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The Effect of Ginseng on Heart Contraction and Sarcoplasmic Reticulum Function(II)

The Effect of Ginseng on $^{45}Ca^{2+}$ Uptake by Sarcoplasmic Reticulum Fragments of Rat Heart

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Abstract □ It was reported from our laboratory that the rate of deterioration of the force of contraction was slower in heart from *Panax ginseng* extract treated rats. Present investigation was designed to elucidate the mechanism of the slow deterioration of contractility of ginseng treated hearts. Therefore, $^{45}Ca^{2+}$ uptake by sarcoplasmic reticulum (SR) isolated from ginseng treated rats and control rats was studied. Rats weighing 150-250g were administered orally with ginseng ethanol extract (100mg/kg) for 10 days. Cardiac SR was isolated by differential centrifugation and $^{45}Ca^{2+}$ uptake was assessed by the Millipore method. Freshly isolated SR from treated as well as control animals did not show any differences, but after incubation for 30 and 60 min at 37°C, $^{45}Ca^{2+}$ uptake of control animal SR was found to be greatly depressed. The SR of treated animal possessed a greater degree of resistance to incubation. Thus it can be concluded that ginseng may have an ability to sustain the normal function of the heart by sustaining-Ca accumulation by SR involved with the excitationcontraction coupling processes.

Keywords □ Ginseng, Heart, Ca^{2+} uptake, Sarcoplasmic reticulum, Rat.

Panax ginseng has been known as a tonic agent in traditional oriental medicine and believed to play an imporant role maintaining the homeostatic mechanism under the stressful condions[1]. It is stated in "Chinese Medicinal Herbs" that ginseng strengthens the function of the heart[2]. We have recently demonstrated a slower rate of deterioration of the force of contraction in heart from ginseng extract treated rats than in untreated rats[3~4]. Present investigation was designed to elucidate the mechanism of the slow deterioration of contractility of ginseng treated hearts. It is well established that contractile force of the heart is regulated by the available intracellular calcium ion concentration, which is profoundly influenced by sarcoplasmic reticulum in the hearts[5~6]. Therefore, we have characterized the calcium uptake of sarcoplasmic reticulum from ginseng treated and control rat hearts.

EXPERIMENTAL METHODS

Ginseng Ethanol Extract

The red ginseng(*Panax ginseng* C.A. Meyer) proffered from Korea Ginseng Research Institute was pulverized and extracted with 70% ethanol at 70°C. The extract was concentrated in rotary vacuum evaporator to make it contain 40% water. Ginseng ethanol extract was dissolved in 0.9% saline solution for oral treatment.

Animals

Male Sprague-Dawley rats weighing 150 to 200g were used and were administered orally with either ginseng ethanol extract (100mg/kg/

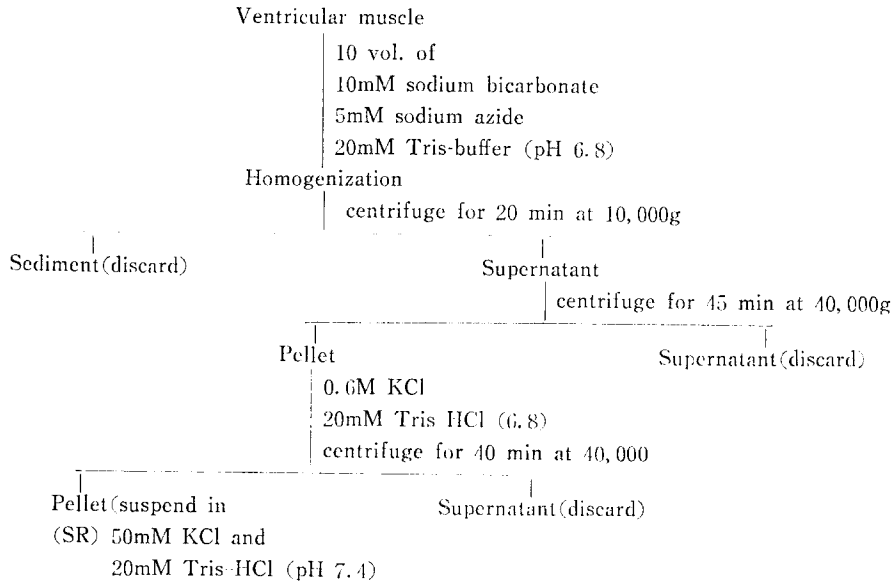

Fig. 1: Flow sheet diagram for preparations of sarcoplasmic reticulum.

day) or an equal volume of normal saline solution for 10 days.

Preparation of Membrane Vesicles from Cardiac Muscle

The fragments of cardiac sarcoplasmic reticulum were prepared from rat hearts by a modification of the method described by Harigaya and Schwartz[7] and Sulakhe and Dhalla[8] (Fig. 1).

The control and ginseng treated rats were killed by a blow on the head and the heart was immediately removed and perfused with ice cold isotonic saline solution through the aorta to eliminate the blood. After removal of fatty and connective tissue, ventricular muscle tissue was cut into small pieces with a scissor and homogenized with 10 volumes of the homogenizing medium containing 10mM sodium bicarbonate, 5mM sodium-azide and 15mM Tris-HCl (pH 6.8). The suspension was homogenized with glass homogenizer with a Teflon pestle for 15 seconds, three times with a rest interval of about 15 to 20 seconds. The homogenate was centrifuged at 10,000×g for 20 minutes. The residue was discarded to remove the cell debris, nuclei, myofibrils and mitochondria and the supernatant fluid was filtered through four layers of gauze and centrifuged at 10,000×g for 20 minutes, yielding a second supernatant fraction which was centrifuged at 40,000×g for 45 minutes. The sediment was suspended in a centrifuging tube with a Teflon pestle in 4 volumes of 0.6M KCl containing 20mM Tris-HCl buffer (pH6.8). The resulting suspension was then centrifuged at 40,000×g for 40 minutes to remove solubilized actomyosin. The harvested pellet was suspended in 0.8ml of 20mM Tris-HCl buffer (pH7.4) containing 50mM KCl.

This suspension is termed SR fraction. The entire procedure was carried out in a cold room at 0~4°C and on the day of preparation.

Protein Concentration Assay

Protein concentration were determined by the method of Lowry using bovine serum albumin as a standard[9].

Assay of $^{45}Ca^{2+}$ Uptake by Sarcoplasmic Reticulum

Millipore filter method—Calcium uptake by SR

fragment from both control and ginseng treated rats was measured. The reaction mixture consisted of 100mM KCl, 10mM $MgCl_2$, 5mM potassium oxalate, 20mM Tris-ATP and 0.2~0.3 mg/ml membrane-protein in a total volume of 6ml. The reaction was started by the addition of ATP and was carried out for 20 minutes at 37°C with constant stirring.

In both group, the test group was subdivided into three groups according to preincubation times, i.e. incubation time before adding the ATP. The preincubation time were 1 minute, 30 minutes, 60 minutes, respectively. $^{45}Ca^{2+}$ uptake was measured by the millipore technique employing $^{45}Ca^{2+}$. Each of aliquot (1ml) removed at selected time after the start of the reaction was filtered through a swinney filter holder containing a 13mm Millipore filter (HA 0.45) connected via flask to a vacuum pump.

The filtration was usually completed in 2~3 seconds. Calcium uptake was estimated from a radioactivity of the filtrate and filter. Both were suspended in a 10ml of liquid scintillation cocktail solution containing naphthalene, PPO (2.5-diphenyloxazole), POPOP (1.4-bis 2-(5-phenyloxazolyl) benzene phenyl-oxazolyl phenyl-) ethylene glycol, absolute methanol, in dioxane, and counted in a Packard liquid Scintillation Spectrometer. $^{45}Ca^{2+}$ uptake was calculated by following formula.

$$Ca^{2+} \times \frac{\text{Exp. CPM}}{\text{control CPM}} \times \frac{6}{1000} \times \frac{1}{\text{total protein in total reaction mixture (mg)}} = \text{n mole/mg protein}$$

RESULTS

The uptake of $^{45}Ca^{2+}$ by sarcoplasmic reticulum is shown in Fig. 2.

Preincubation for 1 Minute

Fig. 2 shows the calcium uptake by the SR of control and treated hearts when ATP was added after the preincubation for 1 minute. Although calcium uptake by SR of the ginseng treated heart at 20min of incubation was increased by about 10.8% of the control, the values were not significantly different ($P<0.05$) from control rat hearts.

Preincubation for 30 minutes

When ATP was added after the preincubation for 30 minutes, the control calcium uptake values are 29.3±6.4, 36.5±7.2, 46.3±5.6 and

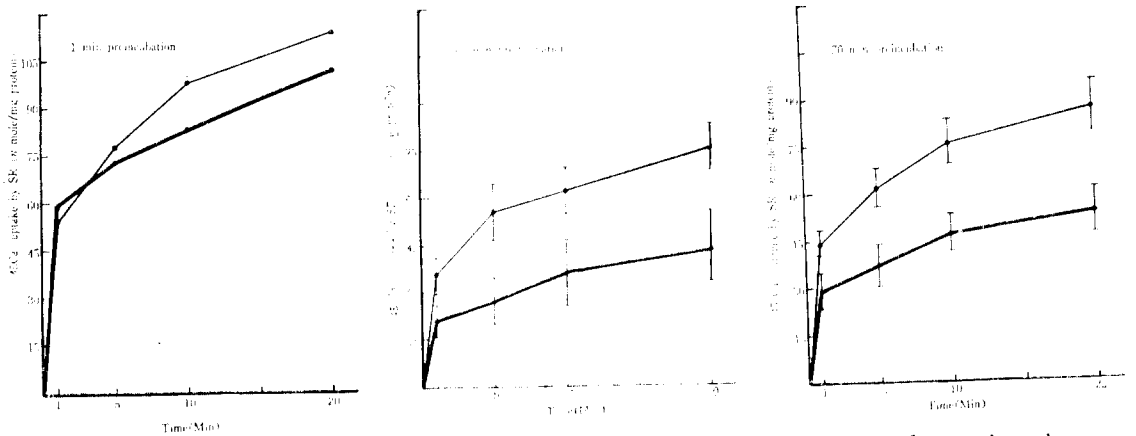

Fig. 2: $^{45}Ca^{2+}$ uptake by SR obtained from ginseng total extract treated rat heart and control rat heart. (incubated for 1, 30 and 60 minute at 37°C). Ginseng treated(—·—). Control(···)

52.8±8.0 nmole/mg protein at selected incubation time, 1, 5, 10, and 20 min, respectively. The calcium uptake value of treated hearts at 1, 5, 10, and 20 min incubation was higher about 40~60% than that of the control. The value of treated heart were 44.3±4.4, 61.7±6.0, 76.0±7.2 and 86.0±8.8 nmole/mg protein at selected time, respectively, (Fig. 2).

Preincubation for 60 Minutes

When ATP was added after the preincubation for 60 minutes, the control calcium uptake values were 21.7±5.2, 26.7±8.0, 35.7±10.8 and 43.0±12.6 nmole/mg protein at selected incubation time; 1, 5, 10 and 20min, respectively. The calcium uptake value of treated hearts at 1, 5, 10, and 20min incubation was higher about 60~80% than that of the control. The value of treated hearts were 35.0±6.0, 54.3±9.2, 61.7±7.8 aud 75.0±7.2 nmole/mg protein at selected time, respectively, (Fig. 2). As a result, preincubation of sarcoplasmic reticulum for 30 and 60 min at 37°C results in the decrease in the $^{45}Ca^{2+}$ uptake in the control rat compared to that in the treated group and the sarcoplasmic reticulum of ginseng treated rats showed a greater degree of resistance in the decrease of calcium uptake.

DISCUSSION

Previous results showed that orally administered ginseng ethanol extract, ginseng total saponin and ginsenoside Rb1 prevent the deterioration of contractile force of the hearts. This seems to indicate that ginseng provides an ability for the heart to maintain or strengthen its celluar integrity. It is well established that contractile force of the heart is regulated by the concentration of intracelluar calcium ions, which is in turn profoundly influenced by sarcoplasmic reticulum (SR) in the heart. Therefore, it is expected that SR obtained from the weakened heart accumulates less amount of calcium than normal heart does. This conclusion is consistent with the results obtained from our experiments.

With the SR freshly isolated, there was no significant differences in the Ca^{2+} uptake of both contol and ginseng treated rats. However, with the SR deteriorated by incubation at 37°C for 30min and 60min, a greater degree of deterioration in the Ca^{2+} uptake was observed in the control heart than SR from the treated rats. Ginseng extracts were found to sustain the capability of SR to accumulate calcium. Thus it is concluded that Ginseng may have an ability to sustain the normal function of the heart. The celluar mechanism of this protective effect appears to reside in the ability of ginseng to sustain Ca^{2+} accumulation by SR involved with the excitation contraction coupling processes.

ACKNOWLEDGEMENT

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