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Effect of Ginseng Extract on Ribonucleic Acid and Protein Synthesis in Rat Kidney

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Administration of the extract from the roots of *Panax ginseng* C.A. Meyer increased the incorporation rate of labeled precursor into rat renal nuclear RNA, this stimulating effect was dose dependent, and the maximum activity was observed at 8 hr after the treatment. The sequential cytoplasmic RNA synthesis was stimulated maximally at 10 hr after the administration. The stimulating effect of the ginseng extract on RNA synthesis resulted in an increased incorporation of labeled leucine into rat renal protein and the maximum activity was observed at 12 hr after the intraperitoneal administration.

Keywords——*Panax ginseng* C.A. Meyer; kidney; RNA; protein; nucleus

We have already reported that administration of the extract from the roots of *Panax ginseng* C. A. Meyer stimulated the incorporation rate of labeled precursors into nuclear and cytoplasmic ribonucleic acid(RNA) in rat liver, and consequently resulted in increased incorporation of radioactive amino acid into rat serum protein.[2] Ginseng radix ("Ninjin" in Japanese) is a crude tonic drug and an important component of prescription in "Kanpo" medicine. It was ascertained that one of the tonic effects of ginseng radix might be an increase of serum protein synthesis. Further investigation was undertaken to determine the distribution of tritium labeled ginseng extract among any organs of the rat after intraperitoneal injection. The radioactivity was detected mainly in the adrenal gland, liver, and kidney. In addition, it became apparent that the radioisotope was associated with these tissues,[3] this fact suggested that the ginseng extract might combine with the rat kidney. In the present work, an attempt was made to clarify the effect of ginseng extract on rat kidney.

Material and Method

Animal——Male Wistar rats weighing *ca.* 100 g were employed throughout all the experiment. The animals were housed in air-conditioned quaters kept at 25° and 60% relative humidity. Rats were fed on laboratory pellet chow (CLEA Japan Inc., Tokyo), and tap water freely. Animals were fasted for 16—18 hr before the experiment.

Chemicals——Orotic acid [5-^{3}H] (17.8 Ci/mM) and L-leucine [4,5-^{3}H] (53 Ci/mM) were purchased from New England Nuclear Corp., U.S.A. All other chemicals used in this study were of analytical or reagent grade and were used without further purification.

Extraction and Partial Purification of Ginseng Radix——Fraction 4 was prepared from the extract of roots of *Panax ginseng* C.A. Meyer produced in Kumsan, Korea, as described previously.[4] Preliminary chemical examinations showed that the fraction 4 was positive to the Liebermann–Burchard reaction and contained 70—75% of hexose which was determined by the Tsugita–Akabori method with glucose as a standard.[5] In addition, this fraction contained 91.7% saponin as determined by the vanillin-H_2SO_4 reaction.[6]

1) Location: *3190 Gofuku, Toyama, 930, Japan.*
2) *a*) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, *Chem. Pharm. Bull.* (Tokyo), **19**, 453 (1971); *b*) H. Oura, S. Hiai, and H. Seno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1598 (1971); *c*) S. Hiai, H. Oura, K. Tsukada, and Y. Hirai, *Chem. Pharm. Bull.* (Tokyo), **19**, 1656 (1971); *d*) H. Oura, S. Nakashima, and H. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 219 (1972); *e*) H. Oura, S. Nakashima, K. Tsukada, and Y. Ohta, *Chem. Pharm. Bull.* (Tokyo), **20**, 980 (1972).
3) H. Oura, S. Hiai, and Y. Hirai, unpublished data.
4) H. Oura, S. Hiai, Y. Odaka, and T. Yokozawa, *J. Biochem.* (Tokyo), **77**, 1057 (1975).
5) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
6) S. Hiai, H. Oura, Y. Odaka, and T. Nakajima, *Planta Med.*, **28**, 363 (1975).

Renal Nuclear and Cytoplasmic RNA Labeling *in Vivo*——Fraction 4 (0.5 ml of saline solution) was administered intraperitoneally to rats and control rats were treated with an equal volume of saline. At designated interval after the treatment, ^{3}H-orotic acid (7 or 20 μCi/rat) was given to animals intraperitoneally 20 min or 1.5 hr before killing them by decapitation.

Separation of Renal Nuclei and Cytoplasmic Ribosomes——Rapidly excised kidneys from three rats were pooled for experimental or control group. The purified renal nuclei were prepared by the same procedure for the separation of hepatic nuclei as described in our previous paper.[2a] The purified renal cytoplasmic ribosomes were prepared by the method of Blobel and Potter for the isolation of rat hepatic ribosomes.[7]

Assay of Specific Radioactivity of Nuclear and Cytoplasmic RNA——The nuclear and cytoplasmic RNA were prepared by the modification of Schmidt–Thannhauser method.[8] To the residue of extracted RNA 0.3 N KOH was added and the sample was hydrolyzed at 37° for 18 hr. The hydrolysate was neutralized with chilled $HClO_4$ and centrifuged. The supernatant fluid was used for the determination of RNA by the orcinol reaction modified by Mejbaum.[9] The radioactivity was determined with the aliquots of this supernatant in the Bray's liquid scintillation mixture,[10] with Packard Tri-Carb liquid scintillation spectrometer, Model 3003, or Aloka liquid scintillation spectrometer, Model LSC-653. Specific radioactivity was estimated as a value of cpm/mg RNA or dpm/mg RNA from the radioassay and colorimetric determination.

Renal Protein Labeling *in Vivo*——Fraction 4 (5 mg/0.5 ml of saline) was administered intraperitoneally to rats, while control rats were treated with an equal volume of saline. At designated interval after the treatment, ^{3}H-leucine (5 μCi/rat) was given intraperitoneally to animals 20 min before killing them by decapitation.

Preparation of Renal Homogenate, and Post-mitochondrial and Mitochondrial Protein——Rapidly excised kidneys from three rats were pooled for experimental or control group, and homogenized with 5 volumes of chilled 0.25 M sucrose in TKM buffer (50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, pH 7.5) with a Potter-Elvehjem device at 10 strokes of motor-driven Teflon pestle, in a cold room at 4°. The homogenate was filtered through 4 layers of gauze. The filtered fraction was taken as the total protein. The filtered homogenate was centrifuged at 25000×*g* for 10 min. The supernatant and precipitate fractions were taken as post-mitochondrial and mitochondrial protein, respectively. To each fraction 10% trichloroacetic acid (TCA) was added to a final 5% concentration. The TCA-insoluble residue was washed three times with 5% TCA. After extraction of nucleic acid with 5% TCA for 20 min at 90°, the residue was washed once with 5% TCA, twice with ethanol, and once with ether. The prepared protein fractions were dried and dissolved in 1 N NaOH.

Assay of Specific Radioactivity of Protein in Each Fraction——The solution containing protein was submitted to the determination of protein by the Lowry method,[11] with bovine serum protein as a standard. The radioactivity was determined in a liquid scintillation mixture, which contained 33% Triton X-100, 0.4% 2,5-diphenyloxazole, and 0.01% 1,4-bis[2-(5-phenyloxazole)]benzene in toluene, with Aloka liquid scintillation spectrometer, Model LSC-653. The specific radioactivity was estimated as a value of dpm/10 mg protein from the radioassay and colorimetric determination.

Results

Stimulating Effect of Ginseng Extract on Rat Renal Nuclear RNA Synthesis

Examination was made to see increased incorporation of labeled precursor into rat renal nuclear RNA 8 hr after administration of ginseng fraction 4. The control and experimental

Table I. Increasing Effect of Graded Dose of Fraction 4 Values at 8 hr after the Treatment on Incorporation of ^{3}H-Orotic Acid into Rat Renal Nuclear RNA

Material	Dose (mg)	Specific radioactivity (cpm/mg RNA)	Per cent of control
Control	—	11000	100
Fraction 4	0.05	15600	141
Fraction 4	0.5	17000	155
Fraction 4	1.0	18700	170
Fraction 4	5.0	25400	231

^{3}H-orotic acid (7 μCi/rat) was given to rats 20 min before killing them by decapitation.

7) G. Blobel and V.A. Potter, *J. Mol. Biol.*, **26**, 279 (1967).
8) G. Schmidt and S.J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945).
9) W. Mejbaum, *Z. Phys. Chem.*, **258**, 117 (1939).
10) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
11) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randal, *J. Biol. Chem.*, **193**, 265 (1951).

rats were given ^{3}H-orotic acid (7 μCi/rat) intraperitoneally 20 min before killing them by decapitation. The stimulating effect of ginseng extract was confirmed when a graded does of fraction 4 was administered to rats. As shown in Table I, the incorporation of labeled precursor into nuclear RNA depended on the amount of fraction 4 and the administration of 5 mg increased it 2.3 fold over that of the control level.

Time Course of the Effect of Fraction 4 on Renal Nuclear RNA Synthesis

To obtain further evidence for the effect of fraction 4 on renal nuclear RNA synthesis, a time course of the effect on the incorporation of labeled precursor into nuclear RNA was examined after administration of 5 mg of fraction 4. As shown in Table II, maximum specific radioactivity of renal nuclear RNA was observed at 8 hr after administration. In addition, this response gradually decreased and again approached the control level 24 hr after the treatment.

TABLE II. Time Course of the Effect of Fraction 4 on the Incorporation of ^{3}H-orotic Acid into Rat Renal Nuclear RNA

Time after fraction 4 treatment (hr)	No. of rats	Specific radioactivity (cpm/mg RNA)	Per cent of control
Control	24	12556± 707	100
1	3	12600	101
2	3	15700	125
4	21	19214±1663	153
6	6	23550± 350	188
8	9	25800± 300	206
12	3	15750	125
16	3	15200	121
24	3	13500	108

Control is regarded as 0 hr, though the value is expressed as a mean ± S.E. of data at each period after saline treatment. The values are indicated as specific radioactivity or its mean ± S.E.

Time Course of the Effect of Fraction 4 on Cytoplasmic RNA Synthesis

Whether or not ginseng extract caused a stimulation of renal cytoplasmic RNA synthesis was also examined. The control and experimental rats were given ^{3}H-orotic acid (20 μCi/rat) intraperitoneally 1.5 hr before killing them by decapitation. Table III shows the increasing effect of fraction 4 treatment on the incorporation of labeled precursor into renal cytoplasmic RNA. At 8, 10, 14, and 18 hr after the administration, ginseng extract respectively increased

TABLE III. Time Course of the Effect of Fraction 4 on the Incorporation of ^{3}H-orotic Acid into Rat Renal Cytoplasmic RNA

Time after fraction 4 treatment (hr)	No. of rats	Specific radioactivity (dpm/mg RNA)	Per cent of control
Control[b)]	15	7185± 707	100
8	12	10956±1233[c)]	152
10	6	13038±2289[c)]	181
14	6	12358±1102[c)]	172
18	3	10307	143

a) The values are expressed as specific radioactivity or its mean ± S.E.
b) Control is regarded as 0 hr, though the value is indicated as a mean of the data at 8, 10, and 18 hr after saline treatment.
c) Significantly different from the value in control, $p < 0.05$.

specific radioactivity by 52%, 72%, 81%, and 43% over that of the control. The maximum specific radioactivity of cytoplasmic RNA was observed at 10 hr after the treatment. This fact demonstrated that the stimulated nuclear RNA induced with ginseng extract was transferred to the cytoplasm without accumulation in the nucleus.

Time Course of the Effect of Fraction 4 on Rat Renal Protein Synthesis

Effect of ginseng extract on renal protein synthesis was examined and Table IV shows the increasing effect of the administration of fraction 4 on renal protein synthesis. Obviously, an

Table IV. Time Course of the Effect of Fraction 4 on the Incorporation of ^{3}H-leucine into Rat Renal Protein

Time after fraction 4 treatment (hr)	No. of rats	Specific radioactivity[a] (dpm/10 mg protein)			Per cent of control		
		Total protein (I)	Post-mitochondrial protein (II)	Mitochondrial protein (III)	I	II	III
Control[b]	18	2765±393	3242±471	2547±347	100	100	100
12	12	3650±354[c]	4777±371[d]	3382±371[c]	132	147	133
16	6	3244±123[e]	3762± 41[e]	2981±103[e]	117	116	117
20	6	2608±199[e]	2998±210[e]	2615±215[e]	94	92	102

a) The values are indicated as a mean ± S.E.
b) Control is regarded as 0 hr, though the value is expressed as a mean ± S.E. of the data at each period after saline treatment.
c) Different from corresponding value in the control, $p < 0.2$.
d) Significantly different from corresponding value in the control, $p < 0.05$.
e) Not significantly different from corresponding value in the control.

increase in specific radioactivities of post-mitochondrial protein and mitochondrial protein over the control level was at 12 hr after the treatment, and stimulation being 47% and 34%, respectively. In addition, this response returned almost to the control level 20 hr after the treatment. As reported previously, the serum protein synthesis was stimulated maximally at 8—12 hr after administration of the ginseng extract.[2e] Although the specific radioactivity of post-mitochondrial protein was affected probably with the contaminated serum protein, whose specific radioactivity was 1587±49 dpm/10 mg protein at 12 hr after the treatment, the contaminated serum protein was less effective on that in present experimental condition. Accordingly, these experimental results support a possibility that the administration of ginseng extract stimulated a net protein synthesis in rat kidney. In the present investigation, increase of RNA induced with ginseng extract resulted in the stimulation of renal protein synthesis.

Discussion

Many hormones, soon after administration, produce in target organs an increase in the specific activity of rapidly labeled nuclear RNA and a stimulation of RNA polymerase activity, and stimulate the synthesis of r-RNA and m-RNA, and increase the content of heavy polysomes in the cytoplasm, whose protein synthesizing activity is increased with newly formed ribosomes and m-RNA resulting from the stimulation of nuclear RNA synthesis.[12]

As reported previously, ginseng extract produced an increase in the incorporation of labeled precursors into rat hepatic nuclear and cytoplasmic RNA,[2a,b] and in DNA-dependent RNA polymerase activity.[2c] Further it was found that the content of heavy polysomes in the

12) J.R. Tata, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 5, Academic Press Inc., New York, 1966, pp. 191—250; J.R. Tata, "Biochemical Actions of Hormones," ed. by G. Litwack, Academic Press Inc., New York, 1970, pp. 89—133.

post-mitochondrial supernatant from liver cells was increased by treatment with ginseng extract.[2d] In addition, there was a spectacular increase in the rough endoplasmic reticulum of hepatocytes and newly produced ribosomes were found to be mainly membrane-bound ribosomes by electron microscopy and from quantitative experimental results, respectively, when ginseng extract was administered orally to rats every day for 4 weeks.[13] Intraperitoneal administration of ginseng extract stimulated rat serum protein synthesis.[2e]

The present work demonstrated that the ginseng extract increased rat renal nuclear and cytoplasmic RNA synthesis, and consequently resulted in the stimulation of renal protein synthesis. As summarized in Fig. 1, the sequential stimulation of biochemical effects in the renal nucleus, cytoplasm, and protein resulted from a single-dose injection of ginseng extract.

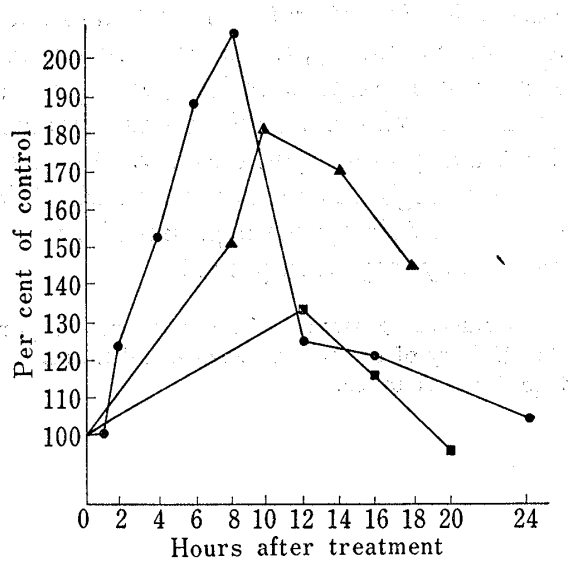

Fig. 1. Summarized Data on the Sequential Stimulation after a Single-dose Administration of Ginseng Extract in Rat Kidney

●, nuclear RNA synthesis; ▲, cytoplasmic RNA synthesis; ■, protein synthesis.
These curves are drawn from data in Tables II, III, and IV.

Williamson[14] found that antinatriuretic activity of aldosterone in rat kidney appeared to be mediated through the induction of RNA synthesis. Forte and Landon[15] reported that aldosterone stimulated the incorporation of labeled precursor into RNA in the kidney of adrenalectomized rat and that maximum stimulated incorporation was observed at 60 min after intravenous treatment, and the incorporation rate again approached that of the control 2—3 hr after the treatment. Avdalovic and Kochakian[16] observed that castration of adult male mice produced a sharp decrease in the RNA polymerase activity of the isolated renal nuclei and that testosterone propionate reversed the effect of castration, whose stimulating effect was evident 2 hr after subcutaneous injection and was dose dependent. On the other hand, it was also found that folic acid and vitamin D metabolites stimulated the RNA synthesis in rat kidney.[17, 18] However, these results are different from our observation that the maximum activity of RNA synthesis was affected at 8 hr after the treatment. Accordingly, it is interesting whether ginseng extract directly stimulates renal nuclear RNA synthesis or the effect of other organs induced by ginseng extract results in an increased synthesis.

Oura, *et al.*[4] reported that seven saponins, which are ginsenoside-Rb_1, -Rb_2, -Rc, -Rc_2, -Rd, -Re, and -Rg_1, were systematically isolated and purified from the biologically active ginseng extract, and that administration of these saponins, except ginsenoside-Rb_1, increased incorporation rate of labeled leucine into mouse serum protein, in which ginsenoside-Rd had the most effective biological activity and the incorporation rate was directly proportional to its dose, and that the increased specific radioactivity in mouse serum protein was not due to a decrease in the pool size of free amino acid in the liver. In the present investigation, ginseng extract stimulated the renal nuclear and cytoplasmic RNA synthesis and resulted in the stimulation of renal protein synthesis, for it was considered that the increase in incorporation of

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14) H.E. Williamson, *Biochem. Pharmacol.*, **12**, 1449 (1963).
15) L. Forte and E.J. Landon, *Biochim. Biophys. Acta*, **157**, 303 (1968).
16) N. Avdalovic and C.D. Kochakian, *Biochim. Biophys. Acta*, **182**, 382 (1969).
17) W. Brade, A. Jarck, and J. Vetter, *Biochem. Pharmacol.*, **21**, 1431 (1972).
18) T.C. Chen and H.F. Deluca, *Arch. Biochem. Biophys.*, **156**, 321 (1973).

labeled leucine into rat renal protein might not be due to a decrease in the pool size of free amino acid in the kidney, as observed in mouse serum protein synthesis. These phenomena were similar to the response induced with ginseng extract in rat liver, though the mode of appearance of these maximum activities was different in each organ. Therefore, it is suggested that the RNA and protein syntheses in rat kidney may be stimulated by these saponins.

Although it is not established what kind of renal function is affected by the stimulation of specific protein synthesis produced by the ginseng extract, it is considered that functions of the kidney may be improved.

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