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Short communication

Modulation of actomyosin ATPase by goniodomin A differs in types of cardiac myosin

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Abstract

Goniodomin A causes the conformational change of actin to modify actomyosin ATPase activity [Furukawa, K.-I., Sakai, K., Watanabe, S., Maruyama, K., Murakami, M., Yamaguchi, K., Ohizumi, Y., 1993. Goniodomin A induces modulation of actomyosin ATPase activity mediated through conformational change of actin. J. Biol. Chem. 268, 26026–26031]. Goniodomin A inhibited the ATPase activities of atrial myofibrils, myosin B and reconstituted actomyosin in a concentration-dependent manner. Interestingly, these ATPase activities of ventricular muscle were enhanced by goniodomin A (3×10^{-8}–3×10^{-7} M), but were decreased when the concentration was further raised. The stimulatory effect of goniodomin A was significantly inhibited by troponin–tropomyosin complex. These results suggest that goniodomin A affects actin to modify cardiac actomyosin ATPase activity, and that this modulation differs in types of cardiac myosin. © 1998 Elsevier Science B.V.

Keywords: Goniodomin A; Actomyosin ATPase; Ventricle; Atrium; Troponin–tropomyosin complex

1. Introduction

The force of muscle contraction is produced by the interaction between myosin and actin molecules in a process that involves cross-bridge cycling coupled with the hydrolysis of ATP. It has been revealed that mammalian cardiac muscle cells contain at least two isozymes of myosin heavy chains, α- and β-myosin heavy chains (Kurabayashi et al., 1988). The details of molecular mechanisms of relaxation–contraction coupling of cardiac muscles are unclear.

In the course of our survey on biologically active substances from marine sources, much attention has been given to compounds affecting the contractile apparatus. Recently, we have isolated actomyosin modulators, such as purealin (Nakamura et al., 1987) and xestoquinone (Sakamoto et al., 1995). Furthermore, goniodomin A (Murakami et al., 1988) has been shown to modulate the skeletal actomyosin ATPase activity mediated through conformational change of actin (Furukawa et al., 1993). However, the effect of goniodomin A on cardiac actomyosin ATPase has not been reported yet. Here, we present the first report indicating that goniodomin A induces largely different modulation of actomyosin ATPase activity between ventricular and atrial muscles.

2. Materials and methods

2.1. Materials

Goniodomin A was purified from a marine dinoflagellate *Goniodoma pseudogoniaulax*, previously reported (Murakami et al., 1988). The grade of purity of goniodomin A was confirmed by using nuclea magnetic resonance analysis. Myofibrils, myosin B and myosin were prepared from cardiac muscle of male guinea pigs as described by Perry and Corsi (1958), Szent-Györgyi (1951)

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and Mueller et al. (1964), respectively. Actin, troponin and tropomyosin were prepared from rabbit skeletal muscle as described by Mommaerts (1951), Kohama (1979) and Ebashi et al. (1971), respectively. Troponin–tropomyosin-free myosin B was prepared as described by Perry et al. (1966).

2.2. Methods

The reaction mixture for each ATPase was as follows (Ojima and Nishita, 1988; Kitada et al., 1989): 0.5 mg/ml myofibrils, 2 mM ATP, 1 mM EGTA, 3 mM $MgCl_2$, 0.49 mM $CaCl_2$, 80 mM KCl and 20 mM imidazol–HCl (pH 7.0) for myofibril ATPase; 0.3 mg/ml myosin B or troponin–tropomyosin-free myosin B, 2 mM ATP, 1 mM EGTA, 2 mM $MgCl_2$, 0.68 mM $CaCl_2$, 50 mM KCl and 20 mM Tris–HCl (pH 6.8) for myosin B or troponin–tropomyosin-free myosin B ATPase; 0.3 mg/ml troponin–tropomyosin-free myosin B, 0.1 mg/ml troponin, 0.1 mg/ml tropomyosin, 2 mM ATP, 1 mM EGTA, 2 mM $MgCl_2$, 0.68 mM $CaCl_2$, 50 mM KCl and 20 mM Tris–HCl (pH 6.8) for the ATPase activity of myosin B reconstituted from troponin–tropomyosin-free myosin B, troponin and tropomyosin; 0.1 mg/ml myosin, 0.1 mg/ml actin, 1 mM ATP, 1 mM EGTA, 2 mM $MgCl_2$, 50 mM KCl and 20 mM Tris–HCl (pH 6.8) for the ATPase activity of actomyosin reconstituted from actin and myosin; 0.2 mg/ml myosin, 0.1 mg/ml actin, 0.1 mg/ml troponin, 0.1 mg/ml tropomyosin, 1 mM ATP, 1 mM EGTA, 2 mM $MgCl_2$, 0.55 mM $CaCl_2$, 50 mM KCl and 20 mM MOPS–Tris (pH 7.0) for the ATPase activity of actomyosin reconstituted from actin, myosin, troponin and tropomyosin; 0.1 mg/ml myosin, 2 mM ATP, 10 mM $CaCl_2$, 500 mM KCl and 50 mM Tris–HCl (pH 6.8) for the Ca^{2+}-ATPase activity of myosin; 0.1 mg/ml myosin, 2 mM ATP, 5 mM $MgCl_2$, 500 mM KCl and 50 mM Tris–HCl (pH 6.8) for the Mg^{2+}-ATPase activity of myosin; 0.1 mg/ml myosin, 2 mM ATP, 5 mM EDTA–Tris, 500 mM KCl and 50 mM Tris–HCl (pH 6.8) for the K^+-EDTA-ATPase activity of myosin. The mixture was preincubated in the absence of goniodomin A and ATP at 30°C for 5 min, followed by the addition of goniodomin A and further preincubation. In the control experiment, dimethyl sulfoxide was added instead of goniodomin A. A final concentration of dimethyl sulfoxide was kept less than 0.5%.

Data were represented as the mean ± S.E., and a statistically significant difference was determined by Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

Goniodomin A caused inhibitory and stimulatory effects on activities of cardiac actomyosin ATPase in myofibrils, myosin B and reconstituted actomyosin. As shown in Fig. 1B, goniodomin A ($> 3 \times 10^{-8}$ M) inhibited atrial myofibril ATPase activity in a concentration-dependent manner. Ventricular myofibril ATPase activity was increased by approximately 20% by goniodomin A at concentration

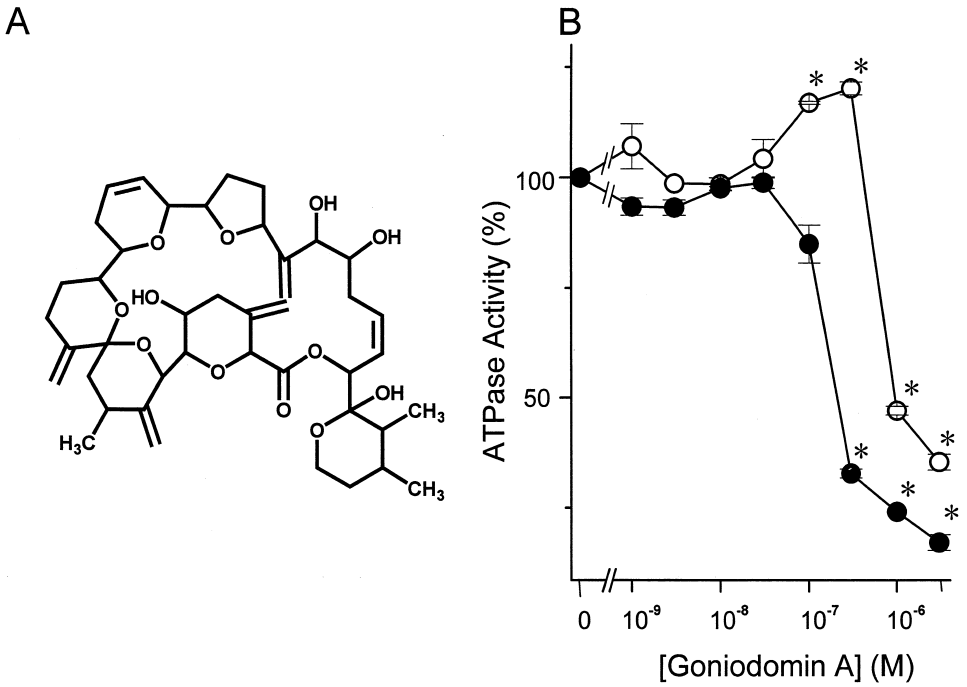

Fig. 1. Chemical structure of goniodomin A (A) and effects of various concentrations of goniodomin A on the actomyosin ATPase activity of ventricular (○) and atrial (●) myofibrils (B). The change was expressed as a percentage against the control activity. Each point represents the mean ± S.E. from three experiments. ∗Significant difference from control.

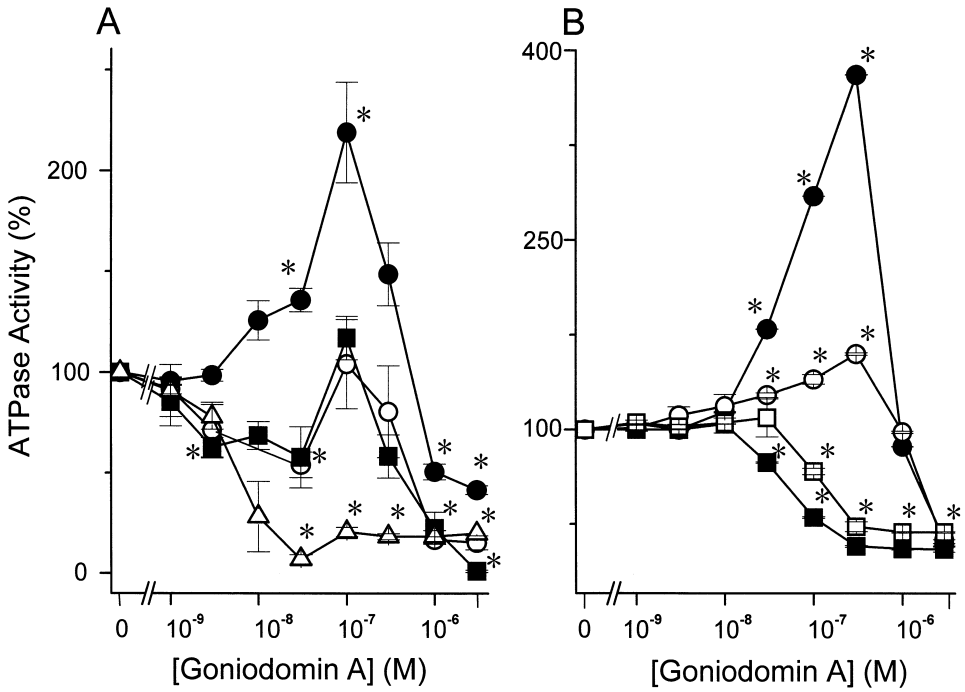

Fig. 2. Effects of various concentrations of goniodomin A on the ATPase activity of cardiac myosin B (A) and reconstituted actomyosin (B). (A) ○: ventricular myosin B, △: atrial myosin B, ■: ventricular troponin–tropomyosin-free myosin B + troponin and tropomyosin, ●: ventricular troponin–tropomyosin-free myosin B. (B) ○: actomyosin reconstituted from ventricular myosin and actin + troponin and tropomyosin, ●: actomyosin reconstituted from ventricular myosin and actin, □: actomyosin reconstituted from atrial myosin and actin + troponin and tropomyosin, ■: actomyosin reconstituted from atrial myosin and actin. The change was expressed as a percentage against the control activity. Each point represents the mean ± S.E. from three experiments. ∗Significant difference from control.

of 3×10^{-7} M, and then decreased by 70% when the concentration of goniodomin A was further raised to 3×10^{-6} M.

Goniodomin A inhibited atrial myosin B ATPase activity at the concentration of 10^{-9} M or more (Fig. 2A). Ventricular myosin B ATPase activity was significantly decreased by goniodomin A in the range of 3×10^{-9}–3×10^{-8} M, and then raised to a control level at the concentration of 10^{-7} M, probably by exertion of the stimulatory effect of goniodomin A. But further increasing the concentration of goniodomin A ($> 3 \times 10^{-7}$ M) again decreased the ATPase activity.

In troponin–tropomyosin-free ventricular myosin B, the ATPase activity was enhanced with an increase in goniodomin A concentration and reached a peak at 10^{-7} M (Fig. 2A). The peak value was 120% higher than that of the control. Further increasing the goniodomin A concentration up to 10^{-6}–3×10^{-6} M decreased the ATPase activity by approximately 60%. Troponin–tropomyosin complex significantly decreased the goniodomin A-induced enhancement of the ATPase activity of troponin–tropomyosin-free myosin B to the level of that of ventricular myosin B in the presence of goniodomin A (Fig. 2A).

Goniodomin A caused a concentration-dependent decrease of the ATPase activity of actomyosin reconstituted from atrial myosin and actin in the presence or absence of troponin and tropomyosin (Fig. 2B). On the other hand, goniodomin A enhanced the ATPase activity of actomyosin reconstituted from ventricular myosin and actin in the range of 10^{-8}–3×10^{-7} M in a concentration-dependent manner, but its ATPase activity was decreased by further increasing goniodomin A concentration (Fig. 2B). The peak value was 300% higher than that of the control. The ATPase activity of actomyosin reconstituted from ventricular myosin, actin, troponin and tropomyosin was enhanced by goniodomin A in the range of 10^{-8}–3×10^{-7} M. Troponin–tropomyosin complex decreased goniodomin A-induced elevation of the actomyosin ATPase activity of actin–myosin reconstituted system (Fig. 2B). In addition, the Ca^{2+}-, Mg^{2+}- or K^{+}-EDTA-ATPase activity of cardiac myosin was not affected by goniodomin A (data not shown).

4. Discussion

The contraction of striated muscles, including the heart, is produced by the cyclical interaction of myosin molecules with ‘regulated’ actin filaments consisting of actin, troponin and tropomyosin. A rise in intracellular Ca^{2+} concentration triggers muscle shortening by initiating the sliding of the myosin and actin filaments relative to each other. Ca^{2+} regulates this process by reversibly binding to

troponin, which is bound to tropomyosin. The troponin–tropomyosin complex regulates actin–myosin interaction by preventing the binding of the myosin cross-bridge to actin in the absence of Ca^{2+} (Tobacman and Adelstein, 1986). It has been previously reported that the conformational change of actin molecules, resulting from stoichiometric binding of goniodomin A to actin monomers in filaments, modifies the interaction between myosin and actin (Furukawa et al., 1993). Goniodomin A at lower concentrations enhanced the ATPase activities of troponin–tropomyosin-free ventricular myosin B and actomyosin reconstituted from ventricular myosin and actin. However, the Ca^{2+}-, Mg^{2+}- or K^{+}-EDTA-ATPase activity of myosin was not affected by goniodomin A, suggesting an elimination of the possible involvement of direct stimulation of myosin ATPase in the mechanism of actomyosin ATPase modulation. It is an important observation that in troponin–tropomyosin-free ventricular myosin B and actomyosin reconstituted from ventricular myosin and actin, troponin–tropomyosin complex nearly abolished goniodomin A-induced enhancement of both the ATPase activities. A probable explanation for these findings is that goniodomin A fails to cause an increase in the ATPase activity in the presence of regulatory protein in muscle contraction such as troponin–tropomyosin complex. These results suggest that goniodomin A-induced modulation of the ventricular actomyosin ATPase activity may be physiologically significant because of the sensitivity to troponin–tropomyosin complex. It is also suggested that goniodomin A affects actin, resulting in the modification of interaction between ventricular myosin and actin and thus changes the actomyosin ATPase activity.

Cardiac muscle is a heterogeneous tissue composed of distinct muscle cell populations (Wobus et al., 1997). Structural studies have revealed the existence of significant differences between ventricular and atrial fibers. It has been reported that atrial myosin differs in structure and enzymatic activity from ventricular myosin (Long et al., 1977). Immunochemical analysis have revealed that α-myosin heavy chains are the predominant isoform in the atrium, whereas β-myosin heavy chains are predominant in the ventricles of animals including human and guinea pig (Tsuchimochi et al., 1984). The ATPase activities of troponin–tropomyosin-free ventricular myosin B and actomyosin reconstituted from ventricular myosin and actin were enhanced by goniodomin A at lower concentrations. Further increasing the goniodomin A concentration decreased the ATPase activity in both the cases. However, goniodomin A decreased the ATPase activity of actomyosin reconstituted from atrial myosin and actin. These results suggest that goniodomin A at lower concentration affect actin to increase actomyosin ATPase activity by enhancing the interaction between actin and ventricular myosin (β isoform). It is also suggested that the different effects of goniodomin A on the actomyosin ATPase activities of ventricular and atrial reconstituted actomyosin result from the difference of the properties between the two types of myosin.

Goniodomin A may be a useful tool for studying the relationships between the structure and function of contractile proteins and the interaction between actin and myosin in cardiac muscle.

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