PHARMACOLOGICAL STUDIES OF NEUTRAL SAPONINS (GNS) OF PANAX GINSENG ROOT

Hiroyuki NABATA, Hiroshi SAITO and Keijiro TAKAGI

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan

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Abstract—Pharmacological studies of neutral saponins (GNS) of Panax Ginseng root, were performed. GNS showed CNS-depressant action in inhibition of spontaneous and exploratory movements, and in potentiation of CNS-depressant. A specific blocking action of conditioned response by GNS was significantly confirmed in small doses and did not produce loss of righting reflex, motor incoordination or myo-relaxation. Analgesic, anticonvulsant and antipyretic effects were recognized. From the tests described above in addition to those of traction, hypothermia, fighting behavior and ratio of two reflexes, GNS appears to have neuroleptic activity. There was neither hemolytic nor vasodilator action on the hind leg or coronary vascular beds.

It is said that the Chinese medicine Ginseng root has some effect on the central nervous system and several reports concerning neuropharmacological properties of Ginseng root have so far been published (1–7). Stimulant action was proved by Brekhman and his co-workers with mice swimming in water (1), and running up an endless rope (2). Petkov (7) also reported the stimulant effect of water-alcohol extracts on the central nervous system and analeptic activity, while on the contrary, antialarm action has also been reported (3–6).

In our laboratory, a number of relatively simple tests were employed to obtain a pharmacological spectrum of the activity of extracts, which had been supplied from Shibata et al. (8). From these results, a crude saponin fraction (G. NO. 3) and GNS, a mixture of neutral saponins, were estimated as having neuroleptic activity (9, 10).

The present study is an attempt to investigate neuroleptic properties of GNS.

MATERIALS AND METHODS

GNS is a mixture of neutral saponins mainly composed of Ginsenoside-Rb1, -Rb2, and -Rc. Details of the separation have been described by Shibata et al. (8). The solution of GNS was prepared with physiological saline with the following methods being employed:

1) Acute toxicity in mice

Male mice (ddy-strain), weighing 18–21 g, were used and the intravenous, intraperitoneal and oral LD50 were determined. Mortality was recorded 72 hr after administration. Intraperitoneal and oral LD50 were calculated by the method of Behrens-Kärber (11). Intravenous LD50 was done by the up and down method.
2) Hemolytic test
The hemolytic effect of GNS was observed using rabbit blood following the method described by Fujita et al. (12). Pure saponin (Merck) was used as a control and hemolytic index was calculated.

3) Motor activity in mice
A hole cross test described by Takagi et al. (13), was employed for the measurements of motor activity. A group of 10 male mice, weighing 18–20 g, was placed in the test cage 30 min after administration of the drug. Counting for a group was continued for 1 hr and recorded as a percent of the control value counted for the same group administered saline i.p. at the same time the day before the test.

4) Climbing test in mice
The effect of GNS on exploratory movement was observed by the method of Sandberg (14). A group of 10 male mice, weighing 18–20 g, was placed in the test cage for 10 min and the number of animals climbing the net was counted. Drugs were given i.p. before the test.

5) Motor incoordination in mice
1) Rotating rod, 2) sliding angle and 3) spring balance tests described by Takagi et al. (15), were employed for the measurements of motor incoordination and relaxation of muscle tone. Male mice in groups of 8, weighing 18–20 g, were tested following i.p. administration.

6) Traction test in mice
Groups of 5 male mice, weighing 18–20 g, were suspended by the forepaws to a metallic wire, and the number of animals incapable of touching the wire with at least one of the hindpaws within 5 sec after placement on the wire was recorded. Tests were done 4 times: 30, 60, 90 and 120 min after i.p. administration of GNS.

7) Potentiation of hypnotic action of hexobarbital in mice
Groups of 10 mice, weighing 22–26 g, were given GNS i.p., and 30 min later 70 mg/kg of hexobarbital sodium was injected via the same route. Duration of loss of the righting reflex was measured.

8) Analgesic tests in mice
i) Writhing induced by 0.7% acetic acid
Male mice in groups of 6, weighing 20–23 g, were given GNS orally, and 30 min later an i.p. injection of 0.7% acetic acid. The number of writhings per mouse was recorded for a period of 10 min, beginning 10 min after administration of acetic acid.

ii) Tail pressure test
The method used was as described by Takagi et al. (16). Groups of 5 male mice, weighing 18–20 g, were tested after i.p. administration of GNS.

9) Anticonvulsant test in mice
i) Convulsions induced by electroshock
Groups of 10 male mice, weighing 18–20 g, were given test substances orally, and 30 min later subjected to the maximum electroshock (Corneal electrodes with 25 mA and
0.17 sec), in order to observe tonic extension of hind legs and death.

ii) Convulsions induced by chemicals

Male mice in groups of 10, weighing 22–26 g, were given the test compound i.p. 30 min before automatic i.v. infusion of pentylenetetrazol (0.05%), strychnine sulfate (0.01%) and nicotine tartarate (0.08%) given at a rate of 0.33 ml/sec. Two end-points were taken: the first appearance of convulsion, and death.

10) Hypothermia in mice

Groups of 10 male mice, weighing 22–26 g with 37–38°C rectal temperature, were given the test substance by the i.p. route and rectal temperature was recorded every 30 min for 2 hr at room temp. of 25°C.

11) Antipyretic test in mice

Male mice in groups of 10, weighing 22–26 g with 37–38°C rectal temperature, were given the test substance i.p. 30 min after i.v. administration of TTG (pyrogen, Fujisawa Chemical Industry Co. Ltd.) in a dose of 100 μg/kg, and rectal temperature was recorded every 30 min for 4 hr. Aminopyrine was used as a control.

12) Ratio of reflexes in mice

This method was described by Witkin et al. (17). Groups of 10 male mice, weighing 18–20 g, were given GNS by the i.p. route. Corneal and pinna reflexes were tested using a pig hair.

13) Conditioned avoidance test in rats

i) Shuttle box test

The apparatus employed was almost similar to that of the Mowrer-Miller type, that is, the box is a small soundproof cage with two compartments, each 25 x 25 cm, separated by a hurdle of 7 cm. The floor of each compartment is made of a grid consisting of stainless-steel bars of 0.6 mm diameter, 10 mm apart. Electroshocks can be given in each compartment individually. In most experiments, single shocks of 0.1 mA of 50 cycle at 35 V, are given at an interval of 1.0 sec. Conditioning stimulus (CS) is the sound from a buzzer attached directly to the top of test box.

Experiment 1.

Male rats (Wistar-strain), weighing 180–200 g, are to move from one compartment to the other at the sound of a buzzer, to avoid shocks. A rat is placed in the box and exposed to the sound of buzzer (1/sec) 10 times, and the same sounds of buzzer with electroshocks (unconditioning stimulus = US, 1/sec) are delivered 10 times, or until the rat escapes punishment by jumping into the other compartment, at which time the stimuli are immediately terminated. This situation is repeated 10 times at an interval of 2 min for 2 hr a day. After 4–5 days the rats jumped into the other compartment in response to the buzzer only. Such conditioned rats were exposed to 6 consecutive trials after i.p. administration of saline as a control, and trained 2 hr a day for three days. GNS was then given i.p., and the rats exposed to 6 consecutive trials.

Experiment 2.

Male rats were employed for the study on process discrimination learning between
two different sounds, a buzzer (CS) and a sound of 1000 c/s (discriminating stimulus = DS), or a sound of buzzer and a white light (DS). A rat is placed in the box 20 min before the test. DS (1/sec) is delivered 10 times. This is repeated 10 times at an interval of one min, then the rats are rested for 5 min. Next, CS is delivered 10 times (1/sec), and US (buzzer plus electroshock, 1/sec) is delivered until the rat moves to the other compartment. This is repeated 10 times at an interval of one min and rats are rested for 5 min. The rat is exposed to this situation twice for 1 hr after oral administration of GNS, 3 consecutive times a day. Ten mg/kg of GNS were given orally once a day, starting 4 days before the test.

ii) Pole climbing test
Details of apparatus and training were as described in a previous report (18). Conditioned male rats in groups of 8, weighing 200–230 g, were exposed to test trials after i.p. administration of GNS. This continued for a week and in the following 3 days tests were continued without administration of GNS.

14) Motor incoordination in rats
1) Rotating rod test and 2) suspension test, described by Takagi et al. (18), were employed for measurements of motor incoordination and myorelaxation.

15) Electrically stimulated fighting behavior in pairs of mice
Details of apparatus and method were described by Tedeschi et al. (19). Selected pairs of male mice, weighing 24–26 g, were tested 30 min after i.p. administration of GNS.

16) Corneal anaesthesia
Male guinea-pigs, weighing approx. 250 g, were used to study local anaesthetic property. Anaesthetic activity was observed after exposure of the cornea to GNS or 2% procaine hydrochloride.

17) Vascular resistance of the hind limb and coronary vessels in the dog
Effects of GNS on coronary (CBF) and femoral blood flow (FBF) were studied in anaesthetized (sodium pentobarbital) and/or open chest artificially respired dogs, weighing approx. 10 kg. CBF and FBF were measured by an electromagnetic flowmeter on the left anterior descending coronary artery and the femoral artery, respectively. Papaverine hydrochloride and pure saponins (Merck) were used as a control.

RESULTS

1) Acute toxicity in mice
I.v., i.p. and oral LD_{50} were 367 mg/kg, 545 mg/kg and more than 5 g/kg, respectively. The following behavioral changes are commonly observed with lethal doses: GNS decreased alertness and grooming, lowered body temp., reduced spontaneous movement, induced piloerection, abolished touch response, pain response, righting reflex and pinna reflex, enlarged pupils and relaxed muscle tone. The mice then died approx. 30 min (i.v. injection) and/or several hr (i.p. injection) after administration. Surviving mice showed no change in behavior the following day.
2) **Hemolytic test**

Hemolytic effect of GNS, in doses of \(10^{-2}\) to \(10^{-3}\) g/ml, was not observed. Hemolytic index of pure saponins (Merck) was approx. 50,000.

3) **Motor activity in mice**

Twenty mg/kg of GNS produced no effect on motor activity, but more than 100 mg/
kg produced a significant decrease in spontaneous movement. ED₅₀ was calculated at 92 mg/kg (Fig. 1).

4) Climbing test in mice

GNS produced a significant inhibition of this exploratory movement (Fig. 2), which was conspicuously observed 1 hr after administration. ED₅₀ was 81 mg/kg.

5) Motor incoordination in mice

No significant changes in sliding angle and spring balance tests was seen after administration of GNS. In the rotating rod test, slight inhibition was seen in a dose of 100 mg/kg, and no mouse was able to stand on the rod with a dose of 400 mg/kg within 1 hr after administration. The ED₅₀ was 160 mg/kg. Four mg/kg of chlorpromazine showed inhibition similar to that of 400 mg/kg of GNS (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Number of animals</th>
<th>Number of mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>8</td>
<td>0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>GNS 100</td>
<td>8</td>
<td>0</td>
<td>0 2 0 0 0 0 0</td>
</tr>
<tr>
<td>GNS 200</td>
<td>8</td>
<td>0</td>
<td>0 5 2 2 1</td>
</tr>
<tr>
<td>GNS 400</td>
<td>8</td>
<td>0</td>
<td>0 6 4 4 4</td>
</tr>
<tr>
<td>CPZ 4</td>
<td>8</td>
<td>0</td>
<td>0 7 7 4 4</td>
</tr>
</tbody>
</table>

*: Number of mice that fell from a rotating rod in 3 min.
CPZ: Chlorpromazine hydrochloride

Table 1. Effect of GNS in rotating rod test.

6) Traction test in mice

No change in the traction test was seen in doses of 100 and 200 mg/kg of GNS however complete inhibition was observed for 2 hr after administration of 400 mg/kg (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Number of mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>5 5 5 5 5 5 5</td>
</tr>
<tr>
<td>GNS 100</td>
<td>5</td>
<td>5 5 5 5 5 5 5</td>
</tr>
<tr>
<td>GNS 200</td>
<td>5</td>
<td>5 5 5 5 5 5 5</td>
</tr>
<tr>
<td>GNS 400</td>
<td>5</td>
<td>1 0 0 0 0 0 1</td>
</tr>
<tr>
<td>CPZ 4</td>
<td>5</td>
<td>0 0 0 0 0 0 1</td>
</tr>
</tbody>
</table>

*: Number of mice which touched the wire at least with one of the hind paws within 5 sec after placement on the wire.
CPZ: Chlorpromazine hydrochloride

Table 2. Effect of GNS in traction test.

7) Potentiation of hypnotic action of hexobarbital in mice

GNS in doses of 100 and 200 mg/kg produced a significant elongation of sleeping time after hexobarbital. The potentiation by 100 mg/kg of GNS was almost similar to that as with 2 mg/kg of chlorpromazine (Fig. 3).
8) Analgesic tests in mice

i) Writhing induced by 0.7% acetic acid

Significant inhibition was seen by GNS. (Table 3a) ED$_{50}$ was 620 mg/kg.

ii) Tail pressure test

Significant increase of maximum pain threshold was seen for 30 min after administration of GNS (Table 3b).

**TABLE 3. Effect of GNS in analgesic tests on mice.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of animals</th>
<th>No. of writhings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>6</td>
<td>39.5±2.6</td>
</tr>
<tr>
<td>GNS</td>
<td>250</td>
<td>6</td>
<td>25.7±5.6**</td>
</tr>
<tr>
<td>GNS</td>
<td>500</td>
<td>6</td>
<td>22.8±3.6*</td>
</tr>
<tr>
<td>GNS</td>
<td>1000</td>
<td>6</td>
<td>14.8±5.0*</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>100</td>
<td>6</td>
<td>17.3±7.2**</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>200</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg, i.p.)</th>
<th>No. of animals</th>
<th>Maximum pain threshold (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>5</td>
<td>73.6±2.6</td>
</tr>
<tr>
<td>GNS</td>
<td>200</td>
<td>5</td>
<td>111.2±10.1*</td>
</tr>
<tr>
<td>GNS</td>
<td>400</td>
<td>5</td>
<td>101.2±7.3*</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>50</td>
<td>5</td>
<td>158.0±10.7</td>
</tr>
</tbody>
</table>

*: Significant difference from control value p<0.01
**: p<0.02

CPZ: Chlorpromazine hydrochloride
9) Anticonvulsant tests in mice

i) Convulsions induced by electroshock

GNS had no effect on inhibition of tonic extensor convulsion but 400 mg/kg decreased mortality.

ii) Convulsions induced by chemicals

GNS in a dose of 200 mg/kg significantly elongated the time to death induced by the three convulsants used. The time to convulsion induced by strychnine, was elongated by GNS in a dose of 200 mg/kg, and by nicotine in a dose of 400 mg/kg (Table 4).

10) Hypothermia in mice

Significant lowering of rectal temp. was seen in a dose of 100 mg/kg, 30 to 120 min after administration of GNS (Fig. 4a).

11) Antipyretic test in mice

Significant antipyretic activity was seen 30 min in a dose of 200 mg/kg, 30 to 120 min after administration of GNS (Fig. 4b).

12) Ratio of reflexes in mice

No changes in corneal reflex were seen with administration of GNS. Disappearance of pinna reflex was significantly observed in a dose of 400 mg/kg (Table 5).

13) Conditioned avoidance tests in rats

i) Shuttle box test

<table>
<thead>
<tr>
<th>Table 4. Inhibitory effects of GNS on convulsion and death induced by infusion of chemicals in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.5% Pentylenetetrazol</strong></td>
</tr>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>GNS</td>
</tr>
<tr>
<td>GNS</td>
</tr>
<tr>
<td>Chlorpromazine</td>
</tr>
</tbody>
</table>

| **0.01% Strychnine**                          |
|____________________________________________|
| **Compound** | **Dose (mg/kg)** | **No. of animals** | **Time to convulsion (sec)** | **Time to death (sec)** |
| Control     | ---                     | 6                 | 33.0±0.7                     | 47.5±1.9               |
| GNS         | 200                     | 6                 | 38.4±1.4**                   | 55.5±2.8*              |
| GNS         | 400                     | 6                 | 39.2±2.2*                    | 57.8±2.4**             |
| Chlorpromazine | 4                     | 6                 | 44.5±2.1**                   | 64.3±1.5**             |

<table>
<thead>
<tr>
<th><strong>0.08% Nicotine</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>GNS</td>
</tr>
<tr>
<td>GNS</td>
</tr>
<tr>
<td>Chlorpromazine</td>
</tr>
</tbody>
</table>

*: Significant difference from control value p<0.05
**: p<0.01
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a) Hypothermia

![Graph showing effect of GNS on rectal temperature of mice.]

b) Antipyresis

![Graph showing effect of GNS on body temperature of mice.]

FIG. 4. Effect of GNS on rectal temp. of mice.

TABLE 5. Effect of GNS on pinna and corneal reflexes of mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Number of mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td>P† C‡ P C P C P C</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>5  5</td>
</tr>
<tr>
<td>GNS 100</td>
<td>5  5</td>
<td>5  5</td>
</tr>
<tr>
<td>GNS 200</td>
<td>5  5</td>
<td>5  5</td>
</tr>
<tr>
<td>GNS 400</td>
<td>5  5</td>
<td>3  5</td>
</tr>
<tr>
<td>CPZ 4</td>
<td>5  5</td>
<td>0  5</td>
</tr>
</tbody>
</table>

*: Number of mice exhibiting positive pinna or corneal reflex in a group of 5.
†: Pinna reflex  ‡: Corneal reflex
CPZ: Chlorpromazine hydrochloride
Experiment 1.

Ten mg/kg of GNS produced a slight specific blocking action of conditioned response (CR). (Fig. 5) Maximal blocking effect on CR occurred approx. 30 min after administration, however these effects on CR were not observed 100 min after. GNS in doses over 30 mg/kg produced a significant specific block of CR for 2 hr after administration (P<0.05). It was found that 30 mg/kg produced a block of unconditioned response (UR) in two animals. A significant non-specific blocking action of UR was seen after administration of 200 mg/kg which simultaneously produced a significant decrease in motor activity, but had no effect on motor incoordination and myorelaxation in rats for 2 hr after administration. 2 rats died in the group of 10, approx. 72 hr after administration of 200 mg/kg.

GNS in doses of 10 and 30 mg/kg was given orally to conditioned rats once a day for one week, starting 4 days before the test.

Ten mg/kg had no effect on either CR nor response latency for 2 hr after administration, however on the 3rd day, 30 mg/kg produced a slight specific block of CR approx. 30 to 60 min after administration.
Experiment 2.

With administration of 10 mg/kg of GNS given 1 hr before the test, no difference was seen in the process of discrimination learning between two different sounds or between CS and a white light.

ii) Pole climbing test

Thirty mg/kg produced a significant specific block of CR on the 6th and 7th days. During the following 3 days GNS was not given and a specific block of CR was not seen. (Fig. 6) Increases in body wt. in animals given GNS during one week were almost similar to that of control animals.

14) Motor incoordination in rats

No significant changes in rotating rod test and suspension test were seen in a dose of 100 mg/kg for 2 hr after administration. Two hundred mg/kg produced slight inhibition in both tests. Rats given 10 and 30 mg/kg i.p. were tested for one week after the pole climbing test, but no changes were observed in either test.

15) Electrically stimulated fighting behavior in pairs of mice

Inhibition was not observed with a dose of 200 mg/kg. 400 mg/kg produced inhibition of fighting behavior in more than half of the pairs (Table 6).

16) Corneal anaesthesia

A slight inhibition of corneal reflex was recognized in 5% solution of GNS 5 to 10 min after administration. No effect was seen in a 2% solution of GNS. 2% procaine
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hydrochloride produced a complete disappearance of corneal reflex approx. 4 to 9 min after administration.

17) Vascular resistance of the hind limb and the coronary vessels in dogs

Close intraarterial injections of GNS at a dose range of 0.2 to 3.2 mg produced no demonstrable changes in blood flow of vascular beds while papaverine and pure saponins (Merck) at a dose range of 0.2 to 1.8 mg produced increases of flow in these beds.

DISCUSSION

In a previous report (9), GNS showed possible neuroleptic, analgesic, central myorelaxant, hypotensive and papaverine-like activities in a blind screening, consisting of 3 tests: 1) neuropharmacological observations in mice, 2) tests on the cardiovascular and respiratory system in rats, and 3) tests on the isolated guinea-pig ileum.

These activities were examined by tests outlined in this paper. GNS produced CNS-depression as revealed by its inhibitory effects on spontaneous and exploratory movements, as well as potentiation of hypnotic action of hexobarbital. The decrease in spontaneous and exploratory movements induced by GNS occurred within doses which did not produce depression on motor coordination and muscle tone in mice.

Analgesic, anticonvulsant, and antipyretic activities were also seen in detailed tests. In relatively small doses, GNS inhibited conditioned avoidance responses in both shuttle box and pole climbing tests. The effects on specific block of CR were transient, and appeared for approx. 1 hr after administration. In the pole climbing test, administration of 30 mg/kg of GNS for one week produced a specific block of CR. These effects disappeared completely when GNS was not administered. Inhibition of CR induced by GNS occurred in doses which did not produce depression of motor coordination and muscle tone in rats.

In addition to these actions, results from the traction test, hypothermia test, fighting behavior test and ratio of the reflexes strongly indicate the neuroleptic activity of GNS.

Possible central myorelaxant activity can be presumed from antagonism against strychnine convulsion and ratio of the reflexes, and anti-inflammatory activity from inhibition of number of writhings and antipyretic activity.

GNS produced no change in either the hind limb nor the coronary blood flows, sug-
gesting that hypotensive activity induced by GNS is not due to its direct vasodilator effect on the peripheral vascular beds. Local anaesthetic activity was found to be very weak, and hemolytic activity was not observed.

Acknowledgements: We are grateful to Prof. S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo and Prof. O. Tanaka, School of Medicine, Hiroshima University for kindly providing GNS.

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