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Effect of Ginseng Total Saponin on Bovine Adrenal Tyrosine Hydroxylase

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Ginseng total saponin (GTS) can modulate dopaminergic activity at both presynaptic and postsynaptic dopamine receptors (Kim *et al.*, 1998). The present study investigated the effect of GTS on the bovine adrenal tyrosine hydroxylase (TH), which catalyze L/tyrosine to DOP. GTS inhibited the bovine adrenal TH by 42.4, 51.5 and 55.3% at concentrations of 40, 80 and 100 μg/ml, respectively. The IC_{50} value of GTS was 77.5 μg/ml. GTS exhibited noncompetitive inhibition with a substrate L-tyrosine. The Ki value was 155 μg/ml.

Key words : Ginseng total saponin, Bovine adrenal, Tyrosine hydroxylase, L-tyrosine, Non-competitive inhibition

INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae) has been used widely as a herbal medicine and saponin is proved to be the main bioactive component of ginseng (Shibata, 1989). Ginseng saponin (ginseng total saponin, GTS) has sedative and tranquilizing effects (Takagi *et al.*, 1972). It has also been reported that GTS modulates dopaminergic hyperactivity induced by morphine at the presynaptic dopamine receptor (Kim *et al.*, 1998) and a single dose treatment of GTS shows an antidopaminergic action at postsynaptic dopamine receptor (Kim *et al.*, 1998).

From these results, it can be hypothesized that antidopaminergic activity of GTS may be related to dopamine biosynthesis. We, therefore, investigated the effect of GTS on bovine adrenal tyrosine hydroxylase (EC 1.14.16.2, TH), the rate-limiting enzyme in the catecholamine biosynthesis, which catalyzes the formation of L-DOPA from L-tyrosine (Nagatsu *et al.*, 1964).

MATERIALS AND METHODS

Materials

GTS, extracted and purified from the root of Panax ginseng, was supplied by Korea Ginseng and Tobacco Research Institute (Taejon, Korea). L-tyrosine, DL-6-methyl-5,6,7,8-tetrahydropterin, catalase, 3,4-dihydroxybenzylamine and alumina were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were reagent grade.

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Bovine adrenal TH

Bovine adrenal was obtained from the Agriculture and Livestock Development Office (Cheongju, Korea). Bovine adrenal TH was purified by the method of Joh and Ross (1983) with minor modification. The bovine adrenal medulla (50 g) was homogenized with 10 mM potassium phosphate buffer (pH 7.0) and then centrifuged at 1,000×g for 5 min. The supernatant was further centrifuged at 105,000×g for 60 min. The sediment was dissolved in 20 mM potassium phosphate buffer (pH 7.0) and the solution was subjected to ammonium sulfate precipitation at 80% saturation. The precipitate was dialyzed against 10 mM potassium phosphate buffer (pH 7.0) and the protein was fractionated, taking the protein which precipitated between 30 and 60% saturation in ammonium sulfate. This fraction was dialyzed against 10 mM the buffer. TH activity obtained from the final enzyme preparation was adjusted to 1.10 nmol/min/mg protein for the experiments.

Assay for TH

TH activity was determined using L-tyrosine as substrate according to a slightly modified procedure of Nagatsu *et al.* (1979) as described previously (Lee and Zhang, 1996). The reaction mixture contained 1.5

M sodium acetate (pH 5.8, 20 μl), 10 mM tyrosine (10 μl), 10 mM 6-methyltetrahydropterin (10 μl), 2 mg/ml catalase (10 μl) and enzyme preparation (50 μl). The enzyme reaction took place at 37°C for 10 min, and the reaction was stopped with 600 μl of 0.5 M perchloric acid containing 100 pmol of 3,4-dihydroxybenzylamine (internal standard). After clean-up of the reaction mixture using alumina cartridges, the eluate was injected into the HPLC system. The conditions of HPLC analysis were same as described previously (Lee and Zhang, 1996).

Data analysis

Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The values of the Michaelis constant (K_m) and the maximum velocity (V_{max}) were obtained by Lineweaver-Burk's plot using various concentrations of L-tyrosine.

RESULTS AND DISCUSSION

GTS inhibited bovine adrenal TH by 42.4, 51.5 and 55.3% at concentrations of 40, 80 and 100 μg/ml, respectively (Table I). The IC_{50} value of GTS was 77.5 μg/ml (20~120 μg/ml). According to the kinetic properties of bovine adrenal TH in this experiment, the values of K_m and V_{max} in terms of the substrate L-tyrosine were 88.0 (7.2 μM (n=4) and 1.01 nmol/min/mg protein, respectively.

Fig. 1 shows the effect of GTS on TH kinetics. This plot indicates noncompetitive inhibition with respect to L-tyrosine according to the definition of Lineweaver-Burk. The V_{max} value was lowered in the presence of GTS (80 μg/ml) to 0.66±0.05 nmol/min/mg protein. The K_i value of GTS was 155 μg/ml. On the other hand, GTS did not inhibit the bovine adrenal dopamine β-hydroxylase, which catalyses the formation of norepinephrine from dopamine (data not shown).

Generally, it has been postulated that the drugs that reduce the availability of catecholamines in the presynaptic neuron or that block the action of the catecholamines on the postsynaptic receptor attenuate the behavioral effects, such as hyperactivity and reinforcing effects, of stimulants in the monkey and rat (Wilson and Schuster, 1972; Pickens *et al.*, 1968). In the previous reports, GTS inhibited dopaminergic hyperactivity induced by morphine, cocaine and methamphetamine at the presynaptic dopamine receptor and also exhibited an antidopaminergic property at the postsynaptic dopamine receptor by inhibiting apomorphine-induced climbing behavior (Kim *et al.*, 1990; 1995; 1996). These findings suggest that GTS may inhibit dopamine biosynthesis in the dopaminergic neuron.

In the present study, GTS inhibited bovine adrenal TH activity. Therefore, the inhibitiory effect of GTS on TH activity might partially contribute to the decrease in dopamine content, and thus might be responsible for the antidopaminergic action of GTS.

Table I. Effect of ginseng total saponin (GTS) on the bovine adrenal TH activity

Drug	TH activity (nmol/min/mg protein) (% of Control)
Control	1.10±0.04 (100.0)
GTS (μg/ml)	
20	0.77±0.06 (69.8)
40	0.63±0.08 (57.6)*
80	0.53±0.06 (48.5)*
100	0.49±0.05 (44.7)**

The control of TH activity, 1.10 nmol/min/mg protein, was taken as 100%. The data were expressed as mean±SEM for 4 experiments. Significantly different from the control value: *P <0.05 and **P<0.01 (Student's t-test).

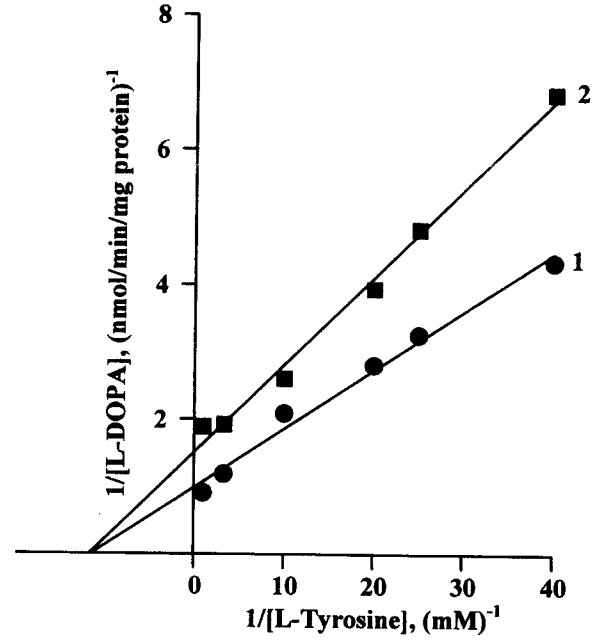

Fig. 1. Inhibition of bovine adrenal TH by GTS added in the enzyme reaction mixture. The data were plotted by linear regression analysis. GTS concentration: 1, 0; 2, 80 μg/ml.

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