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Short Communication

Propyl Gallate, a Strong Antioxidant, Increases the Ca^{2+} Sensitivity of Cardiac Myofilament

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Abstract. Ca^{2+} sensitizers are cardiotonic agents that directly increase the Ca^{2+} sensitivity of cardiac myofilament. To find a novel Ca^{2+} sensitizer, we have screened a group of phenolic compounds by examining their effects on the Ca^{2+}-dependent force generation in cardiac muscle fibers. We found that propyl gallate, a strong antioxidant, increased the Ca^{2+} sensitivity of cardiac myofilament in a dose-dependent and reversible manner. The present study indicates that propyl gallate is a novel type of Ca^{2+} sensitizer with antioxidant activity, which might be more beneficial for the treatment of congestive heart failure associated with oxidative stress than existing Ca^{2+} sensitizers.

Keywords: propyl gallate, Ca^{2+} sensitizer, antioxidant

Ca^{2+} sensitizers are cardiotonic agents that elicit a positive inotropic effect via an increase in the sensitivity of cardiac myofilaments to Ca^{2+} (1, 2). These agents have advantages of avoiding Ca^{2+} overloading and oxidative stress, which could be caused by other cardiotonic agents such as β-receptor agonists through increased myocardial oxygen consumption, in the treatment of congestive heart failure (CHF) (3, 4). Ca^{2+} sensitizers have also been shown to be beneficial for the treatment of dilated cardiomyopathy associated with a decrease in the myofilament Ca^{2+} sensitivity (5). Oxidative stress is involved in myocardial ischemia/reperfusion injury, and antioxidants have been shown to attenuate cardiac dysfunction during post ischemic myocardial stunning (6) and oxidative stress–induced myocardial injury (7) by preventing the accumulation of oxygen free radicals. In this study, we screened a group of phenolic compounds to find a novel type of Ca^{2+} sensitizer.

Membrane permeabilized (skinned) cardiac muscle fibers were prepared from the left ventricular trabeculae of young male albino rabbits (2 – 2.5 kg), and force measurements were performed as described previously (8). Briefly, small bundles (0.5 – 1-mm-wide and 5 – 7-mm-long) of trabeculae tied to glass capillary tubes were skinned with relaxing solution containing 50% glycerol. A small fiber (about 200 μm in diameter) dissected from the stock-skinned trabeculae was mounted in a thermostatically controlled chamber with a capacity of 0.2 ml. The fiber length between hooks was about 1 mm, and the resting sarcomere length was set to 2.3 μm by using laser diffraction. The force generated by skinned muscle fibers was measured at 25°C with a strain gauge (UL-2GR; Minebea, Nagano). The relaxing solution consisted of 50 mM MOPS/KOH (pH 7.0), 100 mM KCl, 6 mM MgCl2, 5 mM ATP, 4 mM EGTA, 0.5 mM DTT, 10 mM creatinine phosphate, and 35 units/ml creatine kinase.

Trolox (water-soluble derivative of tocopherol) was purchased from Sigma (St. Louis, MO, USA). Curcumin, quercetin, chrologenate, gallate monohydrate, dodecyl gallate, and propyl gallate were purchased from Wako Pure Chemical Industries (Osaka). All phenolic compounds were dissolved in dimethylsulfoxide (DMSO) and used at the final concentration of 0.1% DMSO.

Antioxidant activities of phenolic compounds were

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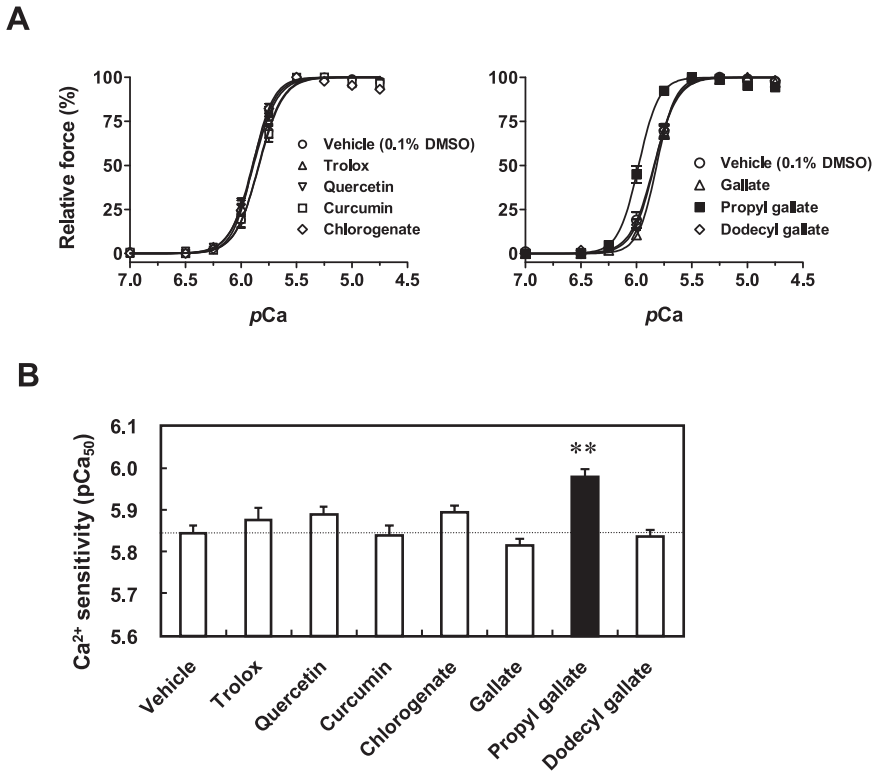

Fig. 1. Effects of phenolic compounds on the force generation in skinned cardiac muscle fibers. A: Force–*p*Ca relationships determined in the presence of 100 μM phenolic compounds. B: Effects of phenolic compounds (100 μM) on the Ca^{2+} sensitivity (pCa_{50}) of force generation. The force–*p*Ca relationship was determined only once for a given muscle fiber by cumulatively increasing [Ca^{2+}] from *p*Ca7 to *p*Ca4.75 in the presence of phenolic compound or vehicle only without pretreatment. Forces were normalized to the maximum force developed by each fiber. Data represent the means ± S.E.M. for 5 – 8 muscle fibers. $^{**}P<0.01$ vs. vehicle (Student's *t*-test).

evaluated by their effects of scavenging stable free radical, 1,1-diphenyl-2-picryl hydrazyl (DPPH), according to the method described by Shirwaikar et al. (9). Briefly, the reaction mixtures containing 10 μM DPPH and various concentrations of phenolic compounds were incubated for 10 min at room temperature in the dark, and then the antioxidant activity was calculated as a percent reduction of the light absorbance of DPPH at 517 nm.

Effects of phenolic antioxidant compounds, trolox, curcumin, quercetin, chrologenate, gallate, dodecyl gallate, and propyl gallate, were examined at 100 μM on the Ca^{2+}-dependent force generation in skinned cardiac muscle fibers (Fig. 1). Propyl gallate was found to shift the force–pCa relationship leftward with a significant increase in the half-maximally activating pCa (pCa_{50}, an index of Ca^{2+} sensitivity), while the other compounds had no significant effects on the force–pCa relationship.

Propyl gallate increased the Ca^{2+} sensitivity of force generation in skinned cardiac muscle fibers in a dose-dependent manner (Fig. 2: A and B), with statistically significant increase in Ca^{2+} sensitivity being detected above 100 μM (Fig. 2C). The Ca^{2+}-sensitizing effect of propyl gallate was reversible and lost immediately after washout (data not shown), and its potency appears to be lower than that of the commercially launched Ca^{2+}-sensitizer pimobendan (cf. supplementary data in ref. 5). All the phenolic antioxidant compounds examined had no significant effects on the maximum force and the slope of force–pCa relationship (i.e., Hill coefficient) in skinned cardiac muscle fibers (data not shown). Propyl gallate had a strong antioxidant activity, which was similar to those of other phenolic compounds, trolox, curcumin, and gallate, when evaluated by their free radical (DPPH) scavenging effects (Fig. 3).

Free radical injury is involved in the pathology of congestive heart failure (CHF); CHF patients have an increased level of plasma lipid peroxide, a marker of oxidative stress, and a decreased activity of glutathione peroxidase, an antioxidant enzyme. Antioxidant supplementation has been shown to improve the myocardial function and survival of CHF patients (10). Clinical therapeutic trials have shown that carvedilol, a nonselective β-adrenergic receptor antagonist with antioxidant activity, reduces the mortality among patients with severe heart failure (11), whereas bucindolol, a nonselective β-adrenergic receptor antagonist with no antioxidant activity, has no favorable effects (12). Propyl gallate is a phenolic antioxidant compound, and its chronic oral administration has been reported to offer significant protection against myocardial oxidative stress–induced injury (7). The present study revealed that this compound also has a Ca^{2+}-sensitizing effect on the cardiac

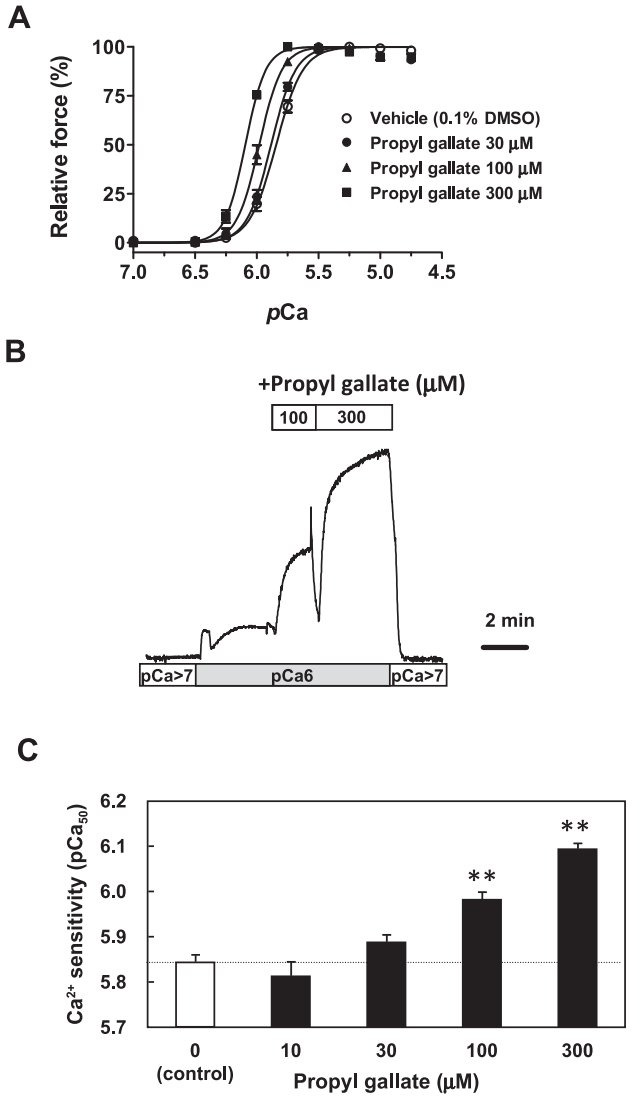

Fig. 2. Effects of propyl gallate on the force generation in skinned cardiac muscle fibers. A: Force–*p*Ca relationships determined in the presence of 0, 10, 30, and 100 μM propyl gallate. B: Force recording showing dose-dependent Ca^{2+}-sensitizing effects of propyl gallate. C: Dose-dependent effects of propyl gallate on the Ca^{2+} sensitivity (pCa_{50}) of force generation. The force–*p*Ca relationship was determined only once for a given muscle fiber by cumulatively increasing [Ca^{2+}] from *p*Ca7 to *p*Ca4.75 in the presence of propyl gallate or vehicle only without pretreatment. Forces were normalized to the maximum force developed by each fiber. Data represent the means ± S.E.M. for 5 – 8 muscle fibers. Statistical significance was determined by ANOVA followed by the post hoc Dunnett's multiple comparison test. ***P*<0.01 vs. control.

myofilament, through which an inotropic effect would be exerted on the heart. Propyl gallate, a Ca^{2+} sensitizer with strong antioxidant activity, thus might be more beneficial for the treatment of CHF than existing Ca^{2+} sensitizers with no antioxidant activities. Further studies would be required to test this possibility.

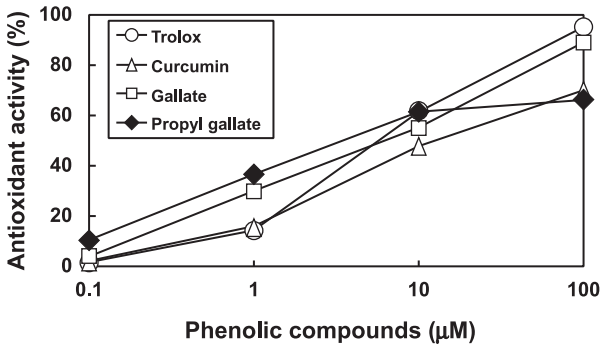

Fig. 3. Antioxidant activities of phenolic compounds. EC_{50} values of free radical (DPPH)-scavenging effects of trolox, curcumin, gallate, and propyl gallate were 8.4, 10.4, 9.0, and 1.3 μM, respectively. Data represent the means ± S.E.M. of 3 determinations.

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