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Novel inhibition of contractility by wortmannin in skeletal muscle

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1 The effects of wortmannin and 2-(4-morpholinyl)-8-phenyl-1[4H]-benzopyran-4-one (LY294002), inhibitors of phosphatidylinositol 3-kinase, on the contractile responses of murine skeletal muscle were studied. Wortmannin (10–100 μM) suppressed twitch and tetanic contraction evoked by field stimulation of diaphragm without causing elevation of muscle tone. The inhibition was quasi-irreversible with $IC_{50} \sim 15$ μM. In contrast, LY294002 increased twitch responses and elevated muscle tone.

2 Wortmannin reversibly depressed the maximal slope of action potential upstroke by ~40% and inhibited the membrane depolarization and spontaneous burst of action potential induced by crotamine, a polypeptide toxin that activates the Na^+ channel of skeletal muscle.

3 Wortmannin inhibited contractures evoked by high K^+, ryanodine and caffeine, but potentiated the contracture induced by rapamycin, which binds to myoplasmic FK506 binding protein, an immunophilin closely associated with the ryanodine receptor. The contractures elicited by cardiotoxin, which disrupts the integrity of sarcolemma and thereby elevates 'myoplasmic' Ca^{2+} level, were suppressed only slightly.

4 In placed left atrium and ventricular strip, wortmannin and LY294002 produced a positive inotropic effect.

5 The results suggest that, in addition to depressing the Ca^{2+} mobilization from sarcoplasmic reticulum, wortmannin exerts a novel inhibitory action on the excitation-contraction coupling in skeletal muscle but not in cardiac muscle.

Keywords: Ryanodine receptor; rapamycin; excitation-contraction coupling; wortmannin; phosphatidylinositol 3-kinase; positive inotropic effect

Introduction

In vertebrates the contraction of skeletal muscle is a complex cascade initiated sequentially by depolarization of the plasma membrane and the invaginated transverse tubular network, release of Ca^{2+} from intrcellular organelle – the sarcoplasmic reticulum (SR), and the binding of Ca^{2+} to a regulatory protein enabling actin-myosin interaction and force generation. The two key elements of the excitation-contraction (E-C) coupling process that relates surface electrical signal to interior Ca^{2+} depot, i.e., the transverse tubule voltage sensor and the Ca^{2+} release channel, are morphologically distinct entities (Marty *et al*., 1994; Franzini-Armstrong & Protasi, 1997; Zucchi & Ronca-Testoni, 1997). At the sarcolemma side, large intramembrane particles are clustered in groups to form tetrads – the putative voltage sensor. In the myoplasm is an electron-dense foot structure spanning a gap that separates the membranes of transverse tubule and SR. The foot structure is the target of ryanodine, a plant alkaloid that opens the Ca^{2+} release channel. The tetrads localize juxta-opposite to the foot structure, suggesting an intimate molecular transduction architecture (Ríos *et al*., 1993; Schneider, 1994).

Recently, a microbial metabolite wortmannin, isolated from a variety of fungal species, has been demonstrated to be capable of blocking contraction of gastro-intestinal and vascular smooth muscles, probably by inhibition of myosin light chain kinase (Burke *et al*., 1996; Takayama *et al*., 1996). The compound also is a potent inhibitor of phosphatidylinositol (PI) 3-kinase, which is essential for glucose transport and for terminal differentiation of skeletal muscle cells (Powis *et al*., 1994; Kaliman *et al*., 1996; Wojtaszewski *et al*., 1996). In view of the difference in the E-C coupling for smooth muscle and skeletal muscle, we investigated its effect on the contractile responses of skeletal muscle.

Methods

Nerve-muscle preparation

Mice (ICR strain, 25–30 g) were stunned and exsanguinated. Phrenic nerve-hemidiaphragms were isolated and bathed in Tyrode solution (composition in mM: NaCl 137, KCl 2.8, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11, NaH_2PO_4 0.33 and dextrose 11.2). Tyrode solution was maintained at 35–37°C and the pH was adjusted to 7.3–7.4 by aeration with a gas mixture of 5% CO_2 in O_2. Preparations were loaded with 5–10 mN resting tension. Indirect contractions were evoked by stimulation of the trunk of phrenic nerve with supramaximal pulses of 0.03 ms duration. Direct contractions were elicited by field stimulation of diaphragm muscle with biphasic pulse of 0.3 ms duration. Twitch responses and tetanic contractions were evoked, respectively, by low (0.01–0.2 Hz) and high (30–100 Hz for 5 s) frequency stimulations. High KCl Tyrode solution was prepared by a direct addition of concentrated KCl solution to the organ bath to make 50 mM (a hypertonic solution) or by a substitution of 120 mM KCl for equi-mole of NaCl (an isotonic solution). The former solution has the advantage that the depolarization-induced acute activation of muscle action potential and the associated contraction are not blocked. Contractile responses were recorded isometrically and quantified by contraction amplitude for twitch responses and by contraction-time integral for tetanic contractions and contractures evoked by chemicals (KCl, ryanodine, caffeine, cardiotoxin or rapamycin). For the latter experiments, preparations were challenged only once with one of the

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contracture-inducing agents for a duration long enough to attain peak and plateau responses.

Cardiac muscle preparations

Left atrium and strips of ventricular muscle were isolated from rats (Wistar strain, 200–300 g), bathed in Tyrode solution and loaded with a resting tension of 2–5 mN. Preparations were driven at 0.5 or 2 Hz with rectangular pulses (40 V, 0.5 ms) delivered through a pair of punctate electrodes.

Electrophysiological studies

The microelectrodes for intracellular recordings were filled with 3 M KCl and had a resistance of 5–10 MΩ. Action potentials of diaphragm muscle were elicited by injections of depolarizing current via the recording electrode. The magnitude of currents (20–80 nA) were adjusted fibre-by-fibre so that, under the control conditions, action potentials could be initiated within 1 ms. Signals were coupled to a differentiator and the maximum slope of action potential upstroke (dV/dt) was calculated. Membrane resistances were estimated by calculating the slope of the current-voltage plots constructed by injecting cathodal currents ranging from 7.5–50 nA which hyperpolarized resting membrane by 5–20 mv.

Statistics

Data were pooled from 3–6 preparations and presented as means ± s.e. Differences between means were analysed by Student's *t* test and a statistical *P* value of less than 0.05 was considered significant. The effects of wortmannin on contracture-time integrals were carried out by paired experiments with a precaution that the control twitch amplitudes of the paired group differed by less than 10%.

Chemicals

Specific chemicals were ryanodine (Research Biochemicals International), rapamycin, wortmannin and 2-(4-morpholinyl)-8-phenyl-1[4H]-benzopyran-4-one (LY294002) (Sigma). Wortmannin and LY294002 were dissolved in dimethylsulphoxide whereas rapamycin was dissolved in ethanol. The final concentration of each vehicle in the organ bath was less than 0.1% and had no signficant effect on the contractile response.

Results

Inhibitions of twitch and tetanic contraction by wortmannin

After equilibration of the nerve-muscle preparation, the direct and indirect twitch forces (0.1 Hz) and the tetanus elicited by train of high frequency direct field stimulation (100 Hz for 5 s every 60 min) maintained steady values for the first 2 h (21 ± 2 mN, 19 ± 1 mN, and 1090 ± 110 mNs, respectively). The peak amplitude of tetanus (200 ± 20 mN) was 9.8 ± 0.4 times that of twitch. Even after protracted incubation for 4 h, contractilities decreased only slightly to 87 ± 3% of control. Since wortmannin inhibits glucose transport of the nerve-muscle preparation, we first investigated the effect of glucose deprivation on the contractility in order to delineate the possible effects of wortmannin on the contractile response contributed by this action. After 1.5 h incubation in the glucose-free or sucrose-substituted Tyrode solution, the direct twitch was well-maintained at 106 ± 4% of control level while the tetanus was reduced to 89 ± 3%. A longer incubation period (3 h) resulted in a moderate decrease of twitch to 82 ± 4% and a severe suppression of tetanic contraction down to 32 ± 4% of control. Hence, the treatment with wortmannin was limited not to exceed 90 min in the experiments in order to separate effects other than the inhibition of glucose transport.

Wortmannin (10–300 μM) progressively suppressed indirect and direct twitches and tetanic contraction in a concentration-dependent manner (Figure 1a,b and c). No steady-state inhibition was attained up to 90 min incubation. Wortmannin appeared to inhibit the indirect twitch more rapidly than the inhibition of direct twitch, suggesting an extra action(s) on the synaptic transmission. However, this issue will not be addressed in the present study. The IC_{50} calculated from the effect at 70 min treatment was 17 ± 3 μM for direct twitch and 11 ± 2 μM for tetanic contraction. An early phase short-term (~3 min) augmentation of twitches up to 11 ± 4% over control was produced when a high concentration (100–300 μM) of wortmannin was applied (Figure 1a). Even after treatment with a very high concentration of wortmannin (300 μM), the resting tension (muscle tone) was not elevated. Wortmannin produced no alterations in the time course of the twitch responses; the 90% contraction duration in control and that after 90% suppression were the same (34 ± 3 *vs* 31 ± 3 ms).

To test whether the inhibition by wortmannin was use-dependent, muscle fibres were field stimulated at a very low frequency of 0.01 Hz in one group, and in the other group, diaphragms were stimulated, in addition to a higher background stimulation at 0.2 Hz, with trains of high frequency pulses (30 Hz for 5 s every 30 s). Under the two stimulation protocols, the times to 90% block of direct twitch response by wortmannin were similar (71 ± 5 *vs* 79 ± 4 min at 30 μM and 49 ± 3 *vs* 55 ± 4 min at 100 μM).

Irreversibility of block

Diaphragms were treated with a high concentration of wortmannin (100 μM for 5 or 50 min), and then preparations were washed with wortmannin-free Tyrode solution every 10–15 min. When the preparations were in contact with wortmannin for a short time period (5 min incubation), the twitch force after washout of wortmannin maintained a steady level for 2 h and declined slowly to 82 ± 4% of control level during 3–4 h, indistinguishable from control preparations not exposed to wortmannin. However, when exposed to wortmannin for 50 min and direct twitches suppressed by ~90%, the contractile responses were not restored after extensive washings for 2 h (Figure 1a).

Inhibition of KCl-contracture

At a moderate concentration of 50 mM, KCl elicited a spike-like contraction followed by a gradual and persistent elevation of muscle tone up to 259 ± 25% of the amplitude of direct twitch response for at least 30 min (Figure 2). The fast contraction spike was suppressed by tetrodotoxin (0.3 μM), whereas the slow contracture was virtually unaltered by the toxin. At a high concentration of 120 mM, KCl elicited a tetrodotoxin-resistant elevation of muscle tone up to 367 ± 31% of the amplitude of direct twitch and then the muscle tone relaxed rapidly (within 10 min) to a low level (87 ± 19% of twitch amplitude), probably caused by the drastic membrane depolarization-induced inactivations of the putative voltage sensor. In the test group that had been preincubated

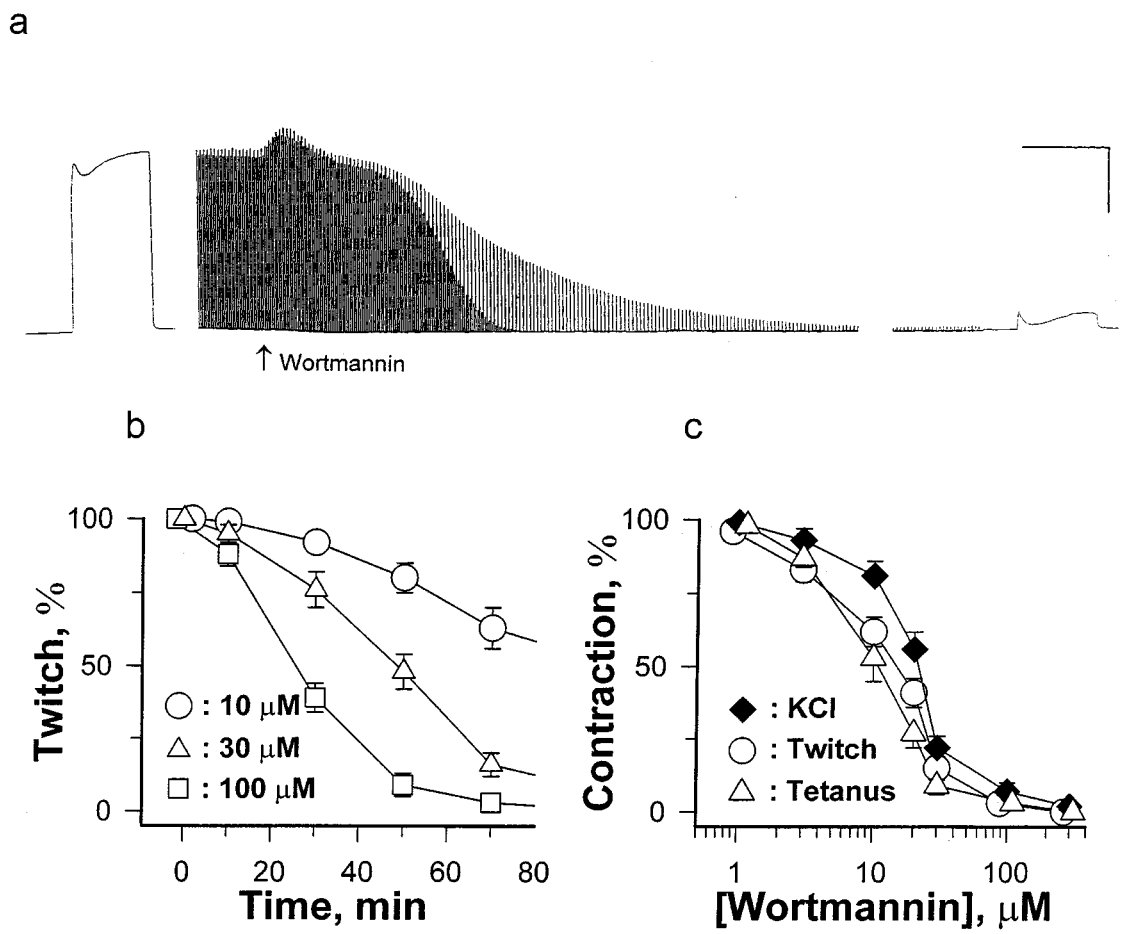

Figure 1 Wortmannin inhibited depolarization-induced contractile responses in mouse diaphragm (a) Left panel: control tetanic contraction evoked by field stimulation (100 Hz for 5 s) of diaphragm. Middle panel: effect of wortmannin on indirect and direct (the larger responses) twitches elicited by alternate stimulations of phrenic nerve and diaphragm at 0.1 Hz. Right panel: twitch and tetanic responses 2 h after washout of wortmannin. Calibrations: 10 mN and 500 s (for twitches), or 100 mN and 5 s (for tetani). (b) Time course of twitch-inhibition by wortmannin. The inhibition continued progressively up to 3 h. However, due to the possible intervention by impairments of glucose transport (see text), only the initial part of the inhibition is illustrated. Error bars, if within symbols are not indicated. (c) Concentration-inhibitory response relationship for wortmannin. The ordinate scale indicates % contractile responses (after 70 min treatment with wortmannin) of direct twitch amplitudes, contraction-time integrals of tetani evoked by 100 Hz for 5 s stimulation of diaphragm, or contractures evoked by 50 mM KCl Tyrode solution for 25 min.

with wortmannin (100 μM) for 50 min, KCl (50 and 120 mM) induced a feeble elevation of muscle tone (cf. Figure 2); the KCl contracture-time integral was suppressed by more than 95%. The effect was concentration-dependent (Figure 1c) and the IC_{50} of wortmannin was 22 ± 3 μM, close to that for inhibition of direct twitch.

Inhibition of ryanodine- and caffeine-induced contracture

In order to minimize possible differences of intervention between the control and wortmannin-treated preparations, resulting from field stimulation-induced use-dependent promotion of the action of ryanodine, tetrodotoxin (0.3–0.4 μM) was added to the control preparations to block the direct twitch response. Ryanodine (1–50 μM) and caffeine (5–75 mM) induced a dose-dependent elevation of muscle tone (Figure 3). The peak amplitudes of ryanodine- and caffeine-contracture reached 413 ± 28% and 483 ± 41%, respectively, that of direct twitch. Wortmannin (100 μM) effectively suppressed the contractures induced by low concentrations, but not those by high concentrations, of ryanodine or caffeine, resulting in a righward shift of concentration-contracture response relationships.

Effects on cardiotoxin-contracture

To see whether wortmannin might directly suppress the contractile machinery, we studied its effect on the contracture elicited by cardiotoxin, which disrupts sarcolemma integrity and thereby increases permeability of membrane to Ca^{2+} (Chang, 1979). As illustrated (Figure 4a), cardiotoxin, at a low concentration of 0.3 μM, elicited a low magnitude and short-term contracture. Wortmannin (100 μM), though greatly suppressing twitch responses, caused only a very slight inhibition (<15%) on either the contracture amplitude or the contracture-time integral evoked by cardiotoxin (Figure 4b) and produced no inhibition of the large contracture induced by a high concentration of cardiotoxin (1.5 μM, not shown).

Potentiation of rapamycin-induced contracture

The ryanodine receptor is tightly associated with an intracellular 12 kD immunophilin, the FK506 binding protein (FKBP), in a stoichiometry of 1:4 (Jayaraman *et al.*, 1992; Timerman *et al.*, 1993). The FKBP-ryanodine receptor interaction is hampered by rapamycin, an immunosuppressant.

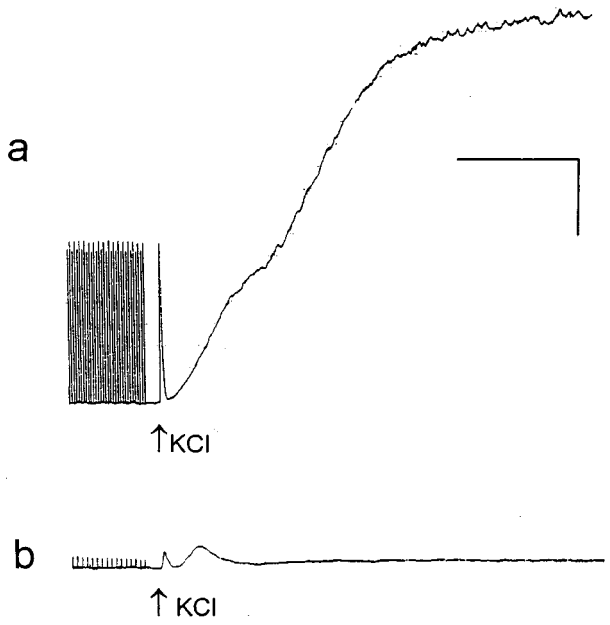

Figure 2 Inhibition of KCl-induced contracture by wortmannin. (a) Control twitch responses and high KCl (50 mM) Tyrode solution-evoked biphasic contracture. (b) The responses in another preparation treated with wortmannin 100 μM for 50 min (traces of the initial 45 min were omitted). Calibrations: 10 mN and 500 s.

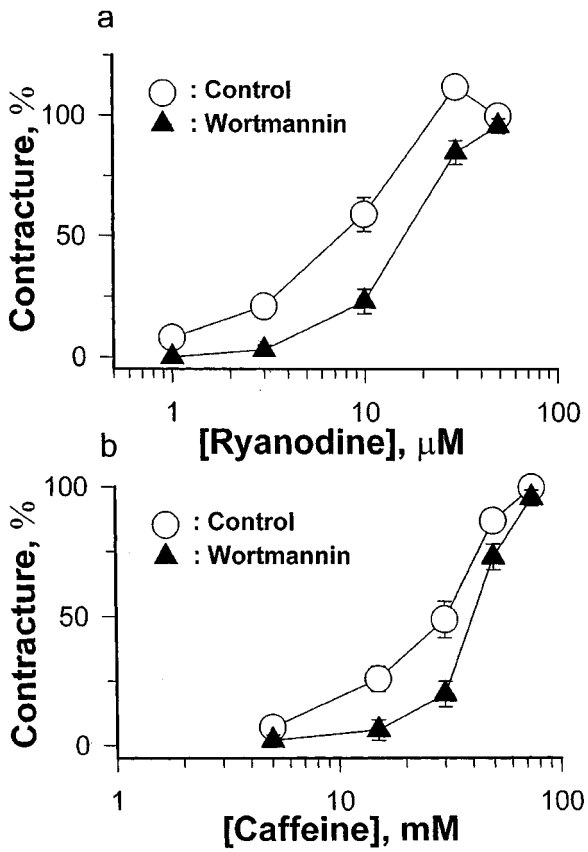

Figure 3 Inhibition of ryanodine- and caffeine-contractures by wortmannin. Concentration-contracture response relationships for ryanodine (a) and caffeine (b). In control, preparations were first treated with tetrodotoxin (0.3–0.4 μM) and the direct twitches depressed by ~90% and then challenged with ryanodine or caffeine. In another group, wortmannin (100 μM) was added for 50 min, which depressed direct twitches to ~10% of control. The contracture-time integral induced by 25 min treatment with ryanodine 50 μM or caffeine 75 mM in the absence of wortmannin was set at 100%.

Rapamycin slowly elevated muscle tone and suppressed twitch responses (Figure 5a). The inhibition of twitch response was not reversed on prolonged washing for 2 h (not shown). Figure 5b depicts the concentration-contracture response relationship for rapamycin. At the highest concentration (30 μM) tested, the contracture reached $163 \pm 19\%$ of twitch amplitude. When preparations were first treated with wortmannin (30–100 μM) and the twitch responses suppressed, subsequent application of rapamycin elicited a greater degree of elevation of muscle tone than in those preparations not pretreated with wortmannin (Figure 5a lower panel, b).

Effects on muscle Na^+ channel

The membrane potential and membrane resistance were not altered by low concentrations (<30 μM) of wortmannin. At high concentrations (100–300 μM), wortmannin elicited a low level of membrane depolarization (-75 ± 3 *vs* -82 ± 2 mV) and caused a decrease of membrane resistance (596 ± 25 *vs* 671 ± 33 kΩ). Figure 6 compares the magnitude of inhibition (at various times after wortmannin) on the amplitude and dV/dt of muscle action potential and on direct twitch. The amplitude and dV/dt of action potential were suppressed at the most by 40%. It is evident that wortmannin produces much greater suppressions of twitch response than of muscle action potential. Moreover, action potential, but not contractility, was restored substantially after washout of wortmannin. The effects of wortmannin on Na^+ channel were further studied by interactions with crotamine, a basic polypeptide that activates the Na^+ channel of mammalian skeletal muscle (Hong & Chang, 1983). Crotamine elicited limited membrane depolarizations by ~20 mV and induced burst discharges of muscle action potential. Wortmannin at 30–100 μM, restored the membrane potential (-76 ± 3 *vs* -62 ± 4 mV) and suppressed action potential burst. After washout of wortmannin and crotamine, the membrane depolarization and spontaneous firings of action potential resumed. These observations suggest that, in contrast to the persistent inhibition on the contractile response, the blockade of the Na^+ channel by wortmannin is reversible. Wortmannin had no effect on the membrane depolarization elicited by high KCl (50 mM) Tyrode solution (-34 ± 3 *vs* -36 ± 3 mV).

Reversible increase of twitch responses by LY294002

The effects of LY294002, which is also a membrane permeable specific inhibitor of PI 3-kinase (Vlahos *et al.*, 1994), on the contractile responses were tested. In contrast to wortmannin, LY294002 (10–300 μM) caused a prominent facilitation of both direct and indirect twitches up to $87 \pm 11\%$ over control and a slight elevation of muscle tone (Figure 7). In preparations pretreated with LY294002, wortmannin still produced a quasi-irreversible inhibition of contraction (not shown).

Positive inotropic effect in paced cardiac muscle

We compared the effect of wortmannin on the E-C coupling of cardiac muscle, another striated muscle preparation. In the paced left atrium and strip of ventricular muscle, wortmannin (10–100 μM) elicited a marked increase in contractile force up

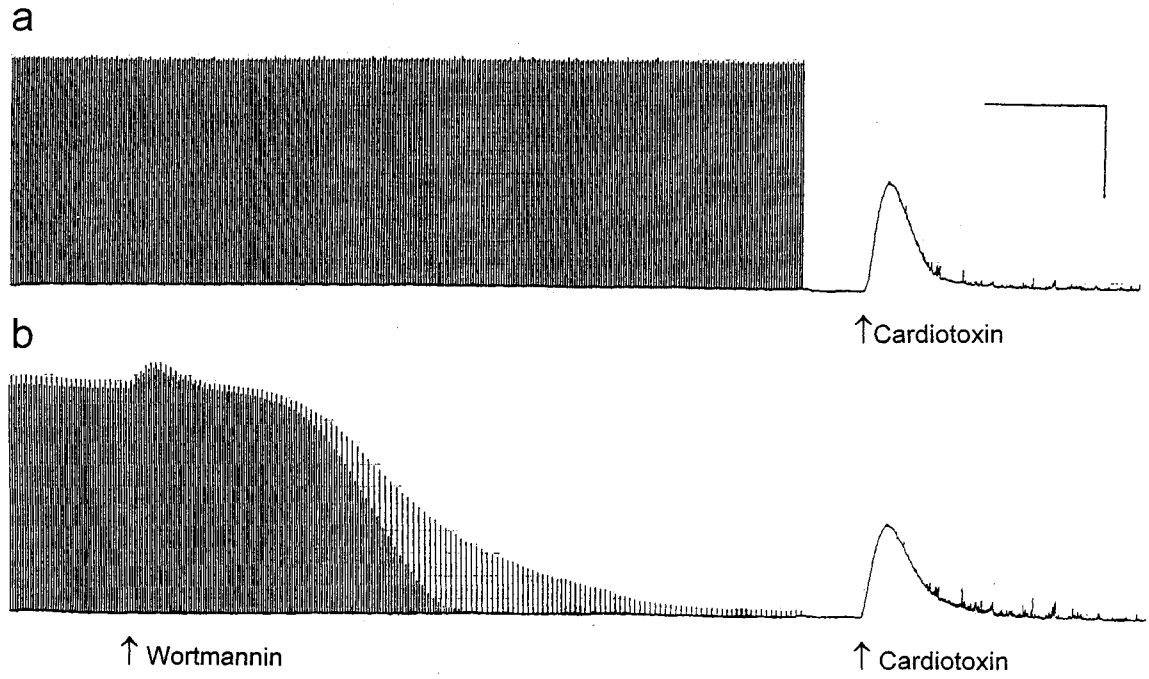

Figure 4 Effects of wortmannin on cardiotoxin-induced contracture. (a) Control twitch response and cardiotoxin (0.3 μM)-induced contracture. (b) Responses in the preparation treated with wortmannin 100 μM for 50 min. Calibrations: 10 mN and 500 s.

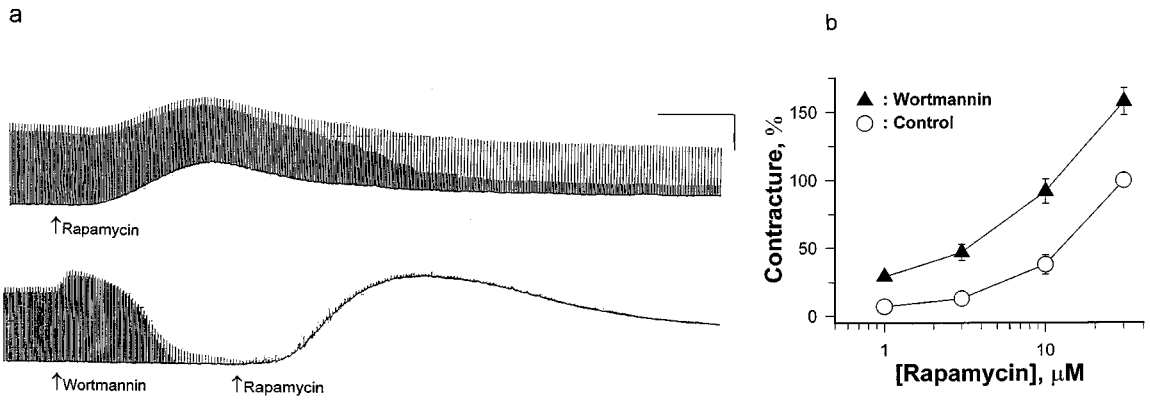

Figure 5 Effects of wortmannin on rapamycin-induced contracture. (a) Tracings showing the effects of rapamycin 10 μM on twitches and muscle tone in the absence (upper panel) or presence of wortmannin 100 μM (lower panel). Calibrations: 10 mN and 500 s. (b) Concentration-contracture response relationship for rapamycin alone, or after treatment with wortmannin (30–100 μM for 20–50 min). The contracture-time integral induced by 25 min treatment with rapamycin 30 μM alone was set at 100%.

to 387±41% of control with no induction of aberrant automaticity (Figure 8). The 90% contraction duration was prolonged slightly (118±4 *vs* 107±3 ms). The positive inotropic effect lasted for hours and was not inhibited by pretreatment with α- and β-adrenoceptor blockers, phentolamine and propranolol (3 μM). In the spontaneous beating right atrium, wortmannin (100 μM) slowed down the heart rate (with concomitant servo-feedback increase of contractility) and induced abrupt cease of beating. LY294002 produced effects similar to those of wortmannin in the cardiac preparations and, in addition, elevated resting tension at a high concentration (100 μM, not shown). The effects of wortmannin and LY294002 were slowly reversible after washout.

Discussion

In murine skeletal muscle, wortmannin inhibited contractions evoked by depolarizations of muscle membrane (field stimulation and high K^+ media) and contractures elicited by direct stimulation of the SR Ca^{2+} channel (ryanodine and caffeine). However, the contractile response evoked by cardiotoxin, which facilitates entry of extracellular Ca^{2+} into myoplasm, were barely changed. Despite the suppression of sensitivity toward ryanodine and caffeine, the maximal contractures induced by the two agents were not reduced. The inhibitory effect was not consequent to inhibition of glucose transport. These results suggest that the E-C coupling, but not the contractile machinery or the SR Ca^{2+} content, is affected by wortmannin.

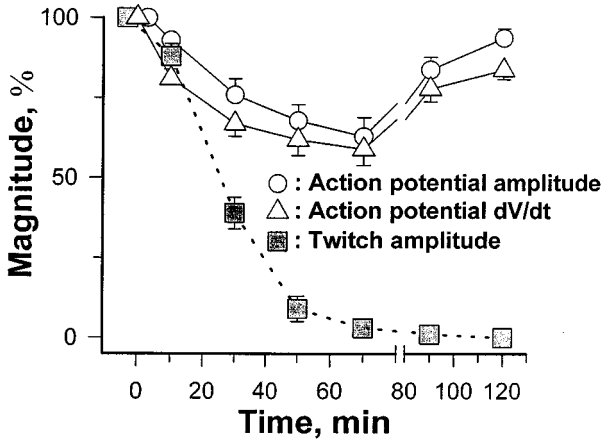

Figure 6 Time courses for inhibition by wortmannin of the amplitude and maximal slope of muscle action potential. Preparations were treated with wortmannin 100 μM for 70 min and then washed out (breaks) with Tyrode solution every 10 min. Muscle action potentials were evoked by injections of depolarizing currents via the recording electrode. The amplitude and maximal slope (dV/dt) of control action potential were, respectively, 113 ± 13 mV and 725 ± 31 V s^{-1}. Inhibitions of direct twitch by wortmannin 100 μM were included for comparison. In the control group, not exposed to wortmannin, action potential amplitude and dV/dt maintained ~95% of control at time 120 min.

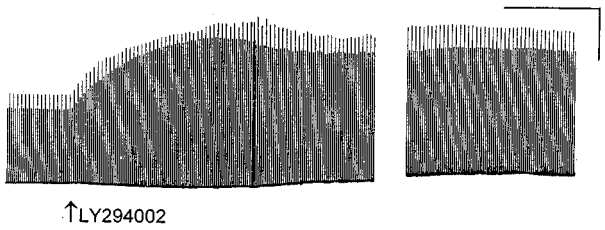

Figure 7 Facilitation of twitch responses by LY294002. Left panel: preparation was treated with LY294002 100 μM. Right panel: twitch responses 2 h later. Calibrations: 10 mN and 500 s.

Figure 8 Increase of rat ventricle contractility induced by wortmannin. Ventricular strip was paced at 2 Hz and treated with wortmannin 100 μM. Calibrations: 10 mN and 100 s, or 0.1 s (for expanded waveforms).

Biochemical studies have revealed that wortmannin inhibits myosin light chain kinase (Nakanishi *et al*., 1992). In smooth muscles, the enzyme, when complexed with Ca^{2+}-calmodulin, catalyzes phosphorylation of myosin light chain which then interacts with the actin filament resulting in cross-bridge formation and force generation (Somlyo & Somlyo, 1994). The cross-bridge formation in the striated muscles is controlled primarily by troponin-Ca^{2+} interaction. Nevertheless, phosphorylations of skeleton muscle myosin light chain might increase the efficiency of E-C coupling during or after tetanic stimulation, suggesting a modulatory, rather than obligatory, function (Sweeney *et al*., 1993). Therefore, it is unlikely that the suppression of twitch response in skeletal muscles by wortmannin is due to inhibition of the enzyme. Additionally, wortmannin is a potent irreversible inhibitor of PI 3-kinase (Arcaro & Wymann, 1993; Powis *et al*., 1994). Since the enzyme plays an important role in cellular signalling pathways (Ui *et al*., 1995) one might speculate that the kinase also participates in the E-C coupling of skeletal muscle. However, the IC_{50} of wortmannin for skeletal muscle contraction was 2–3 orders higher than that for PI 3-kinase. Furthermore, another inhibitor of the kinase, LY294002, produced augmentation of twitch with an elevation of muscle tone– effects opposite to those of wortmannin. These results indicate that the blocking action of wortmannin on the contractile response of skeletal muscle is not causally linked to inhibition of PI 3-kinase although, by way of inhibition of the enzyme-controlled glucose transport, wortmannin could elicit a delayed suppression of contractility.

Site(s) of action of wortmannin

Major steps in the E-C coupling process include activation of sarcolemma voltage sensor and opening of intracellular Ca^{2+} release channel on the SR. Wortmannin, at high concentrations, caused a reversible and partial inhibition of the Na^+ channel and could reduce the activation of the voltage sensor by muscle action potential. This effect, though contributing to the suppression of field stimulation-induced contraction, cannot explain the irreversible inhibition of the E-C coupling activated by K^+-depolarization which by-passes activation of the Na^+ channel.

The voltage sensor bears distinct high affinity binding sites for L-type Ca^{2+} antagonists. The antagonists have been shown to enhance the contractility evoked by transient depolarization (Dulhunty & Gage, 1988; Chang *et al*., 1989), yet, at a low temperature, depress long depolarization-evoked contracture, a use-dependent block (Eisenberg *et al*., 1983; Neuhaus *et al*., 1990). It was proposed that Ca^{2+} antagonists transform the voltage sensor into an inactivated state provided that the voltage sensor is pre-activated by prolonged depolarization (Hui *et al*., 1984). Apparently, occupation of the Ca^{2+} antagonist binding sites on the sarcolemma cannot explain the blocking action of wortmannin, which equi-potently inhibited contractions evoked by either transient or prolonged membrane depolarization without a prerequisite for long-term priming depolarization or lowering of temperature.

The suppression of ryanodine- and caffeine-induced contracture by wortmannin suggests that the Ca^{2+} mobilization from SR is inhibited. The Ca^{2+} release channel on SR is modulated by a cytoplasmic protein FKBP, which coordinates the opening of the Ca^{2+} channel to a maximum conductance state (Brillantes *et al*., 1994; Wagenknecht *et al*., 1996). Selective interruption of the FKBP-ryanodine receptor interaction, for example with the immunosuppressant FK506 or rapamycin, has a profound influence on SR Ca^{2+} release. The depolarization-induced channel conductance and phasic Ca^{2+} release, but not the tonic Ca^{2+} release effected by caffeine, are reduced (Jayaraman *et al*., 1992; Lamb & Stephenson, 1996). In addition to an association with FKBP, rapamycin could directly bind to the ryanodine receptor to cause Ca^{2+} release (Ahern *et al*., 1997). These activities might account for the effects of rapamycin on the intact skeletal muscle: inhibition of twitches with simultaneous elevation of muscle tone. Obviously, wortmannin does not behave as rapamycin does because no elevation of muscle tone was associated with its inhibitory effect on contraction. Interest-

ingly, however, wortmannin potentiated contractures induced by rapamycin, suggesting tht wortmannin does not act simply as an intracellular Ca^{2+} antagonist and that the actions of wortmannin and rapamycin might be closely related. Since wortmannin inhibited depolarization-induced contraction more efficaciously than it did ryanodine-/caffeine-induced contracture (despite the peak amplitude on tetanic stimulation being far larger than that with chemical stimulation), it seems that wortmannin has an additional effect upstream to SR Ca^{2+} release, e.g., it interferes with the depolarization-induced activation of the voltage sensor and/or the subsequent interaction with the cytoplasmic foot structure.

The inhibition of the Na^+ channel and of SR Ca^{2+} release by wortmannin are reminiscent of the actions of local anaesthetics, such as tetracaine (Xu *et al*., 1993), and dantrolene sodium, a hydantoin derivative (Ohta *et al*., 1990). However, local anaesthetics and dantrolene sodium, in contrast to wortmannin, do not augment the contracture induced by rapamycin (unpublished data).

Taken together, wortmannin attacks E-C coupling in a manner not shared by conventional agents. More studies are required to elucidate its novel muscle relaxant actions. It may be anticipated that modified derivatives of wortmannin with specific blocking action on skeletal muscle SR Ca^{2+} release, but devoid of inhibitory actions on PI 3-kinase and myosin light chain kinase, might be of therapeutic benefit in the management of malignant hyperthermia, a pharmacogenetic disease caused by an abnormal increase in the myoplasmic Ca^{2+} level in association with the use of some general anaesthetics and depolarizing muscle relaxants (MacLennan & Phillips, 1992; Mickelson & Louis, 1996).

The positive inotropic effect of wortmannin in cardiac muscle suggests that wortmannin does not inhibit the SR Ca^{2+} release in this tissue. The E-C coupling in cardiac muscle, in contrast to the direct tetrads-food structure interactions in skeletal muscle, is accomplished predominantly by a Ca^{2+}-induced Ca^{2+} release mechanism – the myoplasmic Ca^{2+} enters via the sarcolemma L-type Ca^{2+} channel and gates the opening of the ryanodine-sensitive Ca^{2+} channel (Cannell *et al*., 1995; Howlett & Ferrier, 1997). Additionally, the ryanodine receptors in cardiac and skeletal muscles belong to different isoforms (Sutko & Airey, 1996; Nakai *et al*., 1997). It is possible that these differences might render the cardiac SR Ca^{2+} release channel resistant to inhibition by wortmannin. Further investigations on the sarcolemma Ca^{2+}/K^+ channel, the Na^+, K^+-pump activity, intracellular Ca^{2+} dynamics and the contractile proteins may disclose the mechanism of the positive inotropic action. In view of the similar positive inotropic and negative chronotropic effects produced by wortmannin and LY294002, the role of PI 3-kinase in the E-C coupling of cardiac muscle remains to be elucidated.

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