonism against clonidine-induced analgesia, 100 mg/kg of GS was injected i.p. to mice at various time intervals of 0.5, 1, 2 and 4 hr, before the injection of clonidine(5 mg/kg, i.p.).

To decide the most effective doses of GS, the various doses of GS(25, 50, 100 and 200 mg/kg) were injected i.p. to mice 4 hr before the injection of clonidine(5 mg/kg, i.p.)

Forty μg/body of GS was injected in the mice 3 hr(i.c.)[10] or 2 hr (i.t.)[11] prior to the injection of clonidine(5 mg/kg, i.p.).

Reserpine was injected i.p. to mice 24 hr proir toinjection of clonidine(5 mg/kg, i.p.).

Statistics

The data were expressed as means$\pm$S.E. The differences in the means were analyzed by the Student's t-test.

Results

The analgesic effect and tolerance development of clonidine

Clonidine produced a potent and long-lasting analgesic effect in a dose-dependent manner from 1 upto 5 mg/kg. Tolerance to clonidine developed dose-dependently on the 2nd day after the injection of clonidine, and the degree of tolerance was dependent on the dose of the initial treatment(1, 2.5, 5 and 10 mg/kg of clonidine) (Fig. 1). Thus, for further experiments, the initial dose of clonidine was fixed at 5 mg/kg, and the tolerance to 1 mg/kg of clonidine was estimated on the 2nd and 5th day.

The effects of naloxone on the analgesic effect and tolerance development of clonidince

Naloxone did not antagonize the analgesic effect of clonidine and had no influence on the development of tolerance of both acute and delayed types (Fig. 2).

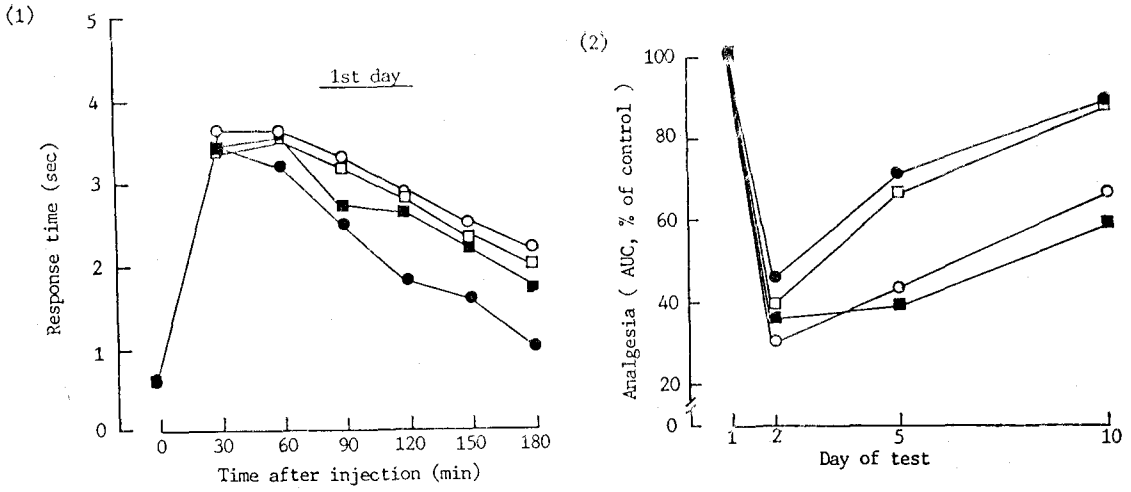

Fig. 1. Analgesic effect (1) and tolerance development (2) of clonidine (CD) in mice. On the 1st day, 1, 2.5, 5 and 10 mg/kg(i.p.) of CD were injected. The analgesic effect of CD was estimated on the 2nd, 5th and 10th day, respectively. The dose of CD was 1 mg/kg, i.p.
●—● CD 1 mg/kg, ○—○ CD 5 mg/kg, □—□ CD 2.5 mg/kg, ■—■ CD 10 mg/kg.

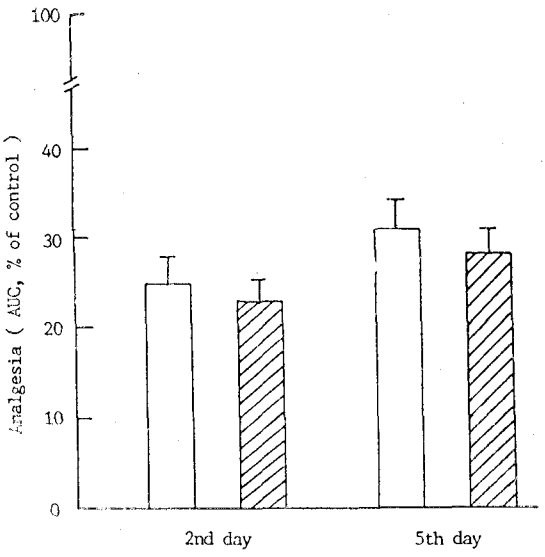

Fig. 2. Effect of naloxone on the development of analgesic tolerance of CD in mice. On the 2nd and 5th day, 1 mg/kg(i.p.) of CD was injected.
□ CD 5 mg/kg,
▨ CD 5 mg/kg+naloxone 10 mg/kg.

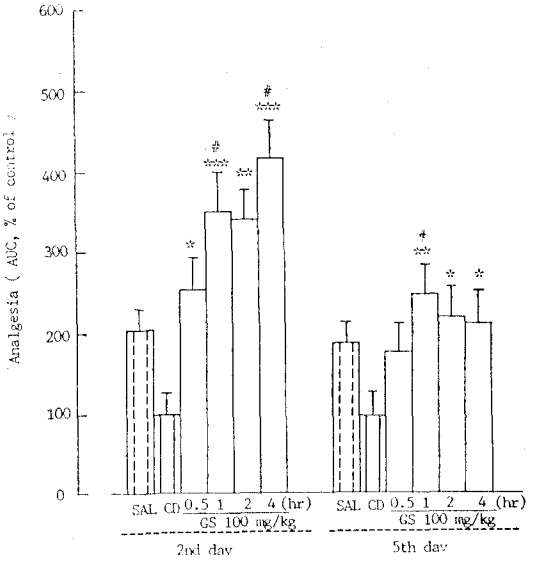

Fig. 3. Effct of pretreatment times of GS on the development of analgesic tolerance of CD in mice. On the 2nd and 5 th day, 1 mg/kg(i.p.) of CD was injected, respectively.
*: $p<0.05$, **: $p<0.05$, ***: $p<0.001$; compared with that of CD-treated group.
#: $p<0.05$; compared with that of saline (SAL)-treated group.

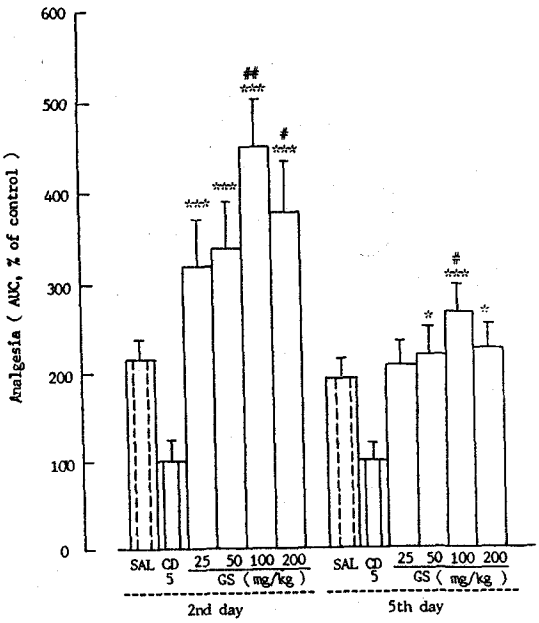

Fig. 4. Effect of the dose of GS on the development of analgesic tolerance of CD in mice. GS was pretreated 4 hr before the injection of CD on the 1st day. A tolerance test was performed on the 2nd and 5th day, respectively.
*:$p<0.05$, ***:$p<0.001$; compared with that of CD-treated group.
#:$p<0.05$, ##:$p<0.01$; compared with that of saline-treated group.

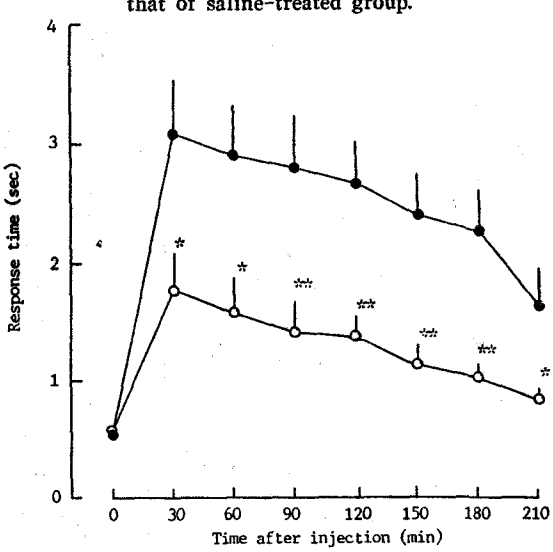

Fig. 5. Effect of intracerebral administration of GS on the analgesic effect of CD. GS was injected at adose of 40 μg/b.w.i.c. 3 hr before the injection of CD(5 mg/kg, i.p.)
*:$p<0.05$, **:$p<0.01$
•—• SAL+CD 5 mg/kg,
○—○ GS 40 μg/body+CD 5 mg/kg.

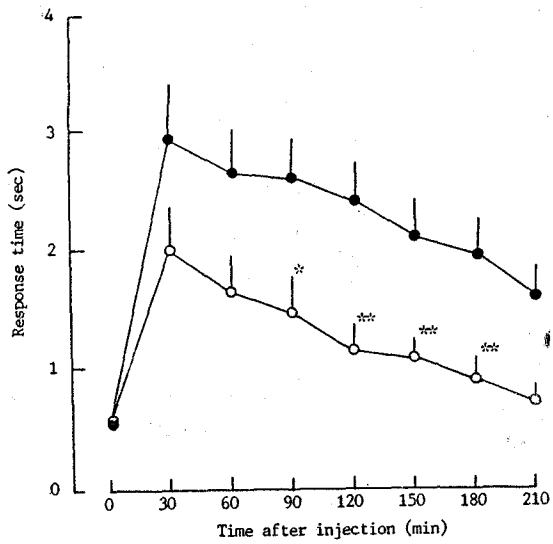

Fig. 6. Effect of intrathecal administration of GS on the analgesic effect of CD. GS was injected at a dose of 40 μg/bow. i.t. 2 hr before the injection of CD(5 mg/kg, i.p.)
*:$p<0.05$, **:$p<0.01$
•—• SAL+CD 5 mg/kg,
○—○ GS 40μg/body+CD 5 mg/kg.

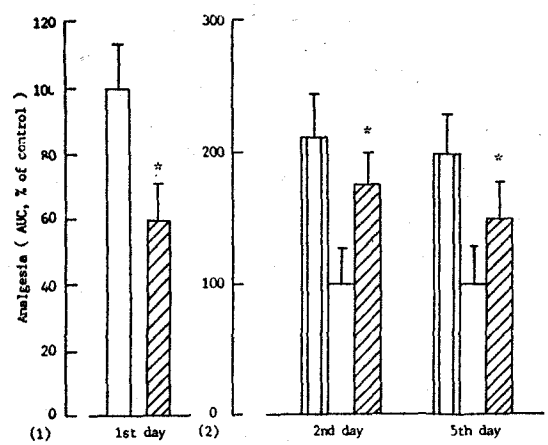

Fig. 7. Effect of reserpine on the analgesic effect(1) and the development of analgesic tolerance (2) of CD in mice. On the 1st day, 0.1 mg/kg(i.p.) of reserpine was injected 24hr before the injection of CD A tolerance test was performed by injecting 1 mg/kg(i.p.) of CD on the 2nd and 5th day, respectively. *:$p<0.05$; compared with that of CD.
□ CD 5 mg/kg. ▨ SAL.
▨ Reserpine 0.1 mg/kg+CD 5 mg/kg.

The effect of pretreatment time intervals and doses of GS on the analgesic effect and tolerance development of clonidine

The degrees of antagonism of GS on the analgesic effect of clonidine were increased with increasing the pretreatment time intervals from 0.5 to 4 hr. The inhibitory effects of analgesic tolerance development were shown to increase 2.5 at 0.5 hr, 3.5 at 1 hr, 3.7 at 2 hr and 4.2 fold at 4 hr, respectively, than that of the clonidine control group on the the 2nd day. It showed less effect but similar tendencies on the 5th day (Fig. 3). Therefore, the pretreatment time interval with GS was fixed at 4 hr for further studies. But the group treated with GS 1 hr in advance enhanced analgesia of 1 mg/kg of clonidine, 2 times on the 2nd day and 1.2 times or so on the 5th day, compared with that of the saline-treated group.

In the same manner, the degrees of antagonism of GS on analgesia of clonidine were dose-dependent from 25 to 100 mg/kg. The inhibition of the tolerance development and the enhancement of clonidine analgesia by GS from 25 to 100 mg/kg showed dose-dependently in the same manner as described above (Fig. 4).

GS, when adminrstered i.c. and i.t. to mice respectively, antagonized the analgesic effect of clonidine (Fig. 5 and 6). Reserpine, at a dose of 0.1 mg/kg. i.p., also showed the antagonism of the analgesic effect and the inhibitory effect in the development of analgesic tolerance to clonidine (Fig. 7).

Discussion

Clonidine, a centrally acting antihypertensive drug, has profound analgesic activity given intracerebroventricularly[12], intrathecally[13] and systemically[14], and this effect apparently involves an adrenergic component[15].

Much attention has been drawn lately to the role of noradrenaline in both narcotic-[16] and non-narcotic-[17] induced analgesia and to the relative contribution of spinal and supraspinal structures to suppressing responses to painful stimulation. These results suggest that non-opioid autoanalgesia is partially mediated by CNS noradrenergic systems. The role of noradrenaline in morphine analgesia is highly controversial. However, there exists an evidence that this effect of morphine is associated with the decreased central noradrenergic activity.[18] It has also been shown that clonidine analgesia might be partly due to an inhibitory action on noradrenergic neurons.[15]

Although the mechanism of clonidine antinociception are not well understood, several reports suggest that the presynaptic alpha-2 noradrenergic receptors involve in its mediation.[15,19] Clonidine has been reported to stimulate central noradrenergic receptors[20], with a preferential affinity for presynaptic alpha-2 synapses.[21]

Much evidence has been put forward for a supraspinal site of action of clonidine in suppressing activity.[22] Recent studies have shown that clonidine may elicit antinociception which activates descending inhibitory systems and directly affects the spinal nociceptive mechanism.[8]

In this experiment, GS, administered i.p., i.c. and i.t. to mice respectively, antagonized the analgesic effect of clonidine (Fig. 3, 4, 5 and 6). These results suggest that GS inhibits the activation of both the spinal descending inhibitory systems and the direct suppressin of pain transmission in the spinal dorsal horn, and GS also inhibits the activation of the supraspinal structures by clonidine. GS, injected i.p., not only inhibited the development of clonidine-induced analgesic tolerance, but also enhanced the analgesia of clonidine on the 2nd and 5th day. (Fig. 3 and 4).

A single dose of clonidine developed tolerance in the analgesic effect. Naloxone, an antagonist of the opiate receptor, did not antagonize the analgesic effect and had no influence on the development of tolerance, both acute and delayed types (Fig. 2). The site of action for naloxone and morphine seems to be different from that of clonidine.[4,15] The present results indicate that the antagonism of clonidine analgesia and the inhibition of the development of clonidine tolerance by GS are not mediated by the opioid mechanism.

Low systemic doses of clonidine produce an analgesic response which is potentiated by reserpine treatments.[15] Svensson and Strömbom have shown that the chronic clonidine treatment leads to an increased responsiveness of postsynaptic central alpha-1 adrenoceptor to clonidine.[23] Supersensitivity is reported to be resultant from functional noradrenergic denervation. This phenomenon is also involved in the development of tolerance to analgesia after long-term clonidine treatment. Owing to the prolonged reduction of the activity of noradrenergic neurons, the postsynaptic alpha-1 adrenoceptor stimulating the properties of clonidine might dominate, and the functional condition of the noradrenergic receptor is unfavorable to analgesia elicited by clonidine.[24]

The inhibition of GS on the development of analgesic tolerance was thought to be resultant from the inhibition of the supersensitivity development of the alpha-1 adrenergic receptor to clonidine(Fig. 3 and 4). So, it is presumed that the functional change of the noradrenergic receptor after the treatment of GS is favorable to the analgesic response elicited or enhanced by clonidine.

GS and reserpine treatments in this study showed no differences in the antagonism of analgesic effect and the inhibition of the development of analgesic tolerance to clonidine (Fig. 7). Reserpine has been known to be the classical intraneuronal storage inhibitor for noradrenaline, other catecholamines and 5-HTP at the synapse. The decreased serotonergic level has been also reported to increase the activity of clonidine on the vocalization during stimulation.[15] Judging from the same results that GS and reserpine pretreatments produced, it is thought that GS and reserpine might share some of common mechanisms at the presynaptic level.

So, we can not exclude the possibility of presynaptic involvement in the bulbar pain response by altering the dopamine, noradrenaline and serotonin activities and by changing the function of the noradrenergic receptor through the administration of GS. And further work is required for the interpretation of the enhancement of clonidine analgesia on the 2nd and 5 day after the pretreatment of GS with clonidin

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