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Effects of ginsenosides on maze performance and brain choline acetyltransferase activity in scopolamine-treated young rats and aged rats

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Abstract

In young adult rats with scopolamine-induced cognitive impairment, choline acetyltransferase activity was increased in the medial septum, but not in the diagonal band, caudate and hippocampus, 30 min after the injection of ginsenosides Rg1 or Re. Rb1 and Rd had no effect on choline acetyltransferase activity. Aged rats showed a smaller number of initial correct responses in the radial-arm maze and a lower choline acetyltransferase activity in the medial septum, diagonal band, caudate and hippocampus than did young adult rats. Repeated i.p. injections of Rg1 increased the number of initial correct responses and the activity of choline acetyltransferase in the medial septum, but not in the diagonal band, caudate and hippocampus, in aged rats. These results suggest that Rg1 and Re may contribute the ameliorative effects through an increase of choline acetyltransferase activity in the medial septum. © 1997 Elsevier Science B.V.

Keywords: Ginsenoside; Choline acetyltransferase; Scopolamine; Maze; Septum, medial

1. Introduction

Systemic injection of scopolamine, a cholinergic receptor antagonist, causes impaired maze performance in young adult rats (Sala et al., 1991; Molchan et al., 1992; Yamaguchi et al., 1995), and the scopolamine-induced impairment is ameliorated by ginsenosides of the protopanaxatriol type, Rg1 and Re, but not ginsenosides of the protopanaxadiol type, Rb1 and Rd (Yamaguchi et al., 1995, 1996). These observations suggest that ginsenosides Rg1 and Re may affect cholinergic systems in the brain to produce their ameliorating effects. In order to explore the mechanisms of the ginsenoside effects, we measured choline acetyltransferase activity in certain brain areas after intraperitoneal (i.p.) injection of ginsenosides in young adult rats injected with scopolamine. So far as we know, brain choline acetyltransferase activity has never been measured in relation to the ameliorative effects of ginsenosides on the impaired performance caused by scopolamine.

It has also been demonstrated that aged rats show a significant impairment in maze performance (Kadar et al., 1990; Luine and Hearns, 1990), and that choline acetyltransferase activity in the brain is reduced (Luine and Hearns, 1990; Gallagher et al., 1990). Since we knew that Rg1 ameliorates this impairment in aged rats, the present studies were designed to examine whether choline acetyltransferase activity is enhanced by Rg1 in the brain of aged rats.

2. Materials and methods

2.1. Animals

Male young adult rats (2 months of age) and male aged rats (21 months of age) of the Sprague-Dawley strain were used. Throughout the choline acetyltransferase study, groups of 3–5 rats were housed in cages in a room maintained at 22°C with a 12-h light/dark cycle. Water and food were available ad libitum. In the maze study, rats were kept individually in another room under the same conditions as above. Feeding was restricted but water was freely available, as shown in Fig. 1.

2.2. Choline acetyltransferase measurement

Choline acetyltransferase activity was assayed as described by Fonnum (1975). After the injection of test drugs, rats were killed by decapitation and brains were removed and immediately placed on an ice-cooled glass

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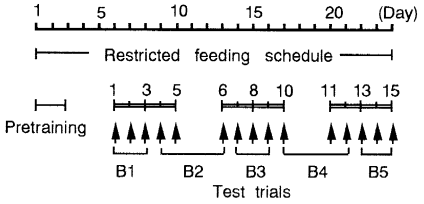

Fig. 1. Experimental schedule for assessing the effect of Rg1 on learning impairment in aged rats. Fifteen test trials were grouped into five blocks (B1–B5).

plate. Brain regions necessary for measurements were dissected out quickly and each tissue was homogenized in a tube containing 400 μl of cold 50 mM phosphate buffer with 10 mM EDTA and 0.5% Triton X-100. The homogenate was used as the enzyme solution. Each enzyme solution was incubated at 37°C in a medium containing radiolabelled [^{14}C]acetyl coenzyme A (Amersham, Tokyo, Japan) to produce acetylcholine, acetyl coenzyme A being combined with choline in the presence of choline acetyltransferase. The amount of radioactive acetylcholine in each tube was measured for 5 min in a scintillation counter. Protein concentration of the sample in each tube was measured by the method of Bradford (1976) with a Protein Assay Kit I (Bio-Rad Laboratories, Hercules, CA, USA) with bovine gamma globulin as the standard. Choline acetyltransferase activity is expressed as nmol acetylcholine synthesized per mg protein per hour.

To determine the optimal time for the measurement of enzyme activity after Rg1 injection, preliminary studies were performed using 49 young adult rats. Rg1 was injected singly i.p. at a dose of 5 mg/kg and choline acetyltransferase activity was measured in the medial septum and the hippocampus 0, 10, 20, 30, 40, 50 and 60 min after the injection. Choline acetyltransferase activity began to increase 20 and 30 min and peaked at 30 ($P < 0.05$) and 40 ($P < 0.05$) min after the injection in the medial septum and hippocampus, respectively (Fig. 2). Therefore, enzyme activity was measured 30 min after the injection of ginsenosides in the following experiments. The statistical difference was evaluated by Student's *t*-test.

2.3. Maze performance of aged rats and Rg1 effect

Maze performance was examined in 17 aged rats and compared with that of 10 young adult rats. Each rat was tested in a standard radial-arm maze (Muromachi Kikai, Tokyo, Japan). Details of the apparatus have been described in a previous paper (Yamaguchi et al., 1995). The experimental schedule is shown in Fig. 1. A restricted feeding schedule and pretraining were begun on the first day of the experiments with both aged and young adult rats. Water was available ad libitum. The weights of rats were reduced to about 80% of the starting weights by reducing the daily ration of food (CE-7, Clea Japan, Tokyo, Japan) at the beginning of test trials for both aged and young adult rats. Subsequently, the body weight of young adult rats, but not of aged rats, was increased by 5 g per week by manipulation of the daily ration of food during the test trials. In order to habituate the rats to the maze, they were subjected to pretraining, which was performed once a day for 3 days (Fig. 1). On the first day of this pretraining, rats were placed in groups (4–6 rats) on the maze without bait for 10 min. On the second and third day, rats of the same groups were placed on the maze with bait (crystallized sugar) and left for 10 min.

In the test trials, rats received injections of test drugs, beginning 3 days after the pretraining. Drugs were injected 15 times (Fig. 1). Each rat was placed in the center of the maze and allowed to visit the wells at the end of eight arms. The wells contained 2 or 3 lumps (approximately 50 mg in total) of crystallized sugar. Rats were required to learn how to obtain food in the well at the end of each arm and to remember not to re-enter those arms that had been visited. Re-entry into an arm that had been visited was scored as an error. The number of consecutive correct choices prior to re-entry into a previously visited arm (the number of initial correct responses) was recorded as an index of performance. Each test trial continued until the bait in all eight wells had been consumed or until 16 choices had been made, or until 10 min had elapsed, whichever occurred first.

Single i.p. injections of different doses of Rg1 suspended in a 1% solution of carboxymethyl cellulose (CMC) or vehicle only were given every day of each test trial 30 min before the rats were placed in the maze (Fig. 1). Doses used were 0, 5 and 10 mg/kg and the injection volume was equivalent to 1 ml/kg. Rats receiving an equal volume of the vehicle served as controls. For each dose, 15 test trials were performed with the same rats. These test trials were divided into the following five blocks, each of which consisted of three test trials: block 1, trials 1–3; block 2, trials 4–6; block 3, trials 7–9; block 4, trials 10–12; block 5, trials 13–15 (Fig. 1). The mean values

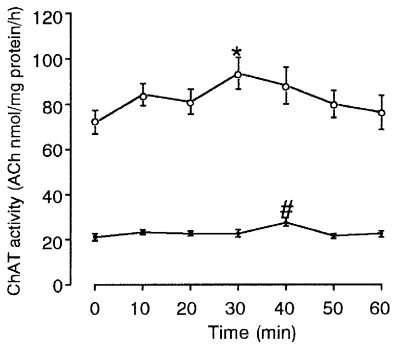

Fig. 2. Time-course curves for the effects of Rg1 on choline acetyltransferase (ChAT) activity in the medial septum (open circles) and the hippocampus (closed circles) of young adult rats. ChAT activity is expressed as nmol acetylcholine (ACh) synthesized per mg protein per hour. $^{*}P < 0.05$, $^{\#}P < 0.05$, compared with respective initial control level.

and standard errors of the three trials in each block were calculated.

The statistical significance of differences between groups was calculated by two-way repeated analysis of variance (ANOVA), which was followed by Dunnett's multiple comparison test. The criterion for significance was $P < 0.05$ in all statistical evaluations.

2.4. Effects of ginsenosides on choline acetyltransferase activity

In the experiments with rats receiving ginsenosides and scopolamine, 64 young adult rats were used. Ginsenosides Rg1, Re, Rb1 and Rd were injected i.p. Doses of ginsenosides were 5 mg/kg in Rg1, Rb1 and Rd, and 0.5 mg/kg in Re. Ten minutes after a single injection of each ginsenoside, scopolamine hydrobromide (Merck, Darmstadt, Germany) dissolved in distilled water was injected i.p. at a dose of 1 mg/kg. Choline acetyltransferase activity was measured 30 min after the injection of ginsenosides.

In the experiments with aged rats, 10 aged rats were used and 5 young adult rats were used for comparison. Single i.p. injection of Rg1 at a dose of 10 mg/kg in CMC or vehicle only was given 15 times to aged and young adult rats. Thirty minutes after the last injection, choline acetyltransferase activity was measured.

The statistical significance of differences in choline acetyltransferase activity was calculated by one-way ANOVA, which was followed by Dunnett's multiple comparison test.

3. Results

3.1. Effects of ginsenosides and scopolamine on choline acetyltransferase activity in young adult rats

Injection of scopolamine alone did not produce any significant changes in the choline acetyltransferase activity in any of the brain regions studied (Figs. 3 and 4).

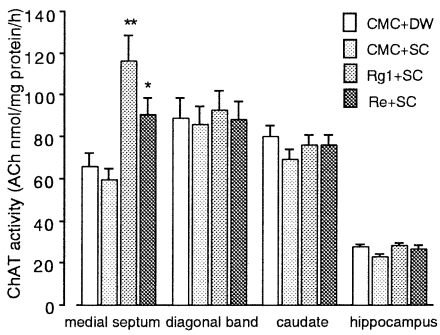

Fig. 3. Effects of i.p. injection of ginsenosides Rg1 and Re on choline acetyltransferase (ChAT) activity in four brain regions of young adult rats. ChAT activity is expressed as nmol acetylcholine (ACh) synthesized per mg protein per hour. $^{**}P < 0.01$, $^{*}P < 0.05$, compared with control receiving CMC (vehicle) + scopolamine (SC).

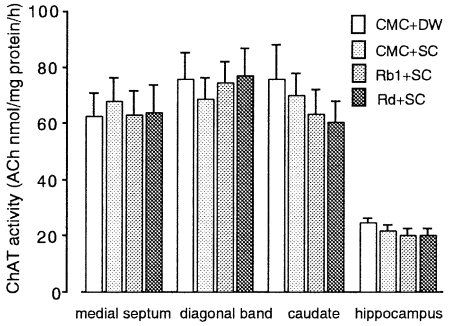

Fig. 4. Effects of i.p. injection of ginsenosides Rb1 and Rd on choline acetyltransferase (ChAT) activity in four brain regions of young adult rats. Neither ginsenoside had an effect on ChAT activity. ChAT activity is expressed as nmol acetylcholine (ACh) synthesized per mg protein per hour.

Significant increases were brought about by Rg1 and Re in the medial septum ($F(2,21) = 10.01$, $P < 0.01$), but not in the diagonal band of Broca, caudate and hippocampus (Fig. 3). Subsequent comparisons showed that Rg1 and Re treatments significantly increased choline acetyltransferase activity in the medial septum ($P < 0.01$ and $P < 0.05$, respectively), as compared with that of scopolamine-treated rats (Fig. 3). Rb1 and Rd did not induce any significant changes in choline acetyltransferase activity in any of the regions investigated (Fig. 4).

3.2. Maze performance and choline acetyltransferase activity of aged rats and Rg1 effects

The aged rats showed a significantly smaller number of initial correct responses (1.9 ± 0.7 to 4.4 ± 0.2, $n = 5$) than did young adult rats in any corresponding block (Fig. 5). In the young adult rats, the number of initial correct responses in five blocks ranged from 4.9 ± 0.3 to 6.7 ± 0.3 ($n = 10$). A two-way repeated ANOVA revealed significant effects of aging ($F(1,13) = 148.06$, $P < 0.01$) and trials ($F(4, 65) = 9.19$, $P < 0.01$) and no interaction effect ($F(4,65) = 0.40$, $P > 0.05$). Rg1 at a dose of 10 mg/kg

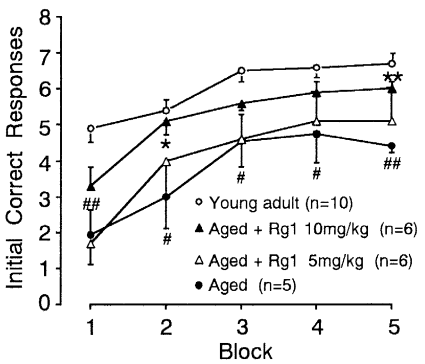

Fig. 5. Ameliorating effects of i.p. injection of Rg1 (10 mg/kg) on the learning impairment of aged rats, as assessed in a radial-arm maze. Each value with standard error of each block represents the mean number of initial correct responses in 3 test trials. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, compared with young adult rats. $^{**}P < 0.01$, $^{*}P < 0.05$, compared with aged rats.

Table 1
Choline acetyltransferase activity in four brain regions of young adult and aged rats

Brain region	Young adult ($n = 5$)	Aged	
		CMC ($n = 5$)	Rg1 ($n = 5$)
Medial septum	59.6 ± 5.0	43.9 ± 4.1^{a}	75.2 ± 4.0^{c}
Diagonal band	84.2 ± 7.1	63.6 ± 3.5^{a}	58.9 ± 6.5
Caudate	78.8 ± 4.2	40.1 ± 3.4^{b}	38.5 ± 2.3
Hippocampus	28.8 ± 2.2	17.2 ± 1.3^{b}	19.7 ± 1.0

Choline acetyltransferase activity is expressed as nmol acetylcholine synthesized per mg protein per hour. $^{a}P < 0.05$, $^{b}P < 0.01$, compared with young adult rats; $^{c}P < 0.01$, compared with aged CMC (vehicle)-treated rats.

increased the number of initial correct responses. A two-way repeated ANOVA revealed significant effects of the treatment ($F(2,14) = 7.86$, $P < 0.01$) and trials ($F(4,70) = 46.68$, $P < 0.01$) and no interaction effect ($F(8,70) = 0.28$, $P > 0.05$) (Fig. 5). Thus, Rg1 (10 mg/kg) mitigated the learning impairment of aged rats.

A significant group effect of age on choline acetyltransferase activity in the medial septum ($F(2,12) = 12.86$, $P < 0.01$), diagonal band ($F(2,12) = 5.22$, $P < 0.05$), caudate ($F(2,12) = 45.65$, $P < 0.01$) and hippocampus ($F(2,12) = 18.81$, $P < 0.01$) was revealed by the analysis of variance (Table 1). Choline acetyltransferase activity in the medial septum, diagonal band, caudate and hippocampus of aged rats was lower by 27% ($P < 0.05$), 24% ($P < 0.05$), 49% ($P < 0.01$) and 40% ($P < 0.01$), respectively, than that of young adult rats. However, Rg1 injection resulted in a significant increase ($P < 0.01$) in the medial septum (71% increase), as compared with that of CMC-treated aged rats (Table 1).

4. Discussion

It is well known that scopolamine, which is a cholinergic receptor antagonist, causes cognitive impairments (Sala et al., 1991; Molchan et al., 1992; Yamaguchi et al., 1995). In the present study, scopolamine treatment did not produce any significant change in choline acetyltransferase activity in any of the regions investigated, even in the basal forebrain and hippocampus, which are important for cognitive processes (for review, see Dutar et al., 1995). These results agree with those of previous reports by other investigators that systemic injection of scopolamine does not change choline acetyltransferase activity in the cortex and hippocampus (Jackson and Soliman, 1996; Hirokawa et al., 1996). It may be concluded, therefore, that the scopolamine-induced performance impairment is not related to choline acetyltransferase activity, but is concerned with ACh receptor mechanisms.

We have already demonstrated that Rg1 and Re, but not Rb1 nor Rd, have ameliorating effects on the impaired performance caused by scopolamine in the radial-arm maze task (Yamaguchi et al., 1995, 1996). In the present study, Rg1 and Re, but not Rb1 nor Rd, caused an increase in choline acetyltransferase activity in the medial septum. These results indicate that only the ginsenosides which have ameliorating effects could bring about an increase in choline acetyltransferase activity in the medial septum. The medial septum cholinergic neurons extend multiple processes in the immediate vicinity and innervate the hippocampus (for review, see Woolf, 1991). In addition, it is well known that the medial septum is a critical site for tasks requiring memory. Lesions in the medial septum cause performance impairment in the radial-arm maze task (Horita et al., 1989; Matsuoka et al., 1991). It has been reported that the cholinergic projection from the medial septum to the hippocampus is necessary for the maintenance of accurate radial-arm maze memory performance (for review, see Levin, 1988). Further, we demonstrated previously that i.p. injections of Rg1 did not mitigate the learning impairment of rats with lesions in the medial septum (Yamaguchi et al., 1995). All these observations suggest that the ameliorative effects of Rg1 and Re on scopolamine-induced impaired performance may be related to the increase in choline acetyltransferase activity in the medial septum. It is likely that enhanced activity of the septal neurons elicited by Rg1 and Re and the accompanying increase in choline acetyltransferase activity stimulate cholinergic neuronal terminals to release acetylcholine for stimulation of the hippocampus, thereby overcoming the effects of scopolamine. It is possible, however, that the increase in choline acetyltransferase activity might have occurred in the cholinergic terminals impinging upon the septum, resulting in an increased production of terminal acetylcholine to stimulate the medial septum and then hippocampus, overcoming the effects of scopolamine. In any case, it is possible that Rg1 and Re caused stimulation of the hippocampus through cholinergic neurons in the septum and the impaired performance was ameliorated. It is known that electrical stimulation of the medial septal nucleus enhances the release of acetylcholine in the hippocampus (Dudar, 1975). Further, iontophoretic administration of acetylcholine in the septal area increases the discharge rate of the majority of septo-hippocampal neurons (for review, see Dutar et al., 1995) and microinjection of carbachol into the medial septal area induces theta field activity in the hippocampus (Lawson and Bland, 1993).

The present studies have demonstrated that aged rats show learning impairment in a radial-arm maze, as reported by other investigators (Luine and Hearns, 1990; Kadar et al., 1990), and that choline acetyltransferase activity is lower in the medial septum, diagonal band of Broca, caudate and hippocampus in aged rats than in young adult rats. It has been reported that aged rats with impaired learning have decreased choline acetyltransferase activity in the basal forebrain, striatum and frontal cortex (Gallagher et al., 1990), diagonal band, striatum and hippocampus (Luine and Hearns, 1990) and medial septum

(for review, see Dutar et al., 1995). Further, treatment with an inhibitor of acetylcholinesterase improves the performance impairment in aged rats (Malin et al., 1991). All these data suggest that the learning impairment of aged rats is at least due to dysfunction of cholinergic systems in regions of the brain necessary for cognitive function.

In the present study, Rg1 improved the spatial learning impairment in aged rats, and increased choline acetyltransferase activity in the medial septum, but it did not change choline acetyltransferase activity in the other regions investigated. As described above, the septo-hippocampal cholinergic systems have very important roles in tasks requiring memory. The improvement of spatial learning elicited by Rg1 in aged rats, which involves cholinergic neurons in the medial septum, may be explained as in the case of young adult rats receiving scopolamine as mentioned above. The only difference is that young adult rats received injections of scopolamine, but the aged rats did not since the aged rats showed an impaired performance without scopolamine.

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