Biochimica et Biophysica Acta 903 (1987) 96–102
Elsevier

BBA 73671

Gingerol, a novel cardiotonic agent, activates the Ca^{2+}-pumping ATPase in skeletal and cardiac sarcoplasmic reticulum

Masaki Kobayashi [a], Noboru Shoji [b] and Yasushi Ohizumi [a]

[a] *Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo and* [b] *Institute of Pharmacy, Tokushima-Bunri University, Tokushima (Japan)*

(Received 14 April 1987)

Key words: Gingerol; ATPase, Ca^{2+}-; (Sarcoplasmic reticulum)

Gingerol, isolated as a potent cardiotonic agent from the rhizome of ginger, stimulated the Ca^{2+}-pumping activity of fragmented sarcoplasmic reticulum (SR) prepared from rabbit skeletal and dog cardiac muscles. The extravesicular Ca^{2+} concentrations of the heavy fraction of the fragmented SR (HSR) were measured directly with a Ca^{2+} electrode to examine the effect of gingerol on the SR. Gingerol (3–30 μM) accelerated the Ca^{2+}-pumping rate of skeletal and cardiac SR in a concentration-dependent manner. The rate of $^{45}Ca^{2+}$ uptake of HSR was also increased markedly by 30 μM gingerol without affecting the $^{45}Ca^{2+}$ efflux from HSR. Furthermore, gingerol activated Ca^{2+}-ATPase activities of skeletal and cardiac SR (EC_{50}, 4 μM). The activation of SR Ca^{2+}-ATPase activity by gingerol (30 μM) was completely reversed by 100-fold dilution with the fresh saline solution. Kinetic analysis of activating effects of gingerol suggests that the activation of SR Ca^{2+}-ATPase is uncompetitive and competitive with respect to Mg · ATP at concentrations of 0.2–0.5 mM and above 1 mM, respectively. Kinetic analysis also suggests that the activation by gingerol is mixed-type with respect to free Ca^{2+} and this enzyme is activated probably due to the acceleration of enzyme-substrate complex breakdown. Gingerol had no significant effect on sarcolemmal Ca^{2+}-ATPase, myosin Ca^{2+}-ATPase, actin-activated myosin ATPase and cAMP-phosphodiesterase activities, indicating that the effect of gingerol is rather specific to SR Ca^{2+}-ATPase activity. Gingerol may provide a valuable chemical tool for studies aimed at clarifying the regulatory mechanisms of SR Ca^{2+}-pumping systems and the causal relationship between the Ca^{2+}-pumping activity of SR and muscle contractility.

Introduction

An important problem to be elucidated in the excitation-contraction coupling of skeletal and cardiac muscles concerns the regulation of sarcoplasmic reticulum (SR) function [1,2]. The Ca^{2+}-ATPase in SR membrane plays a key role in the muscle relaxation by energized Ca^{2+} pumping from the cytoplasm into the lumen of SR [1]. The activity of cardiac SR Ca^{2+}-ATPase is generally accepted to be regulated by cAMP- or Ca^{2+}, calmodulin-dependent phosphorylation of phospholamban [3,4]. It has been proposed that catecholamines accelerate the Ca^{2+}-pumping activity of cardiac SR by phosphorylating phospholamban to increase the amount of stored Ca^{2+} which is releasable in subsequent contraction, and

Abbreviations: SR, sarcoplasmic reticulum; gingerol, [8]-gingerol (5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-dodecanone); HSR, heavy fraction of fragmented skeletal SR; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; Mops, 4-morpholinepropanesulphonic acid; EC_{50}, 50% effective concentration of maximum response.

Correspondence: M. Kobayashi, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan.

thus produce the increased contractility and accelerated relaxation [3], although the details of the final step of this mechanism have not been clarified. On the other hand, in the skeletal muscle SR, little is known about the regulating mechanism of the Ca^{2+}-pumping ATPase activity except a possible involvement of a 53000 dalton glycoprotein [5]. Besides, there are few substances reported to modulate specifically the Ca^{2+}-pumping activity of SR.

In the course of our survey of substances having novel bioactivities from natural sources [6–10], gingerol (Fig. 1) was isolated as a potent cardiotonic agent from the rhizome of ginger *Zingiber officinale* Roscoe [11]. The rhizome of this plant has been widely used not only as a seasoning spice but also as an important Oriental medicine, with stomachic, carminative, stimulant, diuretic, antitussive and antiemetic effects [12]. Here we report the first evidence that gingerol directly and specifically activates the Ca^{2+}-pumping ATPase of both skeletal and cardiac SR. Gingerol may provide a useful chemical probe for studies aimed at clarifying the regulatory mechanisms of SR Ca^{2+} pumps and the causal relationship between the Ca^{2+}-pumping activity of SR and muscle contractility.

Materials and Methods

Materials. Gingerol was obtained from the dried rhizome of ginger *Z. officinale* Roscoe. The purification procedure of gingerol has been published elsewhere [11]. Gingerol was dissolved in dimethylsulfoxide, of which the final concentration was kept less than 1% (v/v) in all experiments. In the control experiments, dimethylsulfoxide was added instead of a solution of gingerol to minimize the effect of the vehicle solvent. Vesicular HSR, purified SR Ca^{2+}-ATPase, sarcolemmal Ca^{2+}-ATPase, myosin and actin were prepared from rabbit skeletal white muscle by the methods of Kim et al. [13], Meissner et al. [14], Seiler and Fleischer [15], as modified by Michalak et al. [16], Perry [17] and Mommaerts [18], respectively. Cardiac SR and myosin were obtained from dog heart muscle according to the methods of Harigaya and Schwartz [19] and Mueller et al. [20]. Bovine heart cAMP-phosphodiesterase was purchased from Sigma.

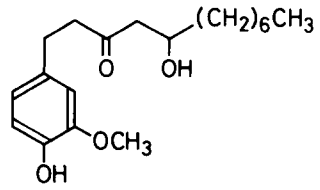

Fig. 1. Chemical structure of gingerol.

Ca^{2+} concentration measurement. The extravesicular Ca^{2+} concentration of SR suspension was measured at 30 or 15 °C with a Ca^{2+} electrode prepared as described previously by Nakamura et al. [8]. The Ca^{2+} electrode showed Nernstian response (slope, 27–29 mV/p*Ca* unit) in the calibration solutions containing Ca^{2+}-EGTA between p*Ca* 3 and 6.5. The time for 90% response was approx. 0.6 s when p*Ca* decreased from 6 to 4. The assay mixture (final volume, 1 ml) for skeletal SR contained 0.05 mM $CaCl_2$, 90 mM KCl, 0.5 mM $MgCl_2$, 50 mM Mops-KOH buffer (pH 7.0), 1.5 mg/ml HSR, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase and 0.5 mM ATP. The mixture for cardiac SR was the same as that for skeletal HSR except that it contained 3 mM $MgCl_2$, 2 mM NaN_3 and 1 mM ATP. The reaction of Ca^{2+} uptake was started by a simultaneous addition of ATP and creatine kinase.

$^{45}Ca^{2+}$ uptake measurement. The $^{45}Ca^{2+}$ uptake of vesicular HSR was measured at 10 °C according to the method of Martonosi and Feretos [21], with modification. The assay mixture (final volume, 1 ml) contained 0.1 mM $^{45}CaCl_2$, 90 mM KCl, 0.5 mM $MgCl_2$, 50 mM Mops-KOH buffer (pH 7.0), 5 mM oxalic acid, 0.05 mg/ml HSR, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase and 0.5 mM ATP. The mixture with gingerol was preincubated in the absence of ATP and creatine kinase at 10 °C for 5 min. The reaction of $^{45}Ca^{2+}$ uptake was started by a simultaneous addition of ATP and creatine kinase. The aliquots (0.15 ml) of the suspension were filtered through Millipore filters (HAWP, 0.45 μm), and washed three times with 0.5 ml of a solution containing 5 mM $LaCl_3$, 90 mM KCl, 5 mM $MgCl_2$ and 50 mM Mops-KOH buffer (pH 7.0). The amount of ^{45}Ca remaining in the HSR vesicles was measured by counting the radioactivity on the washed filters.

The $^{45}Ca^{2+}$ uptake activity was calculated by the difference between the radioactivity before and appropriate time after addition of ATP and creatine kinase.

$^{45}Ca^{2+}$ efflux measurement. The $^{45}Ca^{2+}$ efflux from the vesicular HSR passively preloaded with $^{45}Ca^{2+}$ was measured at 0 °C by the method of Nakamura et al. [8]. After 12 h preincubation of HSR (20 mg/ml) at 0 °C in a solution containing 5 mM $^{45}CaCl_2$, 90 mM KCl and 50 mM Mops-KOH buffer (pH 7.0), the HSR suspension (10 μl) was diluted with 100 vol. of a reaction medium containing 0.4 mM $CaCl_2$, 90 mM KCl, 1.9 mM EGTA and 50 mM Mops-KOH buffer (pH 7.0) in the presence or absence of gingerol. The HSR vesicles in the aliquots (0.15 ml) of the suspension were filtered and washed in the same way as described above for the $^{45}Ca^{2+}$ uptake measurement. The amount of ^{45}Ca remaining in the vesicles was measured by scintillation counting.

Enzyme assay. Each ATPase reaction mixture (final volume, 1 ml) was as follows: 50 mM Mops-KOH buffer (pH 7.0), 90 mM KCl, 5 mM $MgCl_2$, 0.75 mM $CaCl_2$, 1 mM EGTA, 0.01 mg/ml deoxycholate-solubilized SR enzyme and 1 mM ATP for skeletal SR Ca^{2+}-ATPase; 50 mM Mops-KOH buffer (pH 7.0), 90 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM EGTA, 5 mM NaN_3, 5 mM oxalic acid, 0.05 mg/ml cardiac SR enzyme and 1 mM ATP for cardiac SR Ca^{2+}-ATPase; the same solution as that for skeletal SR Ca^{2+}-ATPase supplemented with 3 μM calcium ionophore A23187 for sarcolemmal Ca^{2+}-ATPase; 50 mM Tris-HCl buffer (pH 7.4), 500 mM KCl, 10 mM $CaCl_2$, 0.2 mg/ml skeletal myosin and 2 mM ATP for myosin Ca^{2+}-ATPase; 20 mM Mops-KOH buffer (pH 7.0), 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.2 mg/ml cardiac myosin, 0.2 mg/ml actin and 1 mM ATP for actin-activated myosin ATPase. The mixture was preincubated in the absence of ATP at 30 °C for 5 min, followed by the addition of gingerol and further preincubation for 5 min. The reaction was started by the addition of ATP, and stopped by adding 0.5 ml of cold 10% trichloroacetic acid. The amount of P_i liberated during the 7–15 min incubation (30 °C) was determined by the method of Martin and Doty [22]. ATPase activity was calculated by the difference between the quantity of P_i liberated in the presence and absence of Ca^{2+} added. Cardiac cAMP-phosphodiesterase activity was assayed according to the method of Butcher and Sutherland [23].

Results and Discussion

The Ca^{2+}-pumping activity of fragmented SR can clearly be visualized by monitoring extravesicular Ca^{2+} concentration of HSR directly with a Ca^{2+} electrode (Fig. 2). The semilogarithmic graph (Fig. 2(a)) demonstrates that, upon the addition of ATP, free Ca^{2+} concentrations decreased promptly due to the formation of Ca-ATP complexes and further decreased gradually due to the active Ca^{2+} uptake by skeletal HSR. The profile of net Ca^{2+} uptake exhibited a biphasic time-course which was similar to that reported previously [24]. The initial part of the Ca^{2+} uptake (slow uptake phase) was almost linear in an antilogarithmic plot, and the rate of net Ca^{2+} uptake was about 3–5 nmol/mg per min, a value comparable to the reported one [24]. The slow-uptake phase continued for about 1.5 min and was followed by a fast Ca^{2+} uptake phase. When the Ca^{2+} concentration was reduced to submicromolar levels, the apparent Ca^{2+} uptake slowed. The effect of gingerol was examined on the Ca^{2+}-pumping activity of fragmented SR using this system. After pretreatment with 30 μM gingerol, the slope of the time-course curve of Ca^{2+} uptake at 30 °C (Fig. 2(b)) was much steeper than that of control (Fig. 2(a)). As shown in Fig. 2(c), the slope of the Ca^{2+}-uptake profile suddenly steepened just after application of 30 μM gingerol. No such influence was obtained after addition of the vehicle solvent. Fig. 2(d) shows the time-course curves of Ca^{2+} uptake at 15 °C in the presence of various concentrations of gingerol. As shown in Fig. 2(e), the rate of net Ca^{2+} uptake by HSR was accelerated by gingerol. A similar stimulatory effect of gingerol was observed also by using cardiac SR. These results indicate that gingerol activates the Ca^{2+}-pumping activity of SR from skeletal and cardiac muscles.

The effect of gingerol on the Ca^{2+}-pumping activity of fragmented SR was also examined by measuring the active $^{45}Ca^{2+}$ uptake of vesicular HSR at 10 °C. Following the initial change, the amount of ^{45}Ca remaining in the HSR vesicles

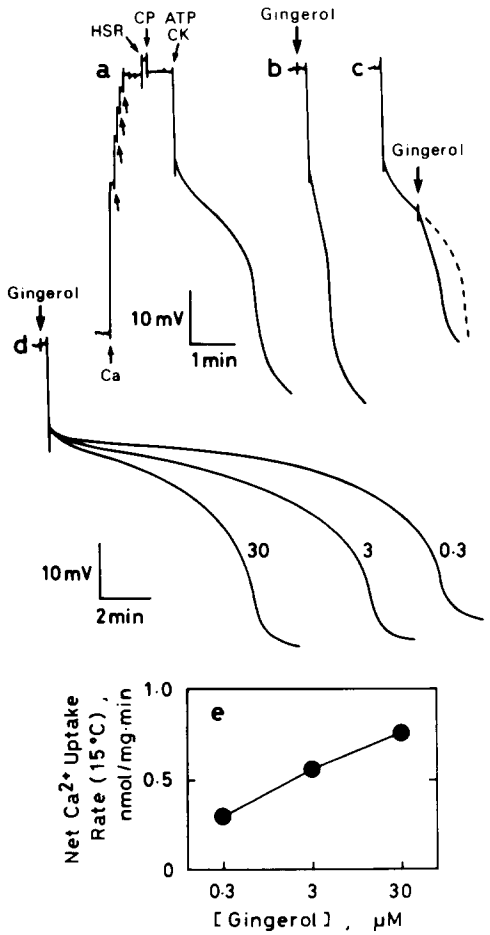

Fig. 2. Stimulatory effect of gingerol on the Ca^{2+}-pumping activity of HSR. The extravesicular Ca^{2+} concentration was monitored with a Ca^{2+} electrode in the mixture containing 1.5 mg/ml HSR, 0.5 mM ATP, 0.05 mM $CaCl_2$, 0.5 mM $MgCl_2$, 90 mM KCl, 5 mM creatine phosphate (CP), 0.1 mg/ml creatine kinase (CK) and 50 mM Mops-KOH buffer (pH 7.0). In (a–d), the vertical calibration bar indicates a response for voltage change of 10 mV (27–29 mV corresponds to 1 p*Ca* unit). At the beginning of each experiment, 0.01 mM $CaCl_2$ was added stepwise five times as the internal standard. In (b–d), the traces are those only after the addition of gingerol or ATP. Temperature: 30 °C (a–c), 15 °C (d). (a) Control, (b) pretreatment with 30 μM gingerol. (c) Application of 30 μM gingerol in mid course of Ca^{2+}-pumping process. The broken line shows the control curve. (d) Pretreatment with various concentrations of gingerol. Each trace was superimposed. The concentration of gingerol (0.3, 3 or 30 μM) is indicated by each trace. The curve after application of 0.3 μM gingerol was almost the same as the control curve. (e) Concentration-dependent acceleration of the net Ca^{2+} uptake rate. Each value was the slope of the initial part of antilogarithmic plot for the curve in (d).

increased linearly (Fig. 3). In the presence of 30 μM gingerol, the $^{45}Ca^{2+}$-uptake rate (62 nmol/mg per min) was larger than that of the control (37 nmol/mg per min). These results corroborate the activating effect of gingerol on the energized Ca^{2+} pump of the SR membrane.

It has been shown that, in the measurement of the Ca^{2+}-pumping activity of HSR with a Ca^{2+} electrode or a Ca^{2+} indicator dye, the slow Ca^{2+} uptake phase is observed which is due to a simultaneous operation of the Ca^{2+}-induced Ca^{2+} release and the energized Ca^{2+} pumping [24]. Thus, there remained a possibility that gingerol accelerated the net Ca^{2+} uptake of HSR by inhibiting the Ca^{2+} efflux through the Ca^{2+}-release channel, just as ruthenium red did [24]. In the measurement with a Ca^{2+} electrode, ruthenium red (0.2 μM) increased the slow uptake rate to eliminate the slow-uptake phase, and the addition of gingerol (30 μM) further increased the Ca^{2+}-uptake rate (not shown). Furthermore, the effect of gingerol on the Ca^{2+} efflux from HSR was examined under the conditions in which the Ca^{2+} pump did not work (0 °C). As shown in Fig. 4, ruthenium red (0.2 μM) markedly decreased the rate of $^{45}Ca^{2+}$ efflux from HSR, whereas gingerol (30 μM) caused no marked effect, or rather a slight increase, in the $^{45}Ca^{2+}$ efflux rate. These observations clearly ruled

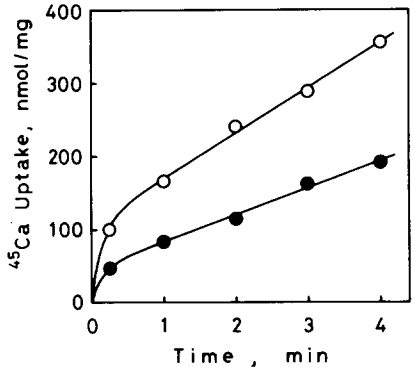

Fig. 3. Stimulatory effect of gingerol on the $^{45}Ca^{2+}$ uptake of HSR. The assay mixture was the same as that for the Ca^{2+} electrode experiment (Fig. 2), except that it contained 0.1 mM $^{45}CaCl_2$, 0.05 mg/ml HSR and 5 mM oxalic acid and that the assay temperature was 10 °C. In the presence (○) or absence (●) of 30 μM gingerol, the time-course of the increase in the ^{45}Ca content in the SR vesicles was measured after simultaneous addition of ATP and creatine kinase. Each value was the mean of two determinations.

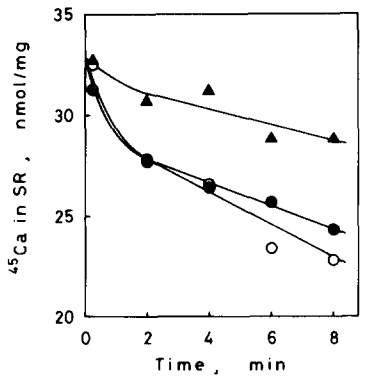

Fig. 4. Effects of gingerol and ruthenium red on the $^{45}Ca^{2+}$ efflux from HSR. The time course of the decrease in the ^{45}Ca content in the vesicles was measured at 0 °C after 100-fold dilution of HSR passively preloaded with $^{45}Ca^{2+}$ into a control assay medium (●) or that containing 30 μM gingerol (○) or 0.2 μM ruthenium red (▲). Each value was the mean of two determinations.

out the possibility that the acceleration by gingerol of the net Ca^{2+} uptake of HSR was due to the inhibition of the Ca^{2+}-releasing activity.

Moreover, gingerol (3–30 μM) activated Ca^{2+}-ATPase activities of skeletal and cardiac SR (Fig. 5), although the higher concentration (100 μM) inhibited these activities nonspecifically. The EC_{50} values of gingerol were 4.0 μM for skeletal SR and 4.3 μM for cardiac SR. These log concentration-response curves were similar to that for cardiotonic

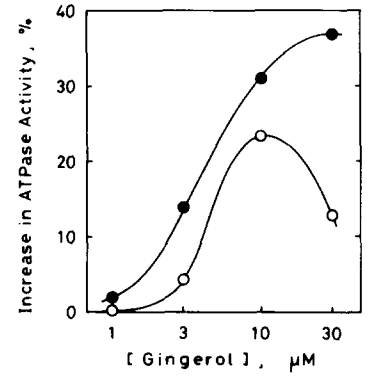

Fig. 5. Effect of gingerol on Ca^{2+}-ATPase activities of skeletal and cardiac SR. ATPase activity was calculated by the difference between the quantity of P_i liberated during the 15-min incubation (30 °C) in the presence or absence of Ca^{2+} added. Each value was the mean of three determinations. In typical experiments without gingerol, specific activities were as follows: skeletal SR Ca^{2+}-ATPase (●), 3.4 μmol/min per mg; cardiac SR Ca^{2+}-ATPase (○), 0.11 μmol/min per mg.

effect of gingerol (not shown). On the other hand, gingerol (3–30 μM) did not greatly affect other enzyme activities that play important roles in muscle contraction, such as sarcolemmal Ca^{2+}-ATPase and myosin Ca^{2+}-ATPase from skeletal muscle, actin-activated myosin ATPase from cardiac muscle and cardiac cAMP-phosphodiesterase (not shown). These observations indicate that the stimulatory effect of gingerol is rather specific to SR Ca^{2+}-ATPase activity. The extent of stimulation by gingerol of Ca^{2+}-ATPase was a little different from that of the Ca^{2+}-pumping activity of fragmented SR, but this may be due to the difference in various conditions. Treatment of skeletal SR Ca^{+}-ATPase with 30 μM gingerol at 30 °C for 15 min resulted in marked activation (48%) of the enzyme activity. However, 100-fold dilution of the mixture containing gingerol (30 μM)-treated enzyme and subsequent incubation reversed completely the stimulatory effect of gingerol on SR Ca^{2+}-ATPase, suggesting that the activation by gingerol is reversible.

In general, activation of the enzyme reaction can be expressed as follows:

$$\begin{array}{ccc} E+S \overset{K_S}{\rightleftharpoons} & & ES \xrightarrow{k_p} E+P \\ + & & + \\ A & & A \\ K_A \Updownarrow & & \alpha K_A \Updownarrow \\ EA+S \overset{\alpha K_S}{\rightleftharpoons} & & ESA \xrightarrow{\beta k_p} EA+P \end{array}$$

where the binding of activator to the enzyme causes the modification of K_S and K_A (dissociation constants of ES and EA, respectively) or k_p (rate constant for the breakdown of ES) by a factor of α or β [25]. The kinetics of activation of gingerol was examined with respect to a Mg-ATP complex or free Ca^{2+} for deoxycholate-solubilized skeletal SR Ca^{2+}-ATPase. It has previously reported that the SR Ca^{2+}-ATPase possesses two kinds of ATP-binding sites, a high-affinity catalytic site and a low-affinity regulatory site [26]. In the present experiments, a double-reciprocal plot (Fig. 6(a)) for the Mg · ATP concentration showed a transition near 0.7 mM Mg · ATP, a similar observation to that reported [26]. In a concentration range of 0.2–0.5 mM, the slope remained nearly constant and both of the intercepts were

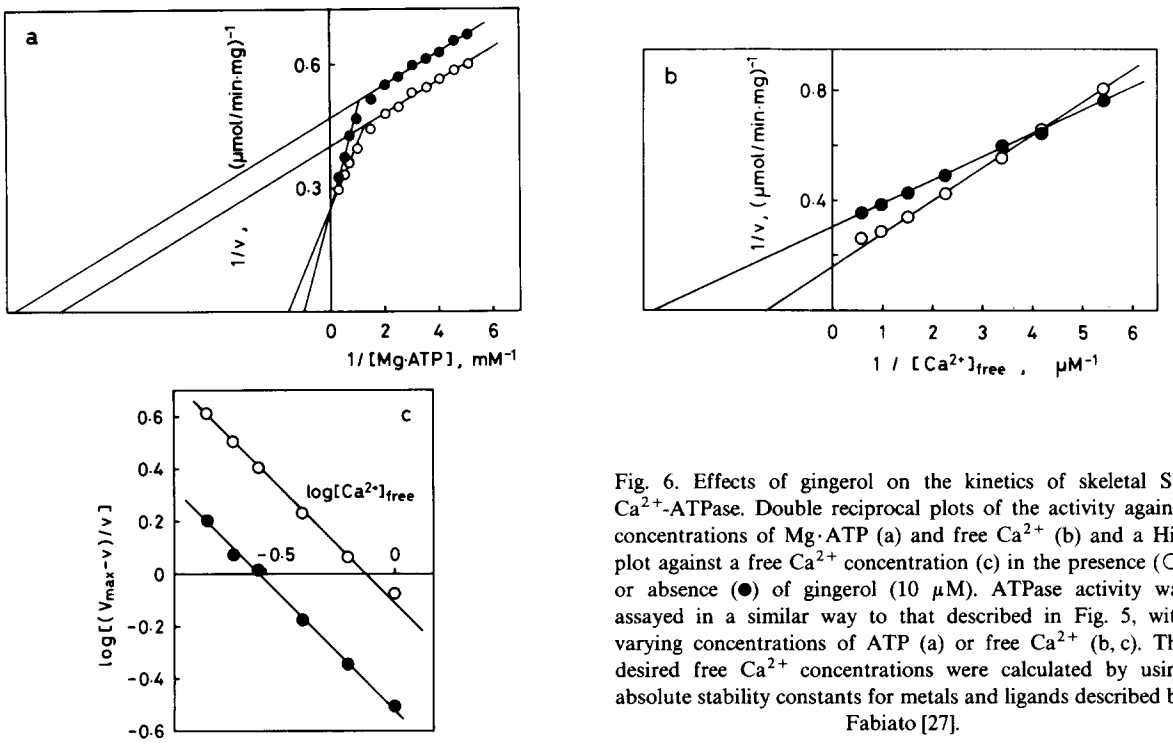

Fig. 6. Effects of gingerol on the kinetics of skeletal SR Ca^{2+}-ATPase. Double reciprocal plots of the activity against concentrations of Mg·ATP (a) and free Ca^{2+} (b) and a Hill plot against a free Ca^{2+} concentration (c) in the presence (○) or absence (●) of gingerol (10 μM). ATPase activity was assayed in a similar way to that described in Fig. 5, with varying concentrations of ATP (a) or free Ca^{2+} (b, c). The desired free Ca^{2+} concentrations were calculated by using absolute stability constants for metals and ligands described by Fabiato [27].

decreased by gingerol (10 μM), and thus the line was almost parallel to each other. This indicates that gingerol shows an uncompetitive activation pattern ($\alpha = \beta > 1$) with respect to the Mg-ATP complex in this Mg · ATP concentration range. At Mg · ATP concentrations above 1 mM, the slope was decreased by gingerol (10 μM) and the two lines crossed at a point on the ventrical axis (Fig. 6(a)). V_{max} of 4.0 μmol/min per mg was constant and K_{ATP} decreased from 1.01 (control) to 0.65 mM. This indicates that gingerol shows a competitive activation pattern ($\alpha < \beta = 1$) with respect to Mg · ATP in the concentration range above 1 mM, suggesting that gingerol may interact with the regulatory ATP-binding site. In a reciprocal plot (Fig. 6(b)) for the free Ca^{2+} concentration ($[Ca^{2+}]_{free}$), the slope was increased but both of the intercepts were decreased by gingerol (10 μM), and the two lines crossed at a point with a positive $1/[Ca^{2+}]_{free}$ value. V_{max} increased from 3.4 (control) to 6.4 μmol/min per mg and K_{Ca} from 0.30 (control) to 0.77 μM. This indicates that gingerol shows a mixed-type activation pattern ($\alpha > \beta > 1$) with respect to free Ca^{2+}. Interestingly, gingerol inhibited SR Ca^{2+}-ATPase at low Ca^{2+} concentrations up to the intersection point (0.24 μM) but it activated the enzyme at higher Ca^{2+} concentrations. These results support the idea that gingerol increases the rate constant for ES complex breakdown, but also markedly decreases the affinity of the enzyme for Ca^{2+}. Moreover, gingerol (10 μM) caused no change in the Hill coefficient ($n = 1$) for Ca^{2+} activation of SR Ca^{2+}-ATPase (Fig. 6(c)), suggesting that the interaction between Ca^{2+}-binding sites of the enzyme remains unchanged in the presence of gingerol. Thus, the SR Ca^{2+}-ATPase is activated by gingerol probably due to the acceleration of product formation in a Ca^{2+} concentration range that is crucial for the pump activation under physiological conditions.

The experiments reported here show that gingerol activates SR Ca^{2+}-ATPase at concentrations in the range of about 3–30 μM, at which gingerol stimulates the Ca^{2+}-pumping activity of fragmented SR and also causes cardiotonic effects on heart muscle, suggesting that the mechanism of stimulatory action of gingerol on the Ca uptake of SR is related to the direct activation of SR Ca^{2+}-ATPase. The Ca^{2+}-pumping ATPase of SR is

generally accepted to be an essential element of the molecular mechanism of a contraction-relaxation cycle in skeletal or cardiac muscle [1]. Despite the availability of information concerning the regulation of SR Ca^{2+}-pumping activity [3–5], knowledge of the underlying mechanism involved is still lacking, especially in skeletal muscle. Furthermore, clear evidence has not yet been reported for the central role of enhanced Ca^{2+}-pumping activity of cardiac SR in the mechanism linking intracellular cAMP to increased contraction and accelerated relaxation [3]. Gingerol may facilitate further understanding of the regulating mechanisms of energized Ca^{2+} pumping through the SR membrane and the causal relationship between the Ca^{2+}-pumping activity of SR and muscle contractility.

Acknowledgements

We are grateful to Ms. Akiko Muroyama, Sumie Kaneko and Hiromi Honda for skillful technical assistance and to Ms. Yoshiko Murakami and Misayo Nakai for secretarial assistance.

References

1 Ikemoto, N. (1982) Annu. Rev. Physiol. 44, 297–317
2 Winegrad, S. (1982) Annu. Rev. Physiol. 44, 451–462
3 Tada, M. and Katz, A.M. (1982) Annu. Rev. Physiol. 44, 401–423
4 Inui, M., Chamberlain, B.K., Saito, A. and Fleischer, S. (1986) J. Biol. Chem. 261, 1794–1800
5 Chiesi, M. and Carafoli, E. (1982) J. Biol. Chem. 257, 984–991
6 Takahashi, M., Ohizumi, Y. and Yasumoto, T. (1982) J. Biol. Chem. 257, 7287–7289
7 Kobayashi, M., Kajiwara, A., Takahashi, M., Ohizumi, Y., Shoji, N. and Takemoto, T. (1984) J. Biol. Chem. 259, 15007–15009
8 Nakamura, Y., Kobayashi, J., Gilmore, J., Mascal, M., Rinehart, K.L., Jr., Nakamura, H. and Ohizumi, Y. (1986) J. Biol. Chem. 261, 4139–4142
9 Ohizumi, Y., Nakamura, H., Kobayashi, J. and Catterall, W.A. (1986) J. Biol. Chem. 261, 6149–6152
10 Takito, J., Nakamura, H., Kobayashi, J., Ohizumi, Y., Ebisawa, K. and Nonomura, Y. (1986) J. Biol. Chem. 261, 13861–13865
11 Shoji, N., Iwasa, A., Takemoto, T., Ishida, Y. and Ohizumi, Y. (1982) J. Pharm. Sci. 71, 1174–1175
12 Perry, L.M. (1980) Medicinal Plants of East and Southeast Asia, p. 443, The MIT Press, Cambridge
13 Kim, D.H., Ohnishi, S.T. and Ikemoto, N. (1983) J. Biol. Chem. 258, 9662–9668
14 Meissner, G., Conner, G.E. and Fleischer, S. (1973) Biochim. Biophys. Acta 298, 246–269
15 Seiler, S. and Fleischer, S. (1982) J. Biol. Chem. 257, 13862–13871
16 Michalak, M., Famulski, K. and Carafoli, E. (1984) J. Biol. Chem. 259, 15540–15547
17 Perry, S.V. (1955) Methods Enzymol. 2, 582–588
18 Mommaerts, W.F.H.M. (1951) J. Biol. Chem. 188, 559–565
19 Harigaya, S. and Schwartz, A. (1969) Circ. Res. 25, 781–794
20 Mueller, H., Franzen, J., Rice, R.V. and Olson, R.E. (1964) J. Biol. Chem. 239, 1447–1456
21 Martonosi, A. and Feretos, R. (1964) J. Biol. Chem. 239, 648–658
22 Martin, J.B. and Doty, D.M. (1949) Anal. Chem. 21, 965–967
23 Butcher, R.W. and Sutherland, E.W. (1962) J. Biol. Chem. 237, 1244–1250
24 Morii, H., Takisawa, H. and Yamamoto, T. (1985) J. Biol. Chem. 260, 11536–11541
25 Segel, I.H. (1975) Enzyme Kinetics, pp. 188–191, 227–231, John Wiley & Sons, New York
26 Dupont, Y. (1977) Eur. J. Biochem. 72, 185–190
27 Fabiato, A. (1981) J. Gen. Physiol. 78, 457–497