EFFECTS OF PANAX GINSENG ROOT ON CONDITIONED AVOIDANCE RESPONSE IN RATS

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Abstract—Pole climbing and shuttle box tests were employed to study conditioned avoidance response (CAR) and discrimination behaviour in Wistar male rats given extracts from Panax Ginseng root intraperitoneally. Neutral saponins (GNS), a water soluble fraction (GF1) which does not contain saponins, and a lipid soluble fraction (GNo. 5) inhibited CAR and discrimination ability between 500 Hz sound with electric shock (S°) and 1000 Hz sound without shock (S%). Small doses of GNo. 5 and ginsenoside Rg fraction (GRg) produced a slight shortening of the response latency (RL) to the conditioned stimulus in CAR. GNo. 5 produced the incorrect response to S°. Significant changes in the extinction of CAR were not evident with any fraction. Data from these tests indicate that Panax Ginseng root contains at least three sedative compounds.

Panax Ginseng root has been in common use for thousands of years in China, Korea and Japan and has been ingested as a panacea for all types of disease, regardless of the severity. In general the compound is considered to have tonic, stimulant and sedative properties. Several authors have reported the pharmacological properties of Panax Ginseng root on the central nervous system (CNS) (1, 2, 3), however, systematic data on the components of Ginseng root were not included. In an attempt to differentiate pharmacologically active substances of Panax Ginseng root on the CNS, we employed the pole climbing and shuttle box tests. Conditioned avoidance response (CAR) in rats has been utilized to evaluate drugs affecting the CNS (4). Investigators have also used the inhibition of CAR as a measure of depression of CNS, and differences in pharmacological properties between various tranquillizers can be determined from the slopes of dose-response curves of both the inhibition of CAR and that of the escape behaviour (5). When the pole climbing and shuttle box tests are employed, it is necessary to determine the effect of given drugs on motor coordination and muscle tone as escape failure often indicates a motor defect when the animal cannot climb the pole or move into another compartment. The rotating rod and suspension tests were thus adopted to assess the exact dose of drugs which, when ingested, produced an ataxia and muscle relaxation.

MATERIALS AND METHODS

1) Pole climbing test

Apparatus  The method described by Cook and Weidley (4), was used with minor modi-
fication. Test apparatus was a wooden box about 30 × 30 cm square and 60 cm in height, has a grid floor, a wooden pole and a speaker. The grids are composed of stainless-steel rods 0.3 cm in diameter. A forty-five cm long wooden pole with a diameter of 3 cm was suspended vertically from the top of the box in the center of the square. The bottom end of the pole did not reach the grid floor. The speaker attached directly to the top of the box gives a 500 Hz sound and serves as the conditioned stimulus (CS). The box was in a soundproof enclosure.

Training Male Wistar rats (7–8 weeks old) weighing 150–170 g were used. At the beginning of the training, the rat was placed in the box for 2 min without CS and electric shock. Then 500 Hz sounds which occurred intermittently once a sec and 0.5 sec in duration, were delivered for 20 sec, and it was confirmed that the rat did not climb the pole. After this confirmation, sounds were delivered as CS for 20 sec; sounds only for the first 10 sec and sounds with electric shock (unconditioned stimulus, 0.1 mA, 35 V, AC) for the remaining 10 sec. Shocks were delivered from the grid at the same time and duration of the sounds. When the rat climbed the pole, stimuli were immediately terminated. Rats were exposed to this situation 60 times a day at an interval of 2 min, for 6 days. Avoidance is defined as climbing during CS only, escape, climbing after electric shock, and escape failure, failure to climb the pole after these stimuli. The response latency (RL) was considered the time from presentation of the CS to the response. For each experiment, only rats which showed CAR with a success rate of over 90% were used.

Procedure

Experiment 1: Trained rats in groups of 10 were administered saline and were tested during 60 trials. The results were regarded as control. During the 3 days after the test, the rats were given 60 trials at the same time of day, and it was confirmed that the behavioural baseline did not change. On the 4th day, the animals were again given 60 trials soon after the administration of drugs. One group was given saline only as a control.

Experiment 2: The procedure here was as in experiment 1, except that the number of trials and the testing time differed. Trained rats in groups of 10 were given 10 trials twice daily: 30 and 100 min after the administration of the drugs.

Experiment 3: Trained rats in groups of 6 were exposed to extinction procedure, in which sounds were delivered without electric shock for 20 sec, 10 trials, 4 times daily; 10, 40, 70 and 100 min after the injection of extracts from Ginseng root, for 3 days.

Experiment 4: Rats were trained to discriminate between the sounds of 500 Hz (S\textsuperscript{D}) and those of 1000 Hz (S\textsuperscript{D'}), which were not followed with electric shock. The correct response was defined as the response to climb the pole with S\textsuperscript{D}, or not to climb with S\textsuperscript{D'}. Other responses were regarded as incorrect. Rats in groups of 8 which showed correct response in rates of over 80% were used. The animals were exposed to 5 trials of S\textsuperscript{D} and those of S\textsuperscript{D'} by turns with 20 trials in all, soon after the injection of saline. The results served as control. During 3 days after the test, the rats were tested at the same time of day in the same manner and it was confirmed that the behavioural baseline did not change. On the 4th day, the animals were tested again soon after the administration of drugs.
2) Shuttle box test

Experimental details of the apparatus and training have already been reported (6).

**Procedure**

*Experiment 5:* Male Wistar rats which could move into another compartment with only CS (intermittent sounds of 500 Hz) with success rates of over 90% were used in groups of 10. These animals weighing 180–200 g (8–9 weeks old) were given saline, tested 60 trials at an interval of 1 min and then served as control. During 3 days after the test, the rats were tested at the same time of day and it was confirmed that the behavioural baseline did not change. On the 4th day, these animals were tested in the same manner soon after the administration of drugs.

*Experiment 6:* Rats were trained to discriminate between 500 Hz (S\(P\)) and 1000 Hz (S\(T\)). The correct response was defined as the ability to move into another compartment with S\(P\), or not to move with S\(T\). Rats in groups of 6 which showed correct responses at the rate of over 80% were used. The rats were exposed to 5 trials of S\(P\) and those of S\(T\), by turns, 20 trials in all at an interval of 1 min, soon after the injection of saline. The results were regarded as control. During 3 days after the test, the rats were tested in the same manner and it was confirmed that the behavioural baseline had not changed. On the 4th day, these animals were tested soon after the administration of drugs.

3) Rotating rod test

A plastic rod, 10 cm in a diameter, was rotated at a speed of 10 rotations per min. Male Wistar rats, weighing 170–200 g, which could remain on the rotating rod for more than 5 min in two successive trials were used in groups of 6. The test was terminated in

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**FIG. 1. Separation of Panax Ginseng Root.**

Ginseng Root

<table>
<thead>
<tr>
<th>extd. with hot MeOH</th>
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<tbody>
<tr>
<td>Filtrate</td>
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<tr>
<td>concd.</td>
</tr>
<tr>
<td>MeOH extd.</td>
</tr>
<tr>
<td>MeOH added</td>
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<tr>
<td>ether added</td>
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</tbody>
</table>

Precipitate

(Crude Saponins)

Neatral Saponins

(GNS)

Precipitate

(GNo. 4)

Silica Gel

Column Chromatography (CHCl\(_3\) Eluant)

GF\(_a\)

Alkaline

Soluble Fraction

Ginsenoside Rg

(GNo.5-1)

GF\(_b\)

Alkaline

Insoluble Fraction

(GNo.5-2)
5 min and was repeated 4 times; 30, 60, 90 and 120 min after the injection of drugs. The number of rats which fell off the rotating rod within 5 min was counted.

4) Suspension test

Male Wistar rats, weighing 160–190 g in groups of 6, were suspended by means of their forepaws to a metallic net of 2 × 5 cm which was attached to the center of a large vertical glass plate. Animals which fell from the net within 5 sec were considered to have a weak muscle tone. This test was performed 4 times; 30, 60, 90 and 120 min after the injection of drugs.

Drugs tested

The preparations of Panax Ginseng root are shown in Fig. 1. Four preparations, namely, neutral saponins (GNS), GNo. 4, ginsenoside Rg (GRg) and GF4, were dissolved in saline. GRg separated from GNo. 4 contains ginsenoside Rg1, Rg2 and Rg3. The fraction GF4 is also obtained from the CHCl3-eluate of the column chromatography of GNo. 4 on silica gel and does not contain saponins. GNo. 5 was suspended in saline with a drop of tween 80. Methamphetamine hydrochloride (MA) and chlorpromazine hydrochloride (CPZ) were used as standard agents. All drugs were given i.p.

RESULTS

1) Pole climbing test

The proportionate number of rats which showed an escape response was considered inhibition of CAR and the proportionate number of animals which showed failure to escape as an inhibition of both CAR and escape response.

Experiment 1: Effects of the preparations are shown in Fig. 2. GNS in doses of

![Fig. 2](image-url)
10, 30 and 90 mg/kg, GNo. 4 (100 mg/kg), GF₄ (25 and 50 mg/kg) and GNo. 5 (90 mg/kg) induced a significant inhibition of CAR. At 20 min intervals for 2 hr after the injection of these fractions, the response of each animal was also observed 6 times successively. The percentage of escape response and escape failure or the mean of RL at the time of peak activity within 2 hr allowed for measurements of potency. The maximum effect of GNS was produced about 30 min after the administration. The inhibitory effect on CAR was not observed 100 min after the treatment. Maximum effect of GNo. 4 occurred within 1 hr after the treatment. At a dose of 50 mg/kg, GNo. 4, there was a slight shortening of RL 60-80 min after the treatment ($p < 0.10$, Student's $t$-test). GR₄ inhibited CAR for 2 hr. GR₅ (10, 30 and 90 mg/kg) did not significantly influence CAR or RL, but 10 mg/kg of GR₅ slightly reduced RL 40-80 min after the treatment ($p < 0.10$). GNo. 5 produced an inhibition of CAR which lasted for 2 hr. Thirty mg/kg of GNo. 5 slightly reduced the RL 20-60 min after the treatment and such was prolonged 80-120 min after the treatment ($p < 0.10$).

Experiment 2: Results are shown in Fig. 3. GNS in doses of 10, 30 and 90 mg/kg produced an inhibition of CAR and prolonged RL at 30 min after the treatment, but at 100 min after that the effect had disappeared completely. GNo. 4 (50 and 100 mg/kg) had the same effect as GNS on both CAR and RL. GF₄ (12.5, 25 and 50 mg/kg) inhibited CAR and prolonged RL at 30 min after the treatment, and also at 100 min after that in doses of 25 and 50 mg/kg. GR₅ had no effect on either CAR or RL, though 10 and 30 mg/kg produced a slight reduction of RL 30 min after the treatment ($p < 0.10$). GNo. 5 (90 mg/kg) inhibited CAR and prolonged RL significantly both 30 and 100 min after the treatment. Ten mg/kg of GNo. 5 slightly reduced RL at 100 min after that ($p < 0.10$).

Experiment 3: Influence of GNS in doses of 5 and 10 mg/kg, GNo. 4 (25 and 50 mg/kg), GF₄ (5 and 10 mg/kg), GR₅ (10 and 30 mg/kg) and GNo. 5 (10 and 30 mg/kg) on the extinction of CAR was also studied. CPZ in a dose of 2 mg/kg produced a significant shortening of extinction on the first and second days (Fig. 4). Regarding other drugs,
there were no significant changes in the extinction, but 10 mg/kg of GNS and GF\textsubscript{4} had a tendency to shorten the extinction in the 3rd or 4th trial on the first day or in the 1st or 2nd trial on the second day. On the other hand with 10 mg/kg of GRg or GNo. 5 and 2 mg/kg of MA there was a tendency for prolongation of the extinction in the 1st or 2nd trial on the second day.

Experiment 4: Rats were administered the following doses; GNS (5 and 10 mg/kg), GF\textsubscript{4} (5, 10 and 20 mg/kg), GRg (10 and 30 mg/kg) and GNo. 5 (10 and 30 mg/kg). The results are shown in Fig. 5. Significant decrease of correct response was observed at the dose of 10 mg/kg of GNS and GF\textsubscript{4}, and 30 mg/kg of GNo. 5. GNS and GF\textsubscript{4} inhibited the pole climbing in response to $S^p$ like CPZ (3 mg/kg), on the other hand with GNo. 5 and MA (2 mg/kg) the rats climbed in response to $S'$. GRg had no effect on discrimination behaviour.

2) Shuttle box test

Data are presented as shown in the pole climbing test.

Experiment 5: Effects of preparations are shown in Fig. 6. CPZ in a dose of 3 mg/kg, GNS (30 mg/kg), GF\textsubscript{4} (50 mg/kg) and GNo. 5 (90 mg/kg) induced a significant inhibition
FIG. 6. Effect of Panax Ginseng root on CAR in the shuttle box test. See Fig. 2 for expression.

FIG. 7. Effect of Panax Ginseng root on discrimination behaviour in the shuttle box test. See Fig. 5 for expression.

of CAR and a significant prolongation of RL. Maximum effect of GNS or GF₄ was produced about 30 min after the treatment, and 60 min after that, the effects of these compounds on CAR and RL had disappeared completely. GRg in doses of 10, 30 and 90 mg/kg had no influence on either CAR or RL, but 10 mg/kg of GRg slightly reduced RL 20-40 min after the treatment (p<0.10). Inhibitory effect of GNo. 5 (90 mg/kg) on CAR was evident 60-80 min after the treatment.

Experiment 6: Effects of GNS in doses of 10 and 20 mg/kg, GF₄ (10 and 20 mg/kg), GRg (10 and 30 mg/kg) and GNo. 5 (10 and 30 mg/kg) on behaviour regarding discrimination between S° and S¹ were also determined (Fig. 7). Significant decrease of correct response was observed with MA (2 mg/kg), CPZ (3 mg/kg), GNS (20 mg/kg), GF₄ (20 mg/kg) and GNo. 5 (30 mg/kg). CPZ, GNS and GF₄ inhibited the response to S°, and administration of MA and GNo. 5 resulted in an incorrect response to S¹. GRg had no effect on discrimination behaviour.

3) Rotating rod and suspension tests

Effects of drugs on motor coordination and muscle tone were studied in relation to each of the doses employed in both pole climbing and shuttle box tests. The results indicated that these fractions had no influence on motor coordination and muscle tone in the doses given herein.

DISCUSSION

In previous work, we confirmed that GNS inhibited CAR (7), and GNo. 4 showed a slight CNS-stimulating action (8). Observation of CAR using pole climbing and shuttle box tests indicated that GNS produced a significant inhibition of CAR, and a significant decrease of correct response to S°. Large doses of GNo. 4 produced a significant inhibition of CAR, and small doses slightly reduced RL to CS. This inhibition or reduction suggests
that the ingredients contained in GNo. 4 may have CNS-depressing or CNS-stimulating activity. GF4 and GRg, the components of GNo. 4 were also examined. GF4 produced a significant inhibition of CAR and prolongation of RL in pole climbing and shuttle box tests as well as producing a significant decrease of correct response to SP. Such findings indicate that CNS-depressing components other than GNS are contained in GF4 which does not contain saponins in itself. On the other hand, with GRg, there was no change in either CAR or discrimination behaviour, though with GRg there was a tendency toward reduction of the RL and a prolongation of the extinction of CAR. Administration of ginsenoside Rg1, the main component of GRg, accelerated the recovery from fatigued states in mice (9) indicating that GRg has CNS-stimulating activity. It is considered from these results that CNS-depressin activity of GNo. 4 was represented by GF4 and CNS-stimulating activity of GNo. 4, by GRg. GNo. 5 produced a slight reduction of RL in small doses and a significant inhibition of CAR in large doses, and in addition, there was a slight prolongation of extinction of CAR and a significant increase of incorrect response to S1. Thus GNo. 5 also contains reverse pharmacologically active substances. In our previous report (9), GNo. 5 was demonstrated to have anti-fatigue activity and in smaller doses may also possess a slight CNS-stimulating activity. It would appear that there are three different substances in Panax Ginseng root which have CNS-depressing activities, and at least two different substances which have CNS-stimulating activities.

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