Effect of *Panax Ginseng* on Age-Related Changes in the Spontaneous Motor Activity and Dopaminergic Nervous System in the Rat

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ABSTRACT—Effects of *Panax ginseng* on the spontaneous motor activity and central dopaminergic systems in old rats were investigated and compared with those in young rats. Oral intake of a 1.8% water extract of *Panax ginseng* for four weeks produced an increase in spontaneous motor activity during the dark period in old rats, while it caused a decrease in the activity in young rats. After the intake of ginseng extract for five weeks, it caused a significantly low dopamine utilization in the daytime in the striatum of old rats, while it produced a high dopamine utilization in the structure of young rats. Concentrations of striatal dopamine D-2 receptors in old rats were significantly lower than that in young rats, although subchronic *Panax ginseng* did not affect the striatal D-1 and D-2 receptors of old rats. These results suggest that subchronic intake of ginseng extract inhibits the activity of nigro-striatal dopamine neurons in the daytime and activates spontaneous motor activity during the dark period in old rats, while it produces opposite effects in young rats.

*Panax ginseng* root produces various neuropharmacological effects such as changes in brain biogenic amines, improvement of learning and memory retention (1), promotion of recovery from fatigue (ref. 2, for review, see ref. 3), and an increase in the capacity for intellectual performance in animals and man (for review, see ref. 4). Little evidence, however, has been reported on the anti-aging effects of ginseng, that had been suggested by ancient literature (5), in experimental animals.

A decrease in motor activity was shown in aged mice in association with an increase in monoamine oxidase activity in the brain (6). Impairment of swimming ability, which was similar to that seen after electrolysis of the lateral hypothalamus including the nigro-striatal dopaminergic neurons, was observed in aged rats (7). Diminution of synthesis, release and re-uptake of dopamine and increase in D-2 dopamine receptors in the striatum were reported in aged animals (8–11).

We investigated the effects of a water extract of *Panax ginseng* root on spontaneous motor activity and nigro-striatal dopaminergic nervous systems in two year-old rats and compared them with those in young rats.

MATERIALS AND METHODS

Young and old male Fischer 344 rats (3 and 24 months old at the end of the experiment, respectively; Nippon Charles River, Tokyo) were used. They were kept under an alternat-
ing 12 hr light and dark regimen (on, 07:00 to 19:00), were maintained at a constant temperature (22 - 24°C) and humidity (50 - 75%), and were given a 1.8% water extract of Panax ginseng root instead of tap water ad libitum.

**Spontaneous motor activity**

Four weeks after oral intake of the Panax ginseng extract, a rat housed in a cage was placed on an Animex (Muromachi Kikai, Osaka) for at least 3 hr and spontaneous motor activity was recorded for the successive 24-hr in order to minimize the effect of arousal in a novel environment. The data were analyzed as total activity during day-light and dark periods, separately.

**Brain dissection**

After the intake of Panax ginseng for five weeks (one week after measurement of spontaneous motor activity), rats were intraperitoneally treated with 250 mg/kg of \( \alpha \)-methyl-\( p \)-tyrosine (\( \alpha \)-MPT), a tyrosine hydroxylase inhibitor, and were killed by decapitation 4 hr after the treatment (13:00 to 15:00). The brain was rapidly removed, placed in a cooled template and cut using razor blades at approximately A 9.8 and 7.1 mm according to a brain atlas (12). The left striatum was excised from the slice for dopamine assay; the right, for receptor binding assay; and the striata were frozen in liquid nitrogen.

**Determination of dopamine**

The striatum was homogenized in 1 ml of 0.25 N perchloric acid containing 40 ng of 3,4-dihydroxybenzylamine (DHBA) as an internal standard and centrifuged at 10,000 \( \times \) g for 15 min. Dopamine was extracted with alumina and determined using HPLC with electrochemical detection (13). Ten-microliter samples were injected into an ODS-5 \( \mu \)m column operated at a flow rate of 1 ml/min. The mobile phase consisted of 0.05 M phosphate buffer solution containing 5% (V/V) methanol, 10 \( \mu \)M EDTA and 0.2 mM sodium octylsulfate (final pH of 3.1). An LC-4B amperometric controller equipped with a graphite electrode was used to monitor the column eluates, and detector potential was maintained at 0.75 V vs. an Ag/AgCl reference electrode.

**Receptor binding assay**

The dissected striata of control rats were homogenized in 50 volumes of ice-cold buffer (50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), pH 7.4) using a Teflon homogenizer, centrifuged three times (50,000 \( \times \) g for 10 min), and finally suspended in the same buffer. Duplicate incubation tubes each received 100 \( \mu \)l of 50 mM Tris-HCl buffer (pH 7.4), \([\text{H}]\)spiperone or \([\text{H}]\)SCH 23390 (0.025 - 1.0 nM) and 1 \( \mu \)M ketanserin, and 700 \( \mu \)l of striatal membranes (0.1 mg protein) (14). Incubations were carried out at 20°C for 90 min and then terminated by rapid filtration (Whatman GF/B filters) under vacuum with three 5-ml rinses with an ice-cold buffer (50 mM ion-free Tris-HCl, pH 7.7). The radioactivity retained on each filter was counted by a liquid scintillation counter. Specific binding was defined as the excess over blanks taken in the presence of 1 \( \mu \)M SCH 23390 for D-1 and 1 \( \mu \)M (-)sulpiride for D-2, respectively.

**Drugs**

One hundred grams of Panax ginseng root (Otane-ninjin, cultivated in Nagano Prefecture, Japan) were extracted two times with 900 and 500 ml of water, respectively, at 100°C for 1 hr; and the extract was combined and freeze-dried under vacuum for two days. The extract of Panax ginseng was prepared at the concentration of 1.8% and given to the rat for drinking. In the present study, oral intake of 1.8% solution/rat/day corresponded to approximately 7 g of dried Panax ginseng/kg/day, and the intake of the solution was not significantly different from that of tap water. \( \alpha \)-MPT methylester hydrochloride (Sigma Chemicals) was dissolved in sterile saline. The chemicals used in the HPLC were analytical grade obtained from commercially available sources.
Data analysis

Data were analyzed using one-way ANOVA with Duncan's multiple comparison test.

RESULTS

Effect on spontaneous motor activity

Control old rats showed significantly lower spontaneous motor activity than control young rats during the dark period (19:00–7:00) (P < 0.05), which corresponded to the results reported previously (11). The intake of 1.8% Panax ginseng extract for four weeks in old rats caused a significant increase in spontaneous motor activity to the level of control young rats during the dark period. In contrast, Panax ginseng in young rats produced a significant decrease in spontaneous motor activity during the dark period. Panax ginseng did not modify the motor activity during the light period (7:00–19:00) either in old or young rats. These results are summarized in Fig. 1.

Effect on striatal dopamine levels after treatment with α-MPT

Striatal dopamine levels in old rats were not significantly different from those in young rats. The intake of 1.8% Panax ginseng extract for five weeks did not affect striatal dopamine levels both in old and young rats. Four hours after treatment of old rats with 250 mg/kg of α-MPT, the striatal dopamine level of the ginseng group was significantly higher than that of the control. On the contrary, in young rats, the Panax ginseng group showed significantly lower striatal dopamine level than the control at 4 hr after α-MPT (Fig. 2).

Effect on dopamine D-1 and D-2 receptors in the striatum

In control old rats, concentrations of D-1 receptors (B_{max}) were not significantly different from those in control young rats, whereas the B_{max} value of D-2 receptors was significantly lower than that in control young rats. The intake of 1.8% Panax ginseng for five weeks did not affect the B_{max} values of D-1 and D-2 receptors. The dissociation constant (K_d) of D-1 and D-2 receptors in old rats was not significantly different from that in young rats and was not affected by Panax ginseng (Table I).

![Fig. 1. Effect of subchronic intake of Panax ginseng on spontaneous motor activity in old (A, n = 10) and young (B, n = 5) rats. Rats were given a 1.8% water extract of Panax ginseng root instead of tap water ad libitum for 4 weeks. Spontaneous motor activity was measured for 24 hr and expressed as total counts in the light and dark periods separately. White column: control group, striped column: ginseng group. Vertical bar is the standard error of the mean. *P < 0.05, **P < 0.01 vs. the respective control.]

![Fig. 2. Effect of subchronic intake of Panax ginseng on dopamine levels in the striatum of old (A) and young (B) rats. Rats were given a 1.8% water extract of Panax ginseng root ad libitum for 5 weeks. Striatal dopamine levels were determined 4 hr after intraperitoneal treatment with α-methyl-p-tyrosine (250 mg/kg). White column: control group, striped column: ginseng group. Vertical bar is the standard error of the mean (n = 3–5). *P < 0.05, **P < 0.01 vs. the respective control.]
Table 1. Effect of subchronic intake of Panax ginseng extract on the binding of \( ^{3}H \)SCH 23390 to D-1 receptors and \( ^{3}H \)spiperone to D-2 receptors in the striatum of old rats

|                | D-1  | D-2  |
|----------------|------|------|
|                | \( B_{max} \) | \( K_d \) | \( B_{max} \) | \( K_d \) |
| Young control  | 938 ± 59 | 0.21 ± 0.01 | 358 ± 16 | 0.12 ± 0.01 |
| Old control    | 875 ± 10  | 0.22 ± 0.01 | 262 ± 10** | 0.12 ± 0.01 |
| Panax ginseng  | 831 ± 87  | 0.24 ± 0.02 | 239 ± 17** | 0.10 ± 0.01 |

Rats were given a 1.8% water extract of Panax ginseng root ad libitum for 5 weeks. Concentrations (\( B_{max} \), fmol/mg protein) and affinities (dissociation constant: \( K_d \), nM) were determined from individual Scatchard plots. Values are the mean of four independent experiments ± standard errors. **\( P < 0.01 \) vs. young control.

DISCUSSION

The present study has shown that subchronic intake of a water extract of Panax ginseng restores spontaneous motor activity during the dark period in old rats, while it diminishes the activity in young rats as compared with the respective control. We have already shown that the intake of 1.8% Panax ginseng extract for 4 months produces an increase in spontaneous motor activity during the dark period in mid-life mature rats (15). These results suggest that the effects of Panax ginseng on motor function in old rats are contrary to those in young rats. The reason why Panax ginseng extract does not affect the motor activity during the light period both in old and young rats is not clear, but it may be connected with the diurnal rhythm of spontaneous motor activity in rats.

Chronic treatment with haloperidol was shown to produce behavioral supersensitivity to dopaminergic agonists and an increase in \( ^{3}H \)spiperone binding to striatal membranes in young adults but not in old rats (16). Intrastriatal injection of dopamine produced more rotational behavior contralateral to the side of the injection in young adults than in senescent rats (17). On the contrary, aged rats showed much stronger sedative effects than young adults following systemic administration of haloperidol (18). Thus, the difference of the effects of Panax ginseng on spontaneous motor activity between old and young rats may be due to age-related change in the sensitivity to the active principles of ginseng.

It has been reported that neutral saponins of Panax ginseng and the ginsenoside Rb1 fraction exerts an inhibitory effect on spontaneous movements, and that the ginsenoside Rg1 fraction increases alertness and spontaneous movements in mice (19-21). The extract of Panax ginseng has been shown to induce a general stimulating effect on the integrative nervous activity in rats (4, 22). Panax ginseng, therefore, has several principles that produce central stimulating and/or suppressing effects in animals. These active principles may have some relation to the age-related changes in the effects of Panax ginseng. It is, however, unclear why the extract expressed opposite effects in young adult and old rats, respectively.

Striatal dopamine levels 2 to 4 hr after the treatment with α-MPT are index of dopamine utilization in dopaminergic neurons (23). In the present study, old rats showed relatively higher dopamine utilization in the striatum than young animals in the daytime. It has been reported that tyrosine hydroxylase activity and dopamine catabolism in the striatum decrease with aging (24, 25). Therefore, the present result appears to be contradictory to those reports. This point is a problem to be solved. It is well-known that the dopamine receptor agonist apomorphine inhibits and the antagonist haloperidol increases dopamine utilization via pre- and postsynaptic dopamine receptors in the nigro-striatal dopamine neurons of the rat (26, 27). Apomorphine produces
hypermotility and stereotyped behavior such as sniffing and gnawing dose-dependently, and haloperidol inhibits them in rats (28). Thus, striatal dopamine utilization has apparently a relation to the drug-induced behavioral changes within the limits of drugs acting on dopamine receptors. The present results suggest that Panax ginseng increases locomotor activity during the dark period and inhibits striatal dopamine utilization in the daytime in old rats, while it inhibits the locomotor activity and activates the utilization in young rats. This may be due to the stimulating and/or blocking effects of Panax ginseng on dopamine receptors in the striatum, although there is a time lag between the measurement of the locomotor activity and the determination of dopamine utilization in the present study. We have previously reported that chronic intake of Panax ginseng for 23 months restored the aging-related decrease in the concentrations of dopamine D-2 receptors in senescent rats (15). We have also reported that subchronic ginseng in mature rats did not modify the concentrations of striatal D-1 and D-2 receptors and their dissociation constants (15). In addition, the present study has shown that subchronic ginseng in old rats does not modify striatal D-1 and D-2 receptors. Hence there appears to be no change in the dopamine receptor mechanism after subchronic intake of Panax ginseng in mature and old rats.

Since 2–3 g/day of Panax ginseng has been used clinically in various traditional prescriptions, the dose used in the present experiment is much higher than the clinical one. Thus, further investigations will be performed using a low dose of Panax ginseng extract in rats, and the locomotor activity and dopamine utilization will also be observed at the same time.

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