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Short communication

Effects of ginsenosides on impaired performance caused by scopolamine in rats

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Abstract

The effects of five structurally different ginsenosides on the performance impaired by scopolamine in rats were studied in a radial-arm maze. Ginsenoside Re had an ameliorating effect, but the ginsenosides, 20-*O*-glucosyl-protopanaxadiol, F2, Rf, and Rh1, did not. We reported previously that ginsenoside Rg1 had an ameliorating effect but that ginsenosides Rb1 and Rd did not (Yamaguchi et al., 1995, Psychoneuroendocrinology 20, 645). The sugar moieties at C(6) and C(20), which are possessed by Re and Rg1, seem to be indispensable for an ameliorating effect on the performance impaired by scopolamine.

Keywords: Ginsenoside; Scopolamine; Maze; (Rat)

1. Introduction

There are several kinds of ginsenosides with similar structures that have been purified from *Panax ginseng* (see Shoji, 1990, for review), but their pharmacological actions may differ. For instance, ginsenosides Rb1, Rd, F2 and Rg1 potentiate the growth of nerve fibers mediated by nerve growth factor (NGF) in cultured dorsal root ganglia of the chick embryo, while other ginsenosides, Rb2, Rb3, Rc, Re, Rf, Rg2 and Rg3, do not (Takemoto et al., 1984). Rg1 stimulates the secretion of adrenocorticotropin from cultured rat pituitary cells, while other ginsenosides, Rb1, Rc, Rd and Re, do not (Odani et al., 1986). Both Rg1 and Rb1 accelerate the acquisition of discrimination-reversal learning in a Y-maze task by rats (Saito, 1990). In our previous experiments, Rg1 improved the impaired performance induced in rats by scopolamine in a radial-arm maze, but Rb1 and Rd did not (Yamaguchi et al., 1995). Thus, the biological effects of ginsenosides differ depending on these structures and on the target.

In the present study, we examined the effects of five ginsenosides, 20-*O*-glucosyl-protopanaxadiol, F2, Re, Rf and Rh1, on impaired performance in a radial-arm maze in rats and the relationship between the structure of ginsenosides and ameliorative effects is discussed.

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2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (8 weeks of age, 270–300 g) were obtained from Charles River (Kanagawa, Japan). They were caged individually in a room maintained at around 22°C with a 12-h light/dark cycle.

2.2. Apparatus

Each rat was tested in a standard radial-arm maze (Muromachi Kikai Co., Tokyo, Japan) that was a slightly modified version of the one used by Olton and Samuelson (1976). Details of the apparatus of the maze have been described in a previous paper (Yamaguchi et al., 1995).

2.3. Experimental procedures

The experimental schedule is shown in Fig. 1a. A restricted feeding schedule and pretraining were started on the first day of the experiment, and the weights of the rats were decreased to about 80% of their starting values by a reduction in the daily ration of food (CE-2, Clea Japan, Tokyo) by the beginning of training trial. Subsequently, the weight of each rat was increased by 5 g per week by manipulation of the supply of food prior to the test trial.

In order to adapt the rats to the maze, they were subjected to pretraining once a day for 3 days. On the first

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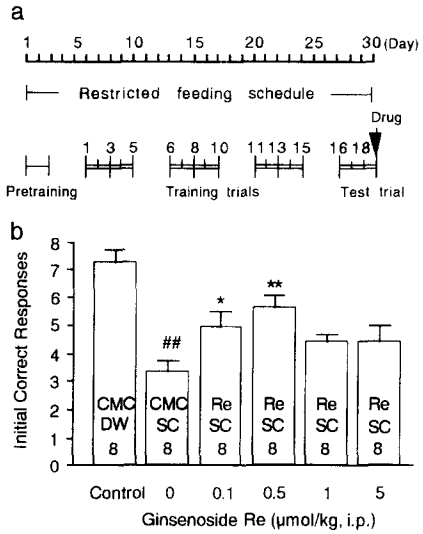

Fig. 1. Experimental schedule for tests of ginsenosides on performance impaired by scopolamine in rats (a). Effects of i.p. injection of Re on impaired performance of rats, as determined in a radial-arm maze (b). Each column represents the mean number of initial correct responses; vertical bars show S.E.M. The number within each column shows the number of rats used. $^{\#\#}P < 0.01$, compared with rats treated with carboxymethyl cellulose (CMC) + distilled water (DW); $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with rats treated with CMC + scopolamine (SC).

day of pretraining, the rats were placed in groups (5–6 rats) on the maze without any bait for 10 min. On the second and third days, rats from the same groups were placed on the maze with the bait (crystallized sugar) scattered on the central hub and arms and left for 10 min.

The training trial started 2 days after the pretraining and was repeated 18 times in order to allow the rats to learn how to perform the radial-arm maze task. On the day after the last training trial, the test trial, in which the rats were injected singly with test drugs or corresponding vehicles, was carried out. For each trial, a rat was placed on the central hub of the maze and allowed to visit the well at the end of each of the 8 arms, which had been baited with 2 or 3 lumps (approximately 50 mg in total) of crystallized sugar. The rats were required to learn how to obtain food in the well and to remember not to re-enter those arms that had been visited once. 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 the index of performance. Each trial continued until the bait in all 8 wells had been consumed, or until 16 choices had been made, or until 10 min had elapsed, whichever occurred first.

2.4. Drugs

The ginsenosides used in the present studies were isolated from *Panax ginseng* by the Natural Products Research Division of our Laboratory and showed approximately 98% purity. These ginsenosides, suspended in a 1% solution of carboxymethyl cellulose (CMC), were injected i.p. at doses of 0, 0.1, 0.5, 1 and 5 μmol/kg. Each injection volume was equivalent to 1 ml/kg. Ten minutes after the injection of ginsenosides, scopolamine hydrobromide (Merck, Darmstadt, Germany) dissolved in distilled water was injected i.p. at a dose of 1 mg/kg; 20 min later the rat was placed on the maze. Control rats received 1% solution of CMC without ginsenosides and distilled water without scopolamine in a manner similar to that used with experimental rats.

A total of 235 rats were used. They were divided into five groups each consisting of 46–48 rats for injections of five different ginsenosides. The rats were given only one injection with a given dose of the ginsenosides or vehicles and were never used twice.

2.5. Statistical analyis

The mean and standard error were calculated and the data were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. The criterion for significance was $P < 0.05$ in all statistical evaluations.

3. Results

The number of initial correct responses decreased significantly in rats injected i.p. with a 1% solution of CMC and scopolamine, as compared with control rats injected with 1% solution of CMC and distilled water in each group ($P < 0.01$, Dunnett's test). However, the number of initial correct responses increased significantly in rats after i.p. injection of Re [$F(4,35) = 3.18$, $P < 0.05$] (Fig. 1b), but not of 20-*O*-glucosyl-protopanaxadiol [$F(4,34) = 0.11$, $P > 0.05$], F2 [$F(4,33) = 0.34$, $P > 0.05$], Rf [$F(4,34) = 1.80$, $P > 0.05$] and Rh1 [$F(4,35 = 1.67$, $P > 0.05$], as compared with rats injected with the 1% solution of CMC and scopolamine in each group. Subsequent comparisons showed that the ameliorative effects of Re were characterized by a significantly improved performance at doses of 0.1 and 0.5 μmol/kg, as compared with the performance of rats receiving CMC and scopolamine ($P < 0.05$ and $P < 0.01$, respectively). The results yielded a bell-shaped dose-response relationship.

4. Discussion

In the present study, a single i.p. injection of Re, but not of 20-*O*-glucosyl-protopanaxadiol, F2, Rf or Rh1, improved the performance impaired by scopolamine in rats. We reported previously that a single i.p. injection of Rg1, but not of Rb1 nor Rd, improved the scopolamine-impaired performance of rats in a radial-arm maze (Yama-

Table 1
Relationship between position of sugar moieties and ameliorative effects of ginsenosides

Ginsenoside	Position of carbon atom			Effect
	C(3)	C(6)	C(20)	
Protopanaxadiol glycosides				
20-*O*-GP [a]	OH	H	*O*-gl	−
F2	*O*-gl [b]	H	*O*-gl	−
Rb1	*O*-gl-gl	H	*O*-gl-gl	− [d]
Rd	*O*-gl-gl	H	*O*-gl	− [d]
Protopanaxatriol glycosides				
Re	OH	*O*-gl-rha [c]	*O*-gl	+
Rf	OH	*O*-gl-gl	OH	−
Rg1	OH	*O*-gl	*O*-gl	+ [d]
Rh1	OH	*O*-gl	OH	−

[a] 20-*O*-glucosyl-protopanaxadiol; [b] glucose; [c] rhamnose; [d] data taken from a previous report (Yamaguchi et al., 1995).

guchi et al., 1995). As shown in Table 1, the ginsenosides examined in the previous and present studies can be classified into two groups: the 20(s)-protopanaxadiol type (20-*O*-glucosyl-protopanaxadiol, F2, Rb1 and Rd) and the 20(s)-protopanaxatriol type (Re, Rf, Rg1 and Rh1). Ginsenosides of the 20(s)-protopanaxadiol type, without sugar attached to C(6), had no effect on performance amelioration. Ginsenosides of the 20(s)-protopanaxatriol type, Re and Rg1, with sugar moieties attached to both C(6) and C(20), had a mitigating effect on impaired performance. Rf and Rh1 of the 20(s)-protopanaxatriol type, with a sugar moiety attached to C(6) but not to C(20), had no effect on the impaired performance. Therefore, it seems that sugar moieties attached to both C(6) and C(20) are indispensable for an ameliorating effect on impaired performance, although it is not known how impaired performance is affected by these structural features.

Since scopolamine is a cholinergic receptor antagonist, the performance impaired by scopolamine may result from dysfunction of central cholinergic mechanisms. Accordingly, the ameliorating effects of Rg1 and Re could be explained mainly by interactions with central cholinergic functions. The interactions may have occurred at least at the medial septum, since the medial septum sends cholinergic fibers to the hippocampus, which is necessary for spatial memory (see Levin, 1988, for review), and the lesions in the medial septum inhibited the mitigating effect of Rg1 (Yamaguchi et al., 1995). Moreover, we have recently found that i.p. injection of Rg1 and Re increased choline acetyltransferase activity of the medial septum in rats (unpublished data).

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