EFFECT OF GINSENG SAPONIN ON PROTEIN SYNTHESIS IN HEART MUSCLE

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1. Perfusion of hearts: Hearts were removed from male rabbits (about 3kg) which were heparinized (sodium heparin, 10 mg/kg, intravenously) and anesthetized with sodium pentobarbital (30 mg/kg, intravenously). Hearts were cooled in ice-cold 0.15 M NaCl solution for 15–20 sec. Perfusion was begun by a modified Langendorff technique using Krebs-Henseleit bicarbonate buffer* gassed with 95 % O_2–5 % CO_2 at 37° C and containing 15 mM glucose and normal plasma levels of all amino acids except phenylalamine. After 10 min. of preliminary perfusion, 200 ml buffer containing 0.08 mM ^{14}C-phenylalamine (0.3×10^6 dpm per μmol) was recirculated for 60 min. at 60 mmHg perfusion pressure. The first 20 ml of radioactive buffer to pass through the heart was discarded to minimize dilution of phenylalamine specific activity.

At the end of perfusion, hearts were cut from cannula into beakers containing 0.15 M NaCl (2–4° C) and prepared as described below.

2. Estimation of incorporated radioactivity into protein: Immediately after perfusion, the heart was opened and a section of 200–250 mg of the lower wall of left ventricle was rinsed rapidly in Krebs-Henseleit bicarbonate buffer, blotted on filter paper and weighed on torsion balance. After homogenizing in 5 % PCA (3–4 ml), the precipitate was spun down (2500 rpm) and washed once with 5 % PCA then once more centrifuged. Excess of non-labelled amino acid was added. The precipitate was then heated for 15 min. in a boiling water bath and washed twice with same solution.

The insoluble material was extracted with 4 ml of 0.3 N NaOH and precipitated with 1 ml of 6 N HCl. The precipitate was then washed twice with 1 % potassium acetate in ethanol and thereafter 3 times with mixture of ether, ethanol, chloroform (1:3:2) and finally twice in ether and dried. The purified protein was dissolved in 2 ml of 1 N NaOH and was heated in a boiling water bath for 25 min. The clear protein solution was obtained. Samples were taken for the protein determination according to the method of Lowry *et al.* For radioactivity measurement, 50 μl of the same clear protein solution was added to 10 ml of Aquasol (New England Nuclear) and counting was carried out by Packard liquid scintillation spectrometer, Model 3375.

3. Results: The rate of incorporation of ^{14}C-phenylalamine into protein of perfused rabbit hearts was 369.6 $\pm$ 35.2 cpm mg protein per hour with the specificity used here but in the hearts perfused with ginseng saponin (10 mg/100 ml perfusion fluid), the rate was 280.7 $\pm$ 12.4 cpm mg protein per hour. The coronary flow of ginseng-perfused hearts was somewhat increased during the perfusion with ginseng extract. Therefore the de-

crease in the protein synthesis can not be due to the decreased coronary blood flow. However the cardiac rate is decreased during the perfusion with ginseng extract and this may have had some effect on the protein synthesis. At present, work is progressing to elucidate the possible mechanism of the inhibitory effect of ginseng on the synthesis of protein in heart muscle.

Note: Ginseng saponin was obtained from Office of Monopoly, Republic of Korea.