Methyl Jasmonate-Induced Stimulation of Sarcoplasmic Reticulum Ca$^{2+}$-ATPase Affects Contractile Responses in Rat Slow-Twitch Skeletal Muscle

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ABSTRACT

The purpose of this study was to determine whether methyl jasmonate, a stimulator of Ca$^{2+}$-adenosine triphosphatase (ATPase) activity of the purified ATPase from fast-twitch skeletal muscle, could affect contractile responses in small bundles of rat isolated slow-twitch (soleus) fibers. In saponin-skinned fibers, sarcoplasmic reticulum (SR) Ca$^{2+}$ loading was performed in pCa 7.0 solution. The amount of Ca$^{2+}$ taken up was monitored by use of the amplitude of contraction following application of 10 mM caffeine. Results indicate that the increased loading rate in the presence of methyl jasmonate is likely due to stimulation of the SR Ca$^{2+}$-ATPase. In Triton-skinned fibers, the myofibrillar Ca$^{2+}$ sensitivity was not changed by methyl jasmonate (50–200 μM). In intact fibers, the amplitude and the time constant of relaxation of twitch and potassium contracture were reversibly reduced after 2 min of application of methyl jasmonate at a concentration of up to 125 μM. At higher concentrations (>150 μM), effects were not reversible. In the presence of methyl jasmonate (100 μM), the relationship between the amplitude of potassium contractures and the membrane potential shifted to more positive potentials, whereas the steady-state inactivation curve was unchanged. These observations suggest that methyl jasmonate has no effect on voltage sensors. Taken together, our results show that methyl jasmonate is a potent, reversible, and specific stimulator of the SR Ca$^{2+}$ pump in slow-twitch skeletal muscle and is an extremely valuable pharmacological tool for improving relaxation and studying calcium-signaling questions.

In mammalian skeletal muscle, triggering of the action potential along the membrane of the T-transverse tubule system increases intracellular Ca$^{2+}$ concentration and produces contraction through the interaction of actin and myosin. Contraction is then terminated through Ca$^{2+}$ uptake by the sarcoplasmic reticulum (SR) via the Ca$^{2+}$ pump. This series of events is referred to as excitation-contraction coupling. During contraction, two key proteins play a major role in the calcium release mechanism: the sarcoplasmal dihydro-pyridine receptor (voltage sensor) and the SR Ca$^{2+}$ release channel (ryanodine receptor). To induce relaxation, Ca$^{2+}$ is removed from the cytosol by extrusion through the sarcolem- mal Na$^{+}$/Ca$^{2+}$ exchanger and Ca$^{2+}$ adenosine triphosphatase (Ca$^{2+}$-ATPase), mitochondria, and by sequestration in the SR mediated by the Ca$^{2+}$-ATPase. However, in slow-twitch muscle, the SR Ca$^{2+}$ pump is the major process responsible for reducing cytosolic Ca$^{2+}$ from a high level to a low resting level during relaxation (Leong and Maclennan, 1998; Lamb, 2000). Pharmacological tools predominantly in-hibiting SR Ca$^{2+}$ uptake, i.e., cyclopiazonic acid, thapsigargin, and 2,5-di-(tert-butyl)-1,4-hydroquinone, have long been used to separate the various cellular mechanisms that regulate the contraction-relaxation cycle in muscle. Other tools that act as specific stimulators of the SR Ca$^{2+}$-ATPase activity of muscle cells could be useful for investigating SR Ca$^{2+}$ uptake and storage.

Biochemical investigations have recently shown that jas- mone, menthone, menthol, and methyl jasmonate are highly selective stimulators of the SR Ca$^{2+}$-ATPase in mammalian skeletal muscle, whereas they have no effect on the properties of the phospholipid bilayer (Starling et al., 1994). Surprisingly, no study has investigated the effects of these substances on skeletal muscle contraction. Several studies have shown that two compounds of the lipoxygenase pathway, i.e., jasmonic acid and methyl jasmonate, regulate wound response in plants (Veronesi et al., 1996). Methyl jasmonate, a linolenic acid-derived cyclopentanone-base, has also been shown to trigger defense reactions in various plants and to be produced in response to wounds and elicitor treatment (Rick- auer et al., 1997).

ABBREVIATIONS: SR, sarcoplasmic reticulum; Ca$^{2+}$-ATPase, calcium adenosine triphosphatase; [K$^+$]o, extracellular potassium concentration; $E_m$, membrane potential; CPA, cyclopiazonic acid; ANOVA, analysis of variance.
Because methyl jasmonate can stimulate ATP-dependent Ca\(^{2+}\) activity of the purified Ca\(^{2+}\)-ATPase from rabbit fast-twitch skeletal muscle (Starling et al., 1994), it seemed of interest to estimate the specificity of methyl jasmonate effects on Ca\(^{2+}\) loading by the SR. Chemically skinned slow muscle fibers (soleus) were chosen for these experiments, as sarcolemmal functions can be eliminated and Ca\(^{2+}\) mobilization greatly simplified. Other experiments investigated whether methyl jasmonate in intact soleus fibers acts at different steps in the excitation-contraction coupling process.

**Materials and Methods**

**General Procedure.** All procedures in this study were performed in accordance with the stipulations of the Helsinki Declarations and with the European current laws for animal experimentation. Adult male Wistar rats (body weight, 400 ± 25 g) were anesthetized by ether vapor flow. After respiratory arrest, soleus was quickly excised and placed in a dissecting dish containing mammalian physiological solution at controlled room temperature (19–20°C).

**Chemically Skinned Fibers.** Short cut bundles containing two to five fibers (150–200 μm in diameter, 10–20 mm in length) were dissected from soleus muscle, and chemical fiber skinnning was carried out immediately. For Triton-skinned fibers, preparations were incubated for 1 h in a relaxing solution (pCa 9) containing 1% Triton X-100 (v/v) to solubilize membranes and then transferred into a relaxing solution without detergent. Fibers were stored at −20°C in relaxing solution containing 50% (v/v) glycerol.

Saponin-skinned fibers were incubated for 30 min in a relaxing solution (pCa 9) containing 50 μg·ml\(^{-1}\) of saponin. This treatment preserves the ability of the SR to accumulate and release Ca\(^{2+}\) (Pano et al., 1989). After skinnning, fibers were transferred and mounted in a manner similar to that of Huchet and Léoty (1993). Preparations were immersed in small chambers (Bioblock tubes; NUNC, Roskilde, Denmark) containing 2.5 ml of solution. Eight chambers were arranged around a disc that could be moved under the muscle to change the solution as required. The disc itself was immersed in a temperature-controlled bath (21°C) positioned on a magnetic stirrer.

Each solution was vigorously stirred at high speed to facilitate diffusion of calcium, EGTA, and substrates into muscle. Fibers were moved between solutions by lifting the transducer assembly fixed to a manipulator, rotating the disc, and lowering the transducer assembly. At the beginning of the experiments, three or four challenges of 10 mM caffeine contracture were performed. Ca\(^{2+}\) uptake by the SR was studied by immersing the preparations sequentially in five different solutions to deplete (solution 1), wash (solution 2), and load (solutions 3 and 4) the SR with Ca\(^{2+}\) before releasing Ca\(^{2+}\) with caffeine (solution 5) to generate transient contracture. At the beginning of the experiments, three or four challenges of 10 mM caffeine contracture were performed. Ca\(^{2+}\) uptake by the SR was determined by an indirect method. The SR of saponin-skinned fibers was preloaded with Ca\(^{2+}\) solution for various periods (25, 35, 65, 95, 125, and 305 s). After the loading period, Ca\(^{2+}\) was released by applying 10 mM caffeine. The caffeine contracture tension that developed showed an increase in amplitude dependent on loading time, which reached its maximum after 5 min of loading. A good fit was achieved by assuming a simple system in which loading resulted from the filling of a two-compartment system (the filling rate for both compartments differed with loading time). This arrangement is roughly similar to that suggested by Chapman and Léoty (1976). For each experimental fiber, the slope of the linear fit provides an estimation of the loading rate. This parameter was evaluated in methyl jasmonate-free medium and in the presence of different methyl jasmonate concentrations (0, 100, and 200 μM).

**Experimental Protocol for Triton-Skinned Fibers.** The tension-pCa relationship (pCa = −log [Ca\(^{2+}\)]) was obtained by exposing the fiber sequentially to solutions of decreasing pCa until maximal tension was reached (in pCa 4.5), after which the fibers were returned to pCa 9. Isometric tension was recorded continuously on chart paper (Linear Bioblock 1200, Reno, NV), and baseline tension was established at the steady state measured in relaxing solution. The relationship between tension and the negative logarithm of [Ca\(^{2+}\)], the pCa, was quantified from the Ca\(^{2+}\) sensitivity curve described by the Hill equation:

\[
T = \left[\frac{[Ca^{2+}]_{0}}{[Ca^{2+}]_{50}}\right]^{(n_{H} - 1)}
\]

Where T is relative tension, K the Ca\(^{2+}\) concentration for half-maximal activation, and n\(_{H}\) the Hill coefficient.

The Hill coefficient and pCa for half-maximal activation, pC\(_{50}\) = (−log K · n\(_{H}\)\(^{-1}\)), were calculated for each experiment using linear regression analysis. The n\(_{H}\) of each type of fiber was calculated as the slope of the fitted straight lines. Resting tension was the tension in pCa 9, and maximal tension was obtained in pCa 4.5. Tension is expressed in millinewton per square meter.

**Isometric Tension Measurement from Intact Fibers.** After removal of connective tissue, small bundles of two to five fibers were dissected along their entire length under the microscope. The preparation was then transferred onto a coverslip in a drop of physiological solution and placed in the experimental chamber. This chamber, with an open-top channel 1.3 × 1.3 × 25.0 mm, has at one end a four-way tap that opens directly into the channel, allowing rapid change of the perfusion solution (<0.2 s), or into a drain so that perfusion by stagnant solutions is avoided. The preparation was mounted as described by Joumsa and Léoty (2001). Briefly, one tendon of the preparation was snared under a fine silver loop and fixed in the open-top channel. The other tendon was fixed to the tip of a force transducer (Kaman KD 2300 0.5 SU displacement measuring system, Colorado Springs, CO).

The preparation was superfused with physiological solution at 20 ml·min\(^{-1}\) and stimulated by square electrical pulses at 0.1 Hz. The preparation was then stretched until twitch reached its maximal amplitude. The experimental system was connected to a chart paper recorder (Goerz, Sevogor 120; Kipp & Zonen, Delft, The Netherlands) and a DTK computer, allowing storage of data and measurement of amplitude, time to peak, and time constant of relaxation. Amplitude was expressed in millinewtons or newtons. All experiments were performed at controlled room temperature (19–20°C).

Potassium contractures were elicited by sudden exposure of fibers to a solution containing a high concentration of potassium (146 mM) in the absence of electrical stimulation. In this solution, the [K+]\(^{-}\) product was kept constant to allow rapid recovery of resting membrane potential (upon return to mammalian physiological solution) and restoration of the amplitude of tension response. For this reason, chloride was replaced by l-glutamate. [K+] concentrations greater than 146 mM were not used because of hypertonicity. After spontaneous relaxation of the contracture, [K+]\(_{o}\) solution was replaced by physiological solution.

**Experimental Protocol.** The activation curve of K\(^{+}\) contracture was obtained by a rapid change from the control solution to one containing an elevated potassium concentration (20–146 mM [K\(^{+}\)]\(_{o}\)). After spontaneous relaxation of the contracture, K\(^{+}\) solution was replaced by physiological solution in which fibers recovered for 15 min before a new contracture cycle was induced. For each experimental fiber, data points were fitted with a Boltzmann equation (Dulhunty, 1991): 

\[
T = T_{\text{max}} \cdot \left[1 + \exp \left(\frac{E_{\text{a}} - E_{\text{c}}}{K_{\text{m}}}ight)\right]^{-1},
\]

where T is the K\(^{+}\) contracture amplitude at a given membrane potential (E\(_{\text{m}}\)), T\(_{\text{max}}\) corresponding to the amplitude of test K\(^{+}\) (146 mM), E\(_{\text{a}}\) the potential at which T = 0.5 T\(_{\text{max}}\), and K\(_{\text{m}}\) a slope factor. Peak K\(^{+}\) contracture tension values were plotted against corresponding mem-
brane potential values and normalized to maximal tension in 146 mM [K+]₀ solution.

The inactivation curve of K⁺ contracture was obtained by measuring test 146 mM [K+]₀ contracture amplitude after submaximal depolarization for 2 min in a conditioning [K+]₀ solution. Peak tension values of test K⁺ contractures were plotted against the corresponding membrane potential and normalized to maximal tension in 146 mM [K+]₀ solution. For each experiment fiber, data points were fitted with a Boltzmann equation: \( T = T_{\text{max}} \left[ 1 + \exp \left( \frac{E_m - E_c}{K} \right) \right]^{-1} \), where \( E_c \) is the potential at which \( T = 0.5 \) \( T_{\text{max}} \) and \( K \) is a slope factor.

**Membrane Potential Recording.** Using a conventional glass microelectrode (10–20 MΩ) connected to an electrometer input-negative capacitance amplifier, membrane potentials (\( E_m \)) were recorded first in physiological solution from bundles containing 20 to 30 fibers and then, after 5 min, in high-potassium solutions (20–146 mM [K+]₀) in the absence and presence of methyl jasmonate.

**Solutions.** Mammalian physiological solution contained 140 mM Na⁺, 6 mM K⁺, 3 mM Ca²⁺, 2 mM Mg²⁺, 156 mM Cl⁻, and 5 mM HEPES. The pH was adjusted to 7.35 by addition of a trisaminomethane solution. A depolarizing solution was prepared by replacing a given amount of Na⁺ with K⁺, and the \([K^+]/[Cl^-]\) product was kept constant by replacing Cl⁻ with l-glutamate.

Relaxing (pCa 9; solution A) and activating (pCa 4.5; solution B) solutions were prepared using the computer program of Godt and Norek (1986). All solutions were calculated to contain 10 mM EGTA, 30 mM imidazole, 30.6 mM Na⁺, 1 mM Mg²⁺, 3.16 mM MgATP, 12 mM phosphocreatine, and 0.3 mM dithiothreitol, with an ionic strength of 160 mM and a pH of 7.10. Solutions of intermediate Ca²⁺ concentrations were prepared by mixing two solutions of extreme concentrations (A and B) in appropriate proportions.

The ionic composition of the five solutions used to study loading time was the same as that of the relaxing solution, except that free magnesium and the concentrations of EGTA and calcium varied as described below. Solution 1: pCa 9, 10 mM EGTA, 1 mM Mg²⁺, and 25 mM caffeine; solution 2: pCa 9, 10 mM EGTA, and 1 mM Mg²⁺; solution 3: pCa 7, 10 mM EGTA, and 1 mM Mg²⁺; solution 4: pCa 7.5, 0.1 mM EGTA, and 0.1 mM Mg²⁺; and solution 5: pCa 7.5, 0.1 mM EGTA, 0.1 mM Mg²⁺, and 10 mM caffeine.

A stock solution of methyl jasmonate (417 mM) was prepared in an absolute ethanol solution (~0.2% for 100 μM methyl jasmonate). In control experiments, no significant effects were related to the presence of ethanol. All chemical products were purchased from Sigma Chemical Co. (S’Quentin Fallavier, France).

**Statistical Analysis.** All values are expressed as means ± S.E.M. for \( n \) observations. Statistical analysis was performed by ANOVA, and Scheffe post hoc analysis was performed when a significant \( P \)-value was obtained. A level of \( P < 0.05 \) indicates statistical significance.

**Results**

**Effects of Methyl Jasmonate on Ca²⁺ Uptake by the Sarcoplasmic Reticulum (Loading Time).** In previous studies, the effect of jasmonate and methyl jasmonate on SR Ca²⁺-ATPase was demonstrated in a series of experiments conducted on the purified Ca²⁺-ATPase from rabbit fast-twitch skeletal muscles (Starling et al., 1994). The purpose of the present study was to confirm these results in slow mammalian skeletal muscle. The effects on loading time of different methyl jasmonate concentrations (50, 100, and 200 μM) were studied on small bundles of saponin-skinned soleus fibers. The results (see Fig. 1a) show that the loading rate was not significantly changed by 50 μM methyl jasmonate, whereas an increase of 41% was found in the presence of 100 μM methyl jasmonate without change in the amplitude of maximum caffeine contracture reached after 5 min of loading. A larger methyl jasmonate concentration failed to increase the loading rate. In the presence of 200 μM methyl jasmonate, the values obtained were similar to control values (control: \( 48.2 \times 10^{-4} \pm 7.5 \times 10^{-4} \) s⁻¹, 200 μM: \( 46.8 \times 10^{-4} \pm 3.9 \times 10^{-4} \) s⁻¹, \( n = 6 \)). Thus, the present results (Fig. 1b), showing a bell-shaped relationship between loading rate and methyl jasmonate effects, are in line with previous findings obtained in the presence of jasmonate, menthone, menthol, and methyl jasmonate (Starling et al., 1994).

In saponin-skinned fibers, application of methyl jasmonate may produce changes in the calcium sensitivity of contractile proteins. To investigate this possibility, experiments were conducted on Triton-skinned fibers.

**Effects of Methyl Jasmonate on Ca²⁺-Activated Tension in Chemically Skinned Fibers.** Triton-skinned rat skeletal muscle fibers from soleus developed force when immersed in solutions of increasing Ca²⁺ concentrations. In
methyl jasmonate-free medium, the tension-Ca\(^{2+}\) relationship was characterized by half-maximal activation (pCa\(_{50}\)) of 6.266 ± 0.039, a Hill coefficient of 1.887 ± 0.086, and a maximal Ca\(^{2+}\)-activated tension (at pCa 4.5) of 91.93 ± 7.09 mN · mm\(^{-2}\) (n = 10). There were no significant differences between the mean values found in the absence and presence of 50, 100, and 200 μM methyl jasmonate (Fig. 2). These results suggest that the reduction in loading time found in the presence of methyl jasmonate was not due to changes in the calcium sensitivity of contractile proteins.

Our data, which indicate that the increase in the methyl jasmonate loading rate in slow skeletal muscle is likely due to methyl jasmonate stimulation of Ca\(^{2+}\)-ATPase, led us to investigate whether Ca\(^{2+}\)-ATPase activation interferes with mechanical activity in intact muscle cells.

**Effects of Methyl Jasmonate on Slow-Twitch Isometric Tension.** In control conditions, the twitch was characterized by an amplitude of 51.5 ± 1.2 mm, a time to peak of 144 ± 7 ms, and a time constant of relaxation of 403 ± 39 ms (n = 10). The application of different methyl jasmonate concentrations (50–200 μM) induced detectable changes in contraction parameters after only 30 s. However, a 2-min exposure was generally required to reach maximal steady-state effect (Fig. 3). All of the results reported here were obtained in steady-state conditions. At methyl jasmonate concentrations between 50 and 125 μM, amplitude, time to peak, and time constant of relaxation were modified and no additional effects were found for exposures longer than 2 min. For instance, in the presence of 100 μM methyl jasmonate, twitch amplitude was decreased by 29% (36.6 ± 1.1 mm; n = 8) (Fig. 4a), and time to peak tension and the time constant of relaxation reached 62 ± 6 ms (n = 8) and 98 ± 11 ms (n = 8), respectively (Fig. 4, b and c). The effects of methyl jasmonate at these concentrations were fully reversible after a 10- to 15-min return into methyl jasmonate-free medium. The effects observed at 125 μM were not potentiated by larger concentrations of methyl jasmonate tested and were not fully reversible. The present results, indicating that twitch characteristics were modified by methyl jasmonate, suggest that this compound may act at different steps in the excitation-contraction coupling process or in the control of myofilament function.

Because potassium contractures have been widely used for studying the depolarization-contraction coupling of skeletal muscle, experiments were conducted to determine whether methyl jasmonate affects the characteristics of [K\(^{+}\)]\(_{o}\) contractures.

**Effects of Methyl Jasmonate on 40 and 146 mM [K\(^{+}\)]\(_{o}\) Contractures.** In skeletal muscle, change from normal to high [K\(^{+}\)]\(_{o}\) solution led to the development of a transient contractile response that reached a maximum and then relaxed in an exponential manner, even when superfusion with a depolarizing solution was maintained (Léoty and Léauté, 1982). The characteristics of the high [K\(^{+}\)]\(_{o}\) contractures were voltage-dependent. At more depolarized membrane potentials, its amplitude was increased, its time to peak was reduced, and its spontaneous relaxation was faster. In methyl jasmonate-free medium, 40 and 146 mM [K\(^{+}\)]\(_{o}\) contractures were characterized by an amplitude of 1.06 ± 0.5 and 1.29 ± 0.4 n, a time to peak of 31.2 ± 2.1 and 14.1 ± 1.2 s, and a time constant of relaxation of 45.2 ± 4.9 and 8.1 ± 0.6 s (n = 8), respectively. The amplitude of [K\(^{+}\)]\(_{o}\) contractures was decreased, and the relaxation phase was greatly reduced by methyl jasmonate in a dose-dependent manner (Fig. 5). Maximum effects after application of 50 to 125 μM methyl jasmonate were obtained in 2 min and were fully reversible after a 12-min return into methyl jasmonate-free medium. In the presence of 100 μM methyl jasmonate, 40 and 146 mM [K\(^{+}\)]\(_{o}\) contractures were characterized by an amplitude of 0.39 ± 0.07 N and 0.83 ± 0.05 N (Fig. 6a), a time to peak of 18.9 ± 1.5 s and 15.4 ± 1.5 s (Fig. 6b), and a time constant of relaxation of 15.3 ± 1.9 s and 5.9 ± 0.5 s (Fig. 6c) (n = 8, P < 0.05), respectively. Increasing the methyl jasmonate concentration (150 μM) produced more marked changes in the parameters of 40 and 146 mM [K\(^{+}\)]\(_{o}\) contractures which showed an amplitude of 0.23 ± 0.08 N and 0.41 ± 0.02 N, a time to peak tension of 21.8 ± 2.4 s and 14.9 ± 1.6 s, and a time constant of relaxation of 21.6 ± 2.7 s and 7.6 ± 0.7 s (n = 8), respectively. In fact, at concentrations between 150 and 200 μM, the effects due to methyl jasmonate application for more than 2 min were not fully reversible.

Nevertheless, to determine whether the change in the relative tension of K\(^{+}\) contracture observed in the presence of methyl jasmonate was correlated with changes in the dependence of the membrane potential relative to [K\(^{+}\)]\(_{o}\) concentrations, membrane potentials were measured in the presence or absence of methyl jasmonate (100 μM).

**Effects of 100 μM Methyl Jasmonate on Membrane Potential.** Membrane potentials (E\(_{m}\)) were recorded on small bundles of intact soleus muscle (20–30 fibers) placed in mammalian physiological solution and high-potassium solutions (20–146 mM [K\(^{+}\)]\(_{o}\)) in the absence or presence of methyl jasmonate (100 μM). Comparative results with control methyl jasmonate (Table 1) indicated that no significant change in membrane potential was recorded for any potassium concentration tested.

Furthermore, it is generally admitted that the time course of K\(^{+}\) contracture tension during prolonged depolarization depends exclusively on activation and inactivation of the process regulating Ca\(^{2+}\) release from the SR. This proposed result led us to analyze whether the activation and inactivation curves of [K\(^{+}\)]\(_{o}\) contractures were affected by methyl...
jasmonate. Appropriate experiments were conducted in the presence of 100 μM methyl jasmonate, a concentration at which changes in twitch characteristics and 146 mM [K\textsuperscript{+}]\textsubscript{o} contracture were maximal and fully reversible.

**Effects of 100 μM Methyl Jasmonate on Voltage-Dependent Activation of K\textsuperscript{+} Contractures.** The relationships between the relative amplitude of K\textsuperscript{+} contracture and E\textsubscript{m} in control and methyl jasmonate conditions are shown in Fig. 7, and the parameters used to plot curves are listed in Table 2a. A comparison of the values showed that a shift to the right (more positive values) and a change in the slope of the activation curves were produced by methyl jasmonate, whereas the activation threshold was not significantly modified. Because this shift could indicate that methyl jasmonate has a direct effect on voltage-sensor properties, this possibility was investigated during voltage inactivation of the K\textsuperscript{+} contracture.

**Effects of 100 μM Methyl Jasmonate on the Time Constant of Relaxation of K\textsuperscript{+} Contracture.** Figure 8 clearly shows that the duration of the relaxation phase was reduced at all membrane depolarization after 2 min of exposure to 100 μM methyl jasmonate. Because the decay of K\textsuperscript{+} contractures could be fitted to a single exponential function, the time constant of relaxation was plotted against [K\textsuperscript{+}]\textsubscript{o}. The results indicate that the time constant of relaxation was significantly reduced by methyl jasmonate and that the effect was more marked at lower membrane depolarization.

The contracture decay produced by steady-state depolarization depends solely on the inactivation of excitation-contraction coupling and is not influenced by the kinetics of contractile proteins and the rate of calcium uptake by the SR (Dulhunty, 1992). Thus, the effect of methyl jasmonate on the time constant of relaxation seems to have been due to inactivation of voltage sensors.

**Effects of 100 μM Methyl Jasmonate on the Inactivation Curve.** Steady-state inactivation was assessed from the amplitude of test 146 mM K\textsuperscript{+} contractures (that reflects maximal activation in soleus muscle) (Fig. 9) after 3-min
equilibration in conditioning solutions containing 20 to 110 mM $[K^+]_o$. The relationships between the relative amplitude of $K^+$ contracture and $E_{m}$ in control and methyl jasmonate conditions are shown in Fig. 4, and the parameters used to plot curves are listed in Table 2b. A comparison of the values calculated showed that exposure to 100 $\mu$M methyl jasmonate induced no significant change in inactivation curves. This suggests that methyl jasmonate did not modify the fraction of voltage-sensitive molecules converted to active state upon depolarization and had no effect on the voltage dependence of the activation of $K^+$ contractures.

**Discussion**

To our knowledge, the results reported here provide a first description of the effects of methyl jasmonate on the contractile responses of intact mammalian skeletal muscle fibers. Soleus muscle was chosen because a stimulating effect of SR Ca$^{2+}$-ATPase was likely to be observed more easily on a slow than a fast-twitch muscle. Moreover, it has been shown that SR Ca$^{2+}$-ATPase plays a rate-limiting role in the twitch relaxation of single slow skeletal muscle. (Chua and Dulhunty, 1988).

Our results for saponin-skinned fibers show that methyl jasmonate concentrations of up to 100 $\mu$M reduced the time required to load the SR with calcium, whereas this effect was less marked at higher concentrations. These results are similar to those previously reported on the ATPase activity of the purified Ca$^{2+}$-ATPase from rabbit fast skeletal muscle (Starling et al., 1994), except that in our experiments, methyl jasmonate concentrations were 100-fold lower. The fact that comparable effects were obtained for calcium loading in both types of muscle suggests that the application of methyl jasmonate resulted in an increase of ATPase activity in slow muscle, with a maximum response at 100 $\mu$M.

The characteristics of the twitch generated by short electrical stimulation were modified by methyl jasmonate. In fact, our results indicate that methyl jasmonate has distinct
dose-dependent effects on the contraction of skeletal soleus muscle cells. Within the range of 50 to 125 μM, methyl jasmonate reversibility decreased twitch amplitude, reduced the time to peak, and accelerated the relaxation phase, whereas 150 to 200 μM methyl jasmonate depressed twitch irreversibly, without producing any further effects on time to peak and the time constant of relaxation. These results may indicate that methyl jasmonate, like jasmonate, menthone and menthol (Starling et al., 1994), has a dose-dependent effect on SR Ca\(^{2+}\)-ATPase in skeletal muscle. Thus, Ca\(^{2+}\) in the cytosol would probably decrease with methyl jasmonate exposure as a result of activation of the SR Ca\(^{2+}\) pump, reducing the Ca\(^{2+}\) ions bound to troponin C. This effect would produce acceleration of the relaxation phase in association with a reduction in twitch amplitude. This scenario is consistent with the results obtained for concentrations of up to 100 μM. However, because the changes due to methyl jasmonate were irreversible at larger concentrations, other
mechanisms involved in excitation-contraction coupling may have been affected. Depolarization-contraction coupling can be studied with the [K\textsuperscript{+}]\textsubscript{0} contracture model. Our results show a great similarity in the effects of methyl jasmonate on twitch and [K\textsuperscript{+}]\textsubscript{0} contractures. In particular, methyl jasmonate decreased tension and reduced time to peak tension and the relaxation phase in the 50 to 125 μM range. With larger concentrations (150–200 μM), methyl jasmonate induced a more marked decrease in tension but prolonged time to peak tension and the relaxation phase, which were still faster than controls. It was then shown that the time course of K\textsuperscript{+} contracture tension during prolonged depolarization depends exclusively on activation and inactivation of the process regulating Ca\textsuperscript{2+} release from the SR. This process relates to the conformational states of voltage-sensitive dihydropyridine receptor molecules in the transverse tubule membrane (Caputo, 1972; Dulhunty, 1991). Moreover, a close similarity between the voltage dependence of tension and charge movement has been observed (Chandler et al., 1976; Rakowski, 1981). A general model for depolarization-contraction coupling has suggested that depolarization sequentially converts a fraction of the resting voltage sensors to an active state and then to an inactive state and that K\textsuperscript{+} contracture tension is proportional to the fraction of voltage sensors in the active state (Caputo, 1972; Dulhunty, 1991).

Although the excitation-contraction coupling mechanism was not affected by methyl jasmonate, the shift in the acti-

**TABLE 2**

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<tr>
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<th>Control</th>
<th>Methyl Jasmonate</th>
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<tr>
<td>E\textsubscript{a}</td>
<td>42.9 ± 0.9</td>
<td>27.9 ± 1.1*</td>
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<tr>
<td>K\textsubscript{a}</td>
<td>6.3 ± 0.8</td>
<td>10.5 ± 1.2*</td>
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<tr>
<td>E\textsubscript{i}</td>
<td>38.8 ± 0.2</td>
<td>39.4 ± 0.1</td>
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<tr>
<td>K\textsubscript{i}</td>
<td>3.3 ± 0.4</td>
<td>2.6 ± 0.5</td>
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* P < 0.05.
vation curve to more positive values, resulting from exposure to methyl jasmonate, could be related to changes in membrane potential and/or the depolarization rate and/or Ca\(^{2+}\) sensitivity for contractile proteins.

No significant differences in membrane potential, in different depolarization [K\(^+\)]\(_0\) solutions, were found for values recorded in methyl jasmonate-free medium and those in the presence of 100 \(\mu\)M methyl jasmonate. Moreover, the rate of change in membrane potential is an important factor in terms of the kinetics of K\(^+\) contractures, particularly because it affects response amplitude. With respect to [K\(^+\)]\(_0\), measurements of tension and membrane potential in depolarizing solutions showed that the depolarization rate was similar in the presence or absence of methyl jasmonate. Thus, it is likely that the shift of the activation curve to the right observed in the present study was not associated with a methyl jasmonate-induced change in membrane potential or depolarization.

Nevertheless, it is possible that the shift in the activation curve in the presence of methyl jasmonate was related to a change in the Ca\(^{2+}\) sensitivity of contractile proteins. Yet, experiments performed on Triton-skinned fibers showed that the application of different methyl jasmonate concentrations (50, 100, and 200 \(\mu\)M) had no significant effect on maximal activated Ca\(^{2+}\) force and the Ca\(^{2+}\) sensitivity of contractile proteins.

To date, it is recognized in mammalian skeletal muscle that the slow decay in tension during prolonged steady-state depolarization depends on the inactivation of excitation-contraction coupling and is not influenced by the kinetics of contractile protein response and the rate of calcium uptake by the SR (Dulhunty, 1992). However, it has recently been reported that the slow decay of tension in frog skeletal muscle during prolonged steady-state depolarization depends not only on inactivation of the process regulating Ca\(^{2+}\) release from the SR, but also on the ability of the SR to pump Ca\(^{2+}\).

Experiments involving potassium contractures showed that the activation curve was shifted to the left by cyclopiazonic acid (CPA), whereas the inactivation curve remained unchanged (Mème and Leoty, 1999). It is now well established that CPA is a specific inhibitor of sarcoplasmic Ca\(^{2+}\)-ATPase in skeletal muscle (Seidler et al., 1989). Thus, the fact that the effect of methyl jasmonate on the activation curve was opposite to that of CPA suggests that methyl jasmonate is a stimulator of sarcoplasmic Ca\(^{2+}\)-ATPase.

In summary, our data show that methyl jasmonate has a similar effect on the characteristics of twitch tension and [K\(^+\)]\(_0\) contractures of slow-twitch mammalian skeletal muscle fibers and that these effects are fully reversible for concentrations less than 125 \(\mu\)M. Moreover, the difference between the contractile responses obtained in the presence and absence of methyl jasmonate could be related to the acceleration of the SR to pump Ca\(^{2+}\) in the absence of any detectable modification in the excitation-contraction coupling process. Finally, methyl jasmonate appears to be a convenient tool for selective activation of SR Ca\(^{2+}\)-ATPase in slow-twitch skeletal muscle.

References


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