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Calciseptine, a Ca^{2+} Channel Blocker, Has Agonist Actions on L-type Ca^{2+} Currents of Frog and Mammalian Skeletal Muscle

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Abstract. Calciseptine is a natural peptide consisting of 60 amino acids with four disulfide bonds. The peptide is a natural L-type Ca^{2+}-channel blocker in heart and other systems, but its actions in skeletal muscle have not been previously described. The aim of this study is to characterize the effects of calciseptine on L-type Ca^{2+} channels of skeletal muscle and on contraction. Whole-cell, patch-clamp experiments were performed to record Ca^{2+} currents (I_{Ca}) from mouse myotubes, whereas Vaseline-gap voltage-clamp experiments were carried out to record I_{Ca} from frog skeletal muscle fibers. We found that calciseptine acts as a channel agonist in skeletal muscle, increasing peak I_{Ca} by 37% and 49% in these two preparations. Likewise, the peptide increased intramembrane charge movement, though it had little effect on contraction. The molecular analysis of the peptide indicated the presence of a local, electrostatic potential that resembles that of the 1,4-dihydropyridine agonist Bay K 8644. These observations suggest that calciseptine shares the properties of 1,4-dihydropyridine derivatives in modulating the permeation of divalent cations through L-type channels.

Key words: Calcium channel — Dihydropyridines — DHP receptors — Excitation-contraction coupling — Muscle — Calciseptine

Introduction

Ca^{2+} channels are especially relevant to the physiology of cells where Ca^{2+} acts as a second messenger. In skeletal muscle, the L-type calcium channel is a complex of five subunits: α_{1s}, β, γ and α_2-δ. The α_{1s} subunit alone can function as a voltage-gated calcium channel and contains the receptor for dihydropyridines (DHP) and other calcium channel antagonists (for a review, *see* Catterall et al., 1993). L-type channels in muscle have two distinct functions: as voltage sensors, they play an essential role in skeletal muscle, regulating the release of Ca^{2+} by the sarcoplasmic reticulum (for reviews *see* Lamb, 1992; Melzer, Hermann-Frank & Lüttgau, 1995); in addition, the channels are permeant to Ca^{2+}, giving rise to very slowly activated Ca^{2+} currents (Sánchez & Stefani, 1983; for a review *see* Melzer et al., 1995). Similar to their action on other excitable cells, DHP antagonists block muscle Ca^{2+} channels (Almers & McCleskey, 1984; Dirksen & Beam, 1995), whereas DHP agonists have the opposite effect (Ildefonse et al., 1985).

In recent years, the actions of calciseptine, a natural peptidic L-type Ca^{2+} channel blocker, have been described (De Weille et al., 1991). Calciseptine blocks L-type currents in heart in a way that resembles that of 1,4-dihydropyridines. In the present experiments, we have described the effects of calciseptine on Ca^{2+} channels of skeletal muscle and have found that in this preparation, the peptide acts as an agonist of Ca^{2+} currents, but has little effect on contraction.

Materials and Methods

Mechanical Recordings

Isometric tension was measured in two different preparations: twitch frog muscle fibers and rat skeletal muscle fibers. Single twitch muscle fibers were dissected from the tibialis anterior muscle of adult specimens of *Rana montezumae* and small bundles of muscle fibers (1–2 mg wet weight) were dissected from flexor digitorum brevis muscles of adult rats (Wistar). The animals were sacrificed by decapitation. Intact fibers were prepared with short tabs of tendon in which pin holes were

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Table 1. Composition of solutions (mM)

	Na^+	K^+	TEA^+	Ca^{2+}	Mg^{2+}	$CH_3SO_3^-$	SO_4^{2-}	Cl^-	Cs^+	$EGTA^-$	Mg^{2+}	Aspartate	Cl^-
Extracellular solutions													
A	117	2.5	—	1.8	—	—	—	123.1	—	—	—	—	—
B	146	5	—	2	1	—	—	152	—	—	—	—	—
C	—	—	106	10	—	126	—	—	—	—	—	—	—
D	—	—	106	—	10	126	—	—	—	—	—	—	—
E	—	—	110	10	—	110	—	20	—	—	—	—	—
F	—	160	—	—	—	—	80	—	—	—	—	—	—
Intracellular solutions													
G	—	—	—	—	—	—	—	—	118	20	1	100	—
H	—	—	—	—	—	—	—	—	140	10	5	140	10

Solutions C–E also contained 3×10^{-7} M tetrodotoxin

made for the insertion of hooks. Isometric tension was measured with a mechanoelectronic transducer (Cambridge Technology, Watertown, MA) in fibers bathed in standard frog Ringer's (solution *A*) or in rodent Ringer's (solution *B*) bubbled with oxygen. The transducer was mounted on a micrometer slide which was used to adjust the fiber length. The fibers were suspended between two stainless-steel hooks, one of which was attached to the recording bath chamber and the other to the force transducer whose output was sampled by a Pentium-based microcomputer. The fiber rest length was adjusted to produce the maximum twitch, which corresponded to a sarcomere length of between 2.0 and 2.2 μm, as measured by a high-magnification water-immersion objective. Muscle cells were stimulated extracellularly with rectangular pulses of 1.5-msec duration, passed through two platinum electrodes placed on each side of the fibers. The amplitude of the pulses was 1.5 times the threshold for mechanical activation.

Cell Culture

Primary cultures were prepared from fore- and hindlimbs of 2- to 3-day old mice. The limb muscles were finely minced and then incubated at 37°C for 1 hr in rodent Ringer's (solution *B* in the Table), which also contained glucose (11 mM), trypsin (0.3%) and DNAase (0.01%), buffered with HEPES at pH = 7.4. After filtration and centrifugation to remove large debris, the cell suspension was preplated for 2 hr in glass to remove rapidly adhering cells and then plated onto 1.8-cm cover slips in plating medium containing (vol/vol) 80% Dulbecco's modified Eagle's medium with 4.5 g/liter glucose (DMEM), 10% horse serum, and 10% calf serum. After 3 days, the plating medium was replaced with maintenance medium: 98% DMEM, 2% horse serum and cytosine arabinoside (10^{-5} M). All culture media contained penicillin (100 U/ml) and streptomycin (100 μg/ml). Cultures were maintained at 37°C in a 95% air, 5% CO_2, water-saturated atmosphere.

Membrane currents were recorded from myotubes 4–7 days after the initial plating. The myotubes selected had a compact, nonbranching geometry. Cell diameters were typically 30 μm and lengths were 100 μm.

Voltage-Clamp Experiments

Membrane currents were measured in two different preparations: single frog skeletal muscle fibers and mouse myotubes. The triple-Vaseline-gap voltage-clamp technique developed by Hille and Campbell (1976) was used to measure membrane currents in cut frog skeletal muscle fibers. Minor modifications to the technique were made as described elsewhere (Arreola et al., 1987). A long segment of a single muscle fiber was dissected in frog Ringer's solution (solution *A* in the Table) and was tested for contractility with a high-K^+ solution (solution *F*). The fiber was pinned down at a sarcomere length of about 2 μm and allowed to contract and then relax spontaneously. The fiber was then laid in a chamber across three Vaseline seals, which divided the chamber into four pools. The ends of the fiber were cut while they lay in the end pools that contained an internal solution. The segment of the fiber lying in one particular inner pool was voltage-clamped; the renewal of the external solution for this segment was ensured by a steady flow of new solution, effectively exchanging the pool volume at least 40 times. Movement artifacts consequent to stimulation were suppressed by a high concentration of intracellular EGTA (solution *G*).

The patch-clamp technique in the whole-cell configuration (Hamill et al., 1981) was used to measure membrane currents in myotubes. Pipettes were double-pulled from hard glass (KIMAX-51, Kimble Glass, Toledo, OH) using a David Kopf 700 D (Tujunga, CA) vertical puller. 70–90% of the series resistance was compensated. Membrane currents were measured with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA).

Data Collection and Pulse Protocol

Twitch and tetanic tension were elicited with the following stimulation protocol: first, a single stimulus was applied to produce one twitch and after that, tetanic tension was elicited by stimulation at 30 Hz during 300 msec. This cycle was applied every 15 sec. In another set of experiments, only twitches were elicited at intervals of 14 sec.

Ca^{2+} currents were elicited by delivering ten command pulses of either 400 msec or 500 msec duration and of increasing amplitude in 10-mV steps. The whole sequence was bracketed by four consecutive hyperpolarizing control pulses, −20 mV from the holding potential (E_h). Linear currents were subtracted 'off line' after the appropriate scaling of membrane currents generated by the −20 mV hyperpolarizing pulses. The holding potential was set at −80 mV. In charge movement experiments, the holding potential was set to −100 mV or to −80 mV and 13 command pulses of 50 msec duration and of increasing amplitude in 10 mV steps were delivered. Linear membrane currents were subtracted off-line by appropriate scaling of membrane currents generated by control pulses.

Analog signals were sampled by a Pentium-based microcomputer and were amplified and filtered with an active four-pole, low-pass Bessel filter with a corner frequency of no more than half the sampling frequency. Data were analyzed by a combination of pCLAMP 6.0 (Axon Instruments, Foster City, CA) and in-house software.

Parameter values given in the text are expressed as mean ± standard error of the mean (SEM). The fitting of numerical formulas to experimental data employed a nonlinear least-squares algorithm. When calculating statistical significance, Student's paired *t*-test was used with significance at the level $p < 0.05$.

SOLUTIONS

The solution employed for measurements of I_{Ca} in Vaseline-gap experiments contained impermeant ions with the exception of Ca^{2+} (solution *C*). In charge movement experiments, Ca^{2+} ions were replaced by Mg^{2+} (solution *D*) to preserve the electrical properties of muscle membranes (Arreola et al., 1987). Both ends of the muscle fibers were bathed in an 'internal' solution containing impermeant ions (solution *G*). Extracellular and intracellular solutions were buffered with 3-*N*-morpholino propanesulphonic acid (MOPS, 4 mM) at pH = 7.2 and 7.1, respectively.

Patch-clamp experiments were performed with the extracellular and intracellular solutions shown in the Table (solutions *E* and *H*, respectively). Intracellular and extracellular solutions were buffered with 5 mM HEPES at pH 7.2.

Calciseptine (Alomone Labs, Israel) was diluted in external solution to the final concentration of 1 μM from a concentrated stock in water, and it was directly perfused onto the cell. Control recordings were obtained prior to testing the effects of calciseptine. This concentration was used because 1 μM calciseptine, obtained from the same source, stops spontaneous contractions of dissociated rat ventricular myocytes and blocks 80% of the Na^+ movements through the L-type Ca^{2+} channel in the absence of Ca^{2+} (De Weille et al., 1991).

MOLECULAR MODELING

All computations were carried out on a Dell OptiPlex GX1 computer using the molecular modeling package Chem-X (Oxford Molecular Group, Oxford, UK). Structures of (-)-S-Bay K 8644 (Ca^{2+} channel agonist), nifedipine (Ca^{2+} channel antagonist) and tetra-peptide *N*-acetyl-Met-Trp-Pro-Tyr-methylamine (a fragment of FS2, *see* below) were built and the geometry of molecules was optimized. The structure of toxin FS2, a homologous 60-amino-acid polypeptide of calciseptine (Kini et al., 1998), was obtained from the Molecular Modeling Database (entry code 2288: coordinates of 20 conformers elucidated by NMR; Albrand et al., 1995). The FS2 structure (conformer 10) was used as a template to define the correct conformation of the tetra-peptide. This was performed by the superposition of tetra-peptide on the toxin residues Met^{45}, Trp^{46}, Pro^{47} and Tyr^{48} (at loop III), which is a region deemed as critical for the interaction with the L-type calcium channels (Kini et al., 1998; Schleifer, 1997). Furthermore, Kini et al. (1998) proposed that the Ca^{2+}-channel binding site in the toxin contains these residues and synthesized an eight-residue peptide that exhibits the L-type Ca^{2+} channel blocking properties of calciseptin, but with a lower potency, suggesting that these residues interact with the channel directly. The tetra-peptide backbone was fixed and a minimization procedure was carried out to find the best orientation for the side chains. Molecular electrostatic potential (MEP) of the tetra-peptide, nifedipine and (-)-S-Bay K 8644 were computed by using the semiempirical method AM1 (Dewar et al., 1985). The superposition of (-)-S-Bay K 8644 and residues Tyr and Pro of the tetra-peptide was performed to identify their common features on the MEPs; the Tyr indol NH was fitted on the DHP NH and the Pro pirrolidine ring was fitted alongside the 2′-trifluoromethylphenyl ring.

Results

EFFECTS OF CALCISEPTINE ON I_{Ca}

The major effect of calciseptine was to increase the amplitude of Ca^{2+} currents. Figures 1*A* and 1*B* show superimposed records of membrane currents from a mouse myotube. The currents were generated by the pulse protocol described in Methods. Figure 1*A* illustrates inward currents under control conditions and Fig. 1*B* shows records obtained from the same experiment after incubation in calciseptine (1 μM). The amplitude of I_{Ca} was larger for all voltage steps. Figure 1*C* shows the relationship between peak I_{Ca} and membrane potential from the same experiment. Open symbols represent results obtained under control conditions and filled symbols, results after the fiber was incubated in calciseptine. The peak amplitude of I_{Ca} increased at all positive potentials by the peptide. This increase was also observed in unsubtracted records and therefore does not depend on changes on the leakage current. There was a distinct increase of about +20 mV in the apparent reversal potential by calciseptine. The relative increase in peak I_{Ca} averaged 1.37 ± 0.09 ($n = 21$). The effect of calciseptine on I_{Ca} develops with a relatively fast time course. Figure 1*D* shows the relative increase in peak I_{Ca} that was observed after the application of calciseptine. Filled symbols represent average results from cells treated with calciseptine, whose application is indicated by the arrow. Each symbol represents the mean increase (±SEM) in the value of peak current from at least four determinations. Open symbols represent the average results from two control experiments. The increase in the amplitude of I_{Ca} fully developed within 5 min and was maintained throughout the duration of the experiment.

Calciseptine also increases I_{Ca} in adult frog skeletal muscle fibers. The increase is similar to that observed in mammalian myotubes. Figure 2*A* shows superimposed records from a fiber incubated under control conditions and panel *B*, after the application of the peptide. Figure 2*C* shows the current-voltage relation from the same experiment. Similar to the effects of calciseptine on mammalian muscle illustrated in Fig. 1*C*, the major effect of calciseptine on frog L-type Ca^{2+} channels was to increase the amplitude of I_{Ca} at all potentials. The mean relative increase was 1.49 ± 0.21 ($n = 7$). However, unlike the results observed in mouse myotubes, calciseptine did not change the reversal potential of I_{Ca}.

EFFECTS OF CALCISEPTINE ON CHARGE MOVEMENT

To assess the effects of calciseptine on charge movement, we measured nonlinear currents in fibers bathed in solutions containing impermeant ions. Figure 3*A* shows traces of nonlinear membrane currents recorded from a

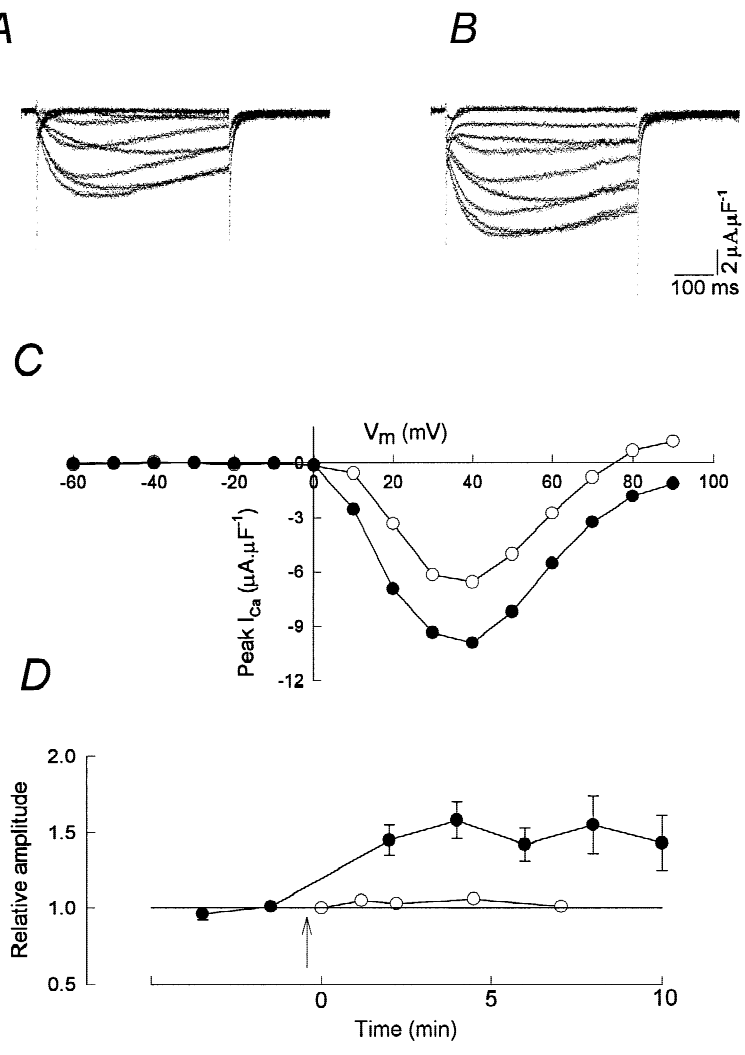

Fig. 1. The increase in the amplitude of Ca^{2+} currents in mammalian myotubes by calciseptine. Panel *A* shows superimposed control current records during voltage steps from $E_h = -80$ mV, to −60 mV and up to +60 mV. Panel *B* shows the effects of calciseptine (1 μM) on I_{Ca} under the same pulse protocol. Panel *C* shows the current-voltage relation of the experiment shown in panels *A* and *B*. (○) peak values of I_{Ca} of the control records; (●) the corresponding values in calciseptine in the same experiment. Panel *D* shows the relative values of peak I_{Ca} during pulses to +20 mV as a function of time. The arrow indicates the application of calciseptine. (●), mean ± SEM of at least four determinations; (○), mean of two control experiments.

single frog skeletal muscle fiber. The currents were obtained under control conditions at the potentials indicated. Nonlinear currents were small at low depolarizations and became larger when the amplitude of the depolarizing pulse increased. Panel *B* shows currents recorded from the same experiment after the application of calciseptine to the bath solution. The amplitude of the currents increased when compared to those recorded under control conditions. The total charge that was moved following the onset of the voltage clamp steps ('on' charge) and the charge moved immediately after the pulse ('off' charge) were obtained by the integration of the areas under the control traces. 'On' and 'off' charges reach very similar values at any given potential under these experimental solutions (Delay, Garcia & Sanchez, 1990). In agreement with this report, we found that 'on' and 'off' values varied less than 10% relative to each other. For this reason, 'on' and 'off' values were averaged. Figure 3*C* shows the relationship between the mobilized charge by the depolarizing pulses as a function of the membrane potential. Open symbols represent results from the control experiment and filled symbols, results after the application of calciseptine. The points were fitted to a two-state Boltzmann distribution (Chandler, Rakowski & Schneider, 1976):

$$Q = Q_{max}/[1 + \exp((-V_m + V)/k)]$$

Where Q_{max} is the maximum charge per unit linear capacitance, V is the mid point and k is a measure of the steepness. The continuous lines represent the best fit of the Boltzmann function to the experimental values. Charge saturated at large depolarizations, reaching the values indicated in the legend. We observed a shift in the midpoint of activation of about −7 mV in the presence of calciseptine. There was also an increase in the value of maximum charge of about 60%. In six similar experiments, the ratio between the maximum charge after application of calciseptine and that under control conditions, averaged 1.53 ± 0.15 ($n = 6$).

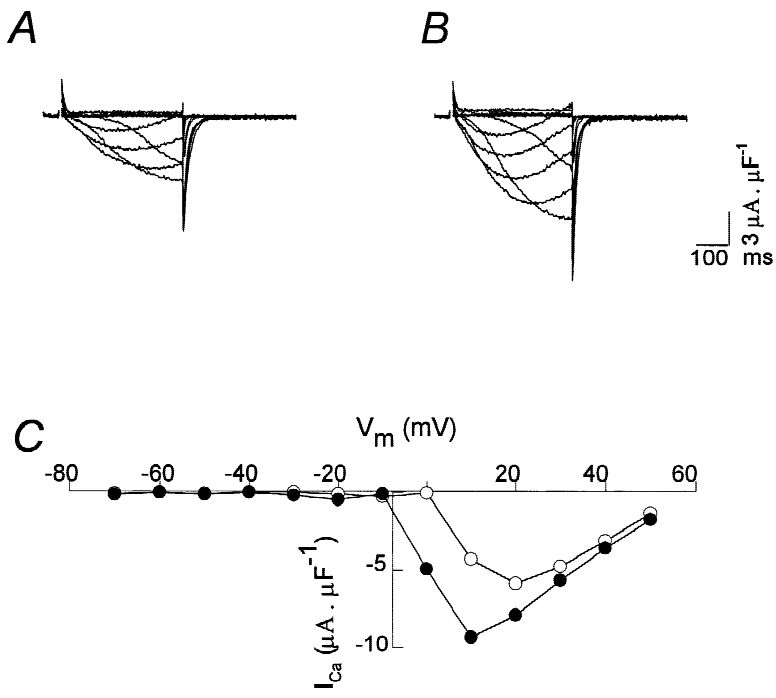

Fig. 2. The effect of calciseptine on Ca^{2+} currents in frog muscle fibers. Panel *A* shows superimposed control current records during voltage steps from $E_h = -80$ mV, to −70 mV up to +50 mV. Panel *B* shows the effects of calciseptine (1μM) on I_{Ca}. Panel *C* shows the current-voltage relation of the experiment shown in panels *A* and *B*. (○), peak values of I_{Ca} of the control experiment; (●) the corresponding values in calciseptine. Same experiment throughout.

Effects of Calciseptine on Twitch and Tetanic Tension

To determine whether the increase in maximum charge by calciseptine potentiates twitch tension, we performed mechanical experiments in intact fibers but found that the peptide had very little effect on contraction. This is shown in Fig. 4*A–C*, which illustrates twitches generated by a small bundle of rat muscle fibers, under control conditions (panel *A*) and 1.9 min (panel *B*) and 3.7 min (panel *C*) after the application of calciseptine (1 μM) to the bath solution. There were very slight changes in twitch tension by calciseptine. Figure 4*D* illustrates, from the same experiment, the values of peak tension as a function of time, relative to the control value. Tension remained within 16% from its control value. In two separate experiments performed in rat-muscle fibers, the average increase in twitch tension was 1.11. Likewise, calciseptine had little effect on twitch frog-muscle fibers. The ratio between twitch tension after application of the peptide and tension under control conditions averaged 1.12 ± 0.06 ($n = 6$, *data not shown*).

To test the possibility that calciseptine might influence contraction during a series of repetitive stimuli that generate tetanus, we measured tension generated by trains of action potentials (*see* Methods). Figure 5*A* shows recordings of the mechanical activity produced by a single stimulus followed by repetitive stimuli that produced tetanus. Records are displayed on a very slow time scale. Calciseptine increased tetanic tension, albeit the effect was relatively small. Figures 5*B* and *C* show representative traces from panel *A*, displayed on a faster time scale. The average tetanic tension after the application of calciseptine, relative to its control value, was 1.12 ± 0.01 ($n = 3$). These results indicate that, even during tetanus, the increase in charge movement or the increase in the influx of Ca^{2+} through the L-type channels caused by calciseptine does not greatly affect contraction.

Discussion

Comparison with Previous Work

The electrical activity of excitable cells is greatly influenced by second messengers and other modulators that act within a period of seconds or minutes. In many cases, ion channels change their unitary behavior as a result of the interaction with modulatory influences. L-type calcium channels are targets of dihydropyridines, highly potent and selective compounds, that have been used to probe these channels in a variety of cell types. In skeletal muscle, nifedipine, a 1,4-dihydropyridine antagonist, blocks the Ca^{2+} current completely (Lamb & Walsh, 1987) and it suppresses in part the asymmetric charge movement in normally polarized cells (Lamb & Walsh, 1987; Rios & Brum, 1987). In contrast to the effects on Ca^{2+} currents and charge movement, the effects of dihydropyridines on tension are controversial (Gallant & Goettl, 1985; Lamb, 1986; Dulhunty & Gage, 1988).

In spite of the abundant literature on the actions of

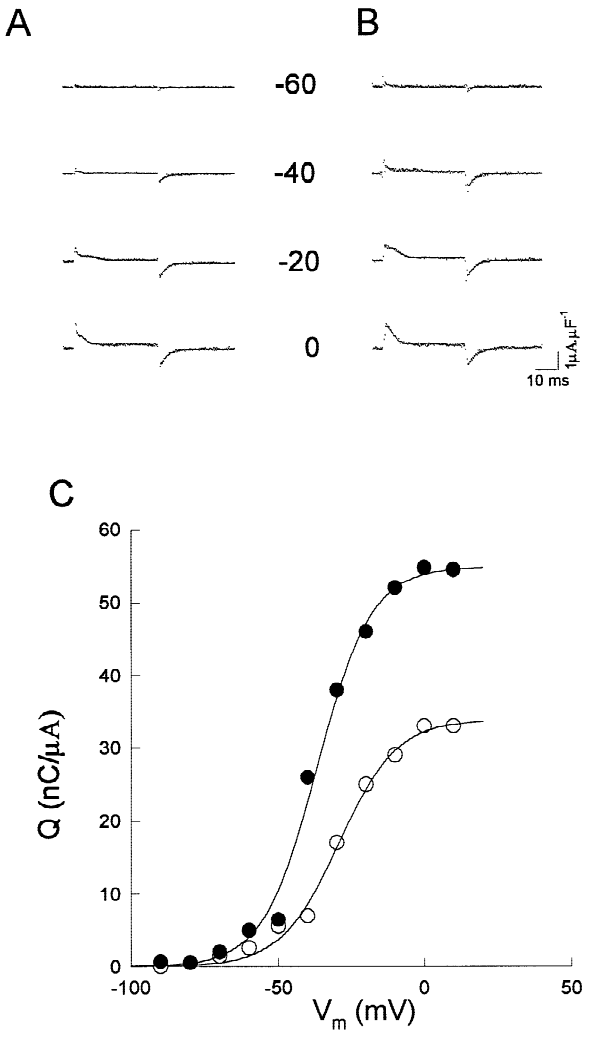

Fig. 3. The effect of calciseptine on charge movement in frog muscle fibers. (*A*) Current records during voltage steps to the potentials indicated (in mV) after subtraction of linear membrane components, E_h = −80 mV. (*B*) Effect of calciseptine (1 μM) on membrane currents in the same experiment at the same potentials as in *A*. E_h = −80 mV. (*C*) Nonlinear charge movement as a function of voltage. (○) and (●) represent the average values of charge displaced by 'on' and 'off' transients from the experiment shown in *A* and *B*, respectively. The continuous lines are the best fit of a Boltzmann function with Q_{max} = 33.9 nC/μF, V = −29.4 mV, k = 10.0 mV for (○) and Q_{max} = 54.9 nC/μF, V = −36.8 mV, k = 9.3 mV for (●).

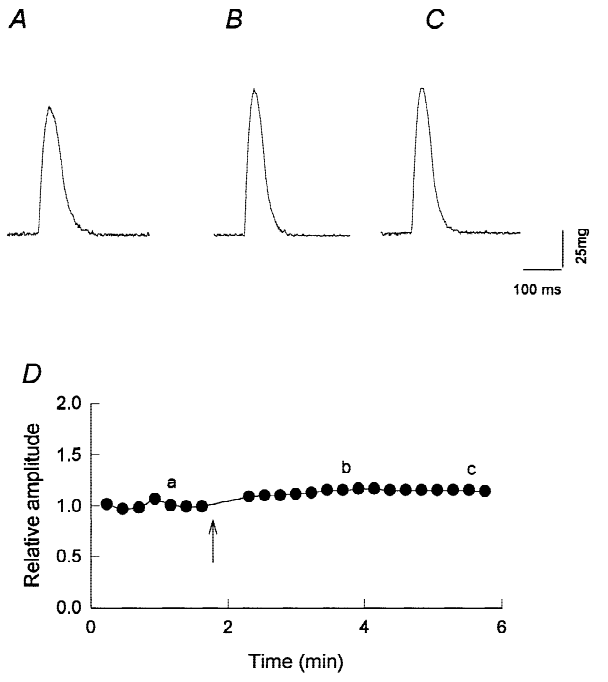

Fig. 4. The effect of calciseptine upon twitch tension in rat muscle. (*A*) A single twitch under control conditions. (*B*) and (*C*) 1.9 min and 3.7 min after the application of calciseptine (1 μM). Panel *D* shows the relative values of peak tension as a function of time. The arrow indicates the application of calciseptine. *a*, *b* and *c* indicate the peak values of the twitches shown in *A*, *B* and *C*, respectively.

dihydropyridines and other synthetic compounds on L-type calcium channels (for a review *see* Rampe & Triggle, 1993), it is only recently that a natural blocker of these channels has been found. Calciseptine is a 60-amino-acid peptide contained in the venom of the black mamba (De Weille et al., 1991). Like dihydropyridines, it is inactive on N-type or T-type channels. Furthermore, it shares the properties of 1,4-dihydropyridine derivatives in the mechanism of binding (Yasuda et al., 1993). Calciseptine competitively inhibits the specific binding of [^{3}H] nitrendipine to the α_1 subunit of the calcium channel and it modulates allosterically the binding of [^{3}H] diltiazem (Yasuda et al., 1993). The physiological effects of calciseptine on muscle preparations, other than skeletal muscle, have been previously characterized. Thus, calciseptine acts as a smooth muscle relaxant, blocking spontaneous contractions of rat portal vein, aorta and uterus (De Weille et al., 1991; Kuroda et al., 1992; Watanabe et al., 1995) and it also blocks contraction of rat cardiac preparations (De Weille et al., 1991). The inhibitory effects of calciseptine on smooth muscle and heart are most likely related to the blocking of L-type calcium channels. Thus, calciseptine blocks L-type calcium currents of aortic cells, cardiac ventricular myocytes and invertebrate muscle fibers (De Weille et al., 1991; Tsutsui et al., 2000). In this regard, the Ca^{2+} channel of skeletal muscle is unique since we found that calciseptine has agonist-like actions in this preparation.

Although calciseptine increases charge movement, contraction is only very slightly affected by this peptide. Several possibilities may help to explain these results. It is possible that calciseptine increases the movement of a charge unrelated to DHP receptors. If this is true, it would indicate a nonspecific action of calciseptine. Alternatively, calciseptine might cause changes in the charge movement generated by DHP receptors that are

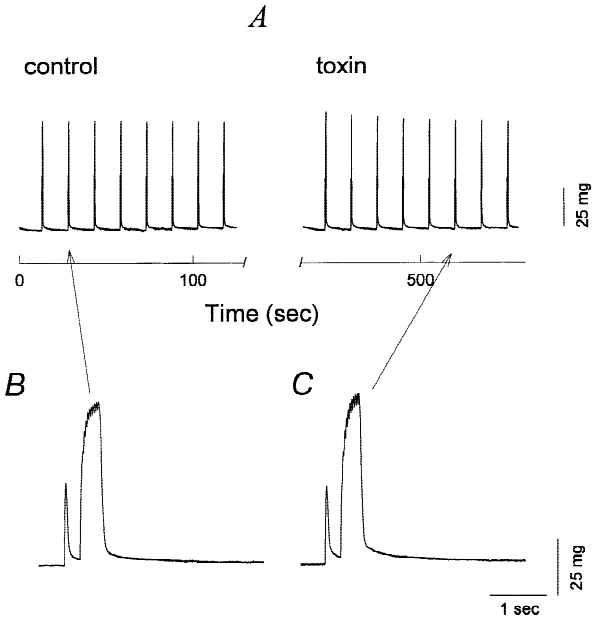

Fig. 5. The effect of calciseptine upon twitch and tetanic tension in a single frog muscle fiber. (*A*) Consecutive records of a single twitch followed by a tetanus at 30 Hz under control conditions and in the presence of calciseptine. (*B*) and (*C*) are records from the indicated segments (*arrows*) in panel *A*, displayed with a faster time scale.

not related to ryanodine-receptor opening. A dual role for DHP receptor in muscle, as a Ca^{2+} channel and as the voltage sensor for E-C coupling, has been proposed (for a review, *see* Lamb, 1992). Finally, it should be pointed out that a considerable amount of charge may be moved before a significant change in Ca^{2+} release takes place (Melzer et al., 1986). This occurs when the charge moved by a depolarizing pulse is only a fraction of the available charge during long but small depolarizing pulses. This subthreshold charge may correspond to charge moved in preliminary transitions preceding release (Melzer et al., 1986). The nonlinear charge moved by an action potential is relatively small because its duration is quite brief. Charge movement during action potentials has been estimated by using a distributed model of the transverse tubular system of the frog muscle fiber (Huang & Peachey, 1992). These calculations reveal that the charge moved by a single action potential is only 25–50% of the available charge, depending on which charge movement component is considered. In addition, no significant buildup of charge transfer occurs with trains of action potentials that would normally cause tetanus (Huang & Peachey, 1992). Therefore, it is plausible that changes in charge movement by calciseptine during action potentials, beyond the subthreshold charge, are relatively small, thus producing only minor changes in Ca^{2+} release and contraction. The increase in Ca^{2+} influx through L-type channels by calciseptine is also expected to have negligible effects on contraction since in this preparation contraction does not depend on Ca^{2+} entrance (Melzer et al., 1995).

Calciseptine as an Agonist of Skeletal Muscle Calcium Channels

Based on the fact that (-)-S-Bay K 8644 is a dihydropyridine agonist (Franckowiak et al., 1985), we decided on a theoretical approach aimed at understanding the agonist nature of calciseptine in skeletal muscle. The molecular properties of the ligands are important for the interaction with their receptor site. In particular, the electrostatic field surrounding each molecule plays a crucial role in the recognition process that precedes the formation of the final interaction complex (Höltje & Folkers, 1996). The display of computed MEPs for (-)-S-Bay K 8644, tetra-peptide *N*-acetyl-Met-Trp-Pro-Tyr-methylamine and nifedipine is shown in Fig. 6. There are several common features between the tetra-peptide and the 1,4-dihydropyridine derivatives. Nifedipine and (-)-S-Bay K 8644 display two negative fields generated by oxygens of esters (or nitro group) at C3 and C5 (red clouds *b* and *a*, respectively). Similar negative fields are displayed by the Trp carbonyl oxygen and carbonyl oxygens of Pro and Tyr on the tetra-peptide. Also, there is a positive field in common (blue cloud *c*) for the 1,4-dihydropyridines (hydrogen of N1) and the tetra-peptide (hydrogen of indol nitrogen).

The MEPs displayed by nifedipine and the tetra-peptide found by us were similar to those obtained by Schleifer (1997), who used a different approach (the *ab initio* 6–31G* method) to evaluate MEPs for nifedipine and the toxin fragment *N*-acetyl-Gly-Trp-Pro-Ala-methylamine. We found that the tetra-peptide fragment *N*-acetyl-Gly-Trp-Pro-Ala-methylamine displays a pronounced negative potential induced by Pro and Tyr carbonyl oxygens (red cloud *a*, Fig. 6). The corresponding potential derived by the ester oxygens of nifedipine is much smaller, whereas the one derived by the nitro group of the (-)-S-Bay K 8644 molecule is larger than that of nifedipine and resembles the negative potential of the tetra-peptide fragment. A distinctive characteristic of agonist dihydropyridines like (-)-S-Bay K 8644 is the presence of a pronounced negative potential on the area of the nitro group, a potential that is smaller in antagonist dihydropyridines (Goldman & Stoltefuss, 1991; Höltje, 1992). This potential presumably attracts a positive charge of the Ca^{2+} channel and prolongs the time that the channel spends in the open state (Goldman & Stoltefuss, 1991). Therefore, we propose that the similarity between the tetra-peptide *N*-acetyl-Met-Trp-Pro-Tyr-methylamine and (-)-S-Bay K 8644 explains the agonist behavior of calciseptine in skeletal muscle. However, the comparison with Bay K 8644 has to be made with caution since this dihydropyridine agonist has no action on charge movement (Lamb & Walsh, 1987), in contrast

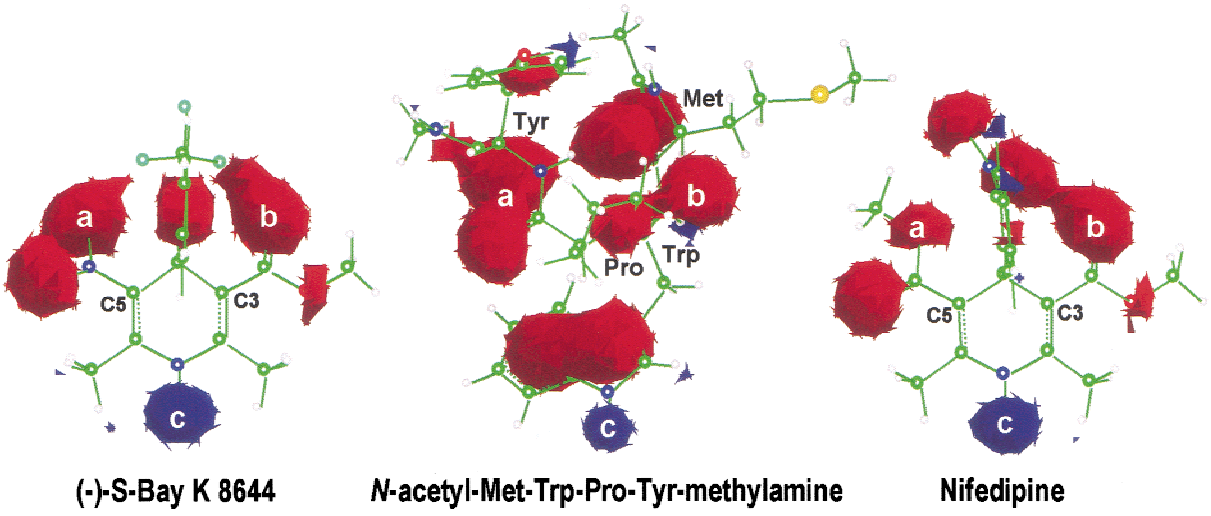

Fig. 6. Visualization of the molecular electrostatic potential of (-)-S-Bay K 8644, toxin fragment *N*-acetyl-Met-Trp-Pro-Tyr-methylamine and nifedipine. Red and blue clouds indicate negative and positive fields, respectively, contoured at ±50 kcal mol^{-1}. Common negative (regions *a* and *b*) and positive potentials (region *c*) are labeled.

to the effects of calciseptine described here. The fact that I_{Ca} and charge movement show an increase in peak current and maximum charge values, respectively, along with a negative shift in the voltage dependence of activation in frog muscle, might be explained if calciseptine alters the local transmembrane field near the gating S4 domain and thereby changes the steady-state distribution of channels between unavailable and available states. This in turn would promote an increase in functional DHP receptors. Why calciseptine antagonizes I_{Ca} in other tissues remains to be explained. Although we do not have an explanation for these opposite effects, it is interesting to note that 1,4-dihydropyridines have mixed effects on Ca^{2+} channels. Thus, the well known 1,4-dihydropyridine antagonist, nitrendipine, also has agonist-like actions in heart cells (Hess, Lansman & Tsien, 1984; Brown, Kunze & Yatani, 1986; Coulombe et al., 1989), and the Ca^{2+} channel activating effect of the 1,4-dihydropyridine derivative 202–791 can turn over into a blocking effect (Kokubun et al., 1986). In these examples, the holding potential plays a critical role since the antagonist effects prevail when cells are maintained at depolarized potentials. However, in the case of calciseptine, a more elaborate explanation has to be put forward since the holding potential in our experiments was the same as that used by De Weille et al. (1991) in heart muscle where calciseptine blocks cardiac I_{Ca}. It is conceivable that calciseptine binds to different sites in the L-type channel of cardiac and skeletal muscle, promoting different effects on the channel. However, we have no evidence to support this idea since the binding sites for the toxin have not been identified yet, though calciseptine may interact with the sites that bind dihydropyridines in the L-type channel. This is because calciseptine inhibits the binding of nitrendipine (Yasuda et al., 1993). If the binding site for calciseptine is, indeed, the same as that for dihydropyridines, then the possibility of different binding sites is very remote because the amino-acid composition of the regions of α_{1s} and α_{1c} that are essential for the binding of dihydropyridines is quite similar (Striessnig et al., 1998; Hockerman et al., 1997).

The fact that calciseptine has opposite effects on the Ca^{2+} channel of skeletal muscle (this paper) as compared to that of heart (De Weille et al., 1991) makes this peptide a potentially useful tool to differentiate the physiological role of the cardiac Ca^{2+} channel (α_{1c} subunit) in cells where both channels coexist. For example, in addition to α_{1s}, slow skeletal muscle and diaphragm express significant levels of the α_{1c} subunit (Péréon et al., 1997a,b) and the α_{1c} subunit is upregulated when fast skeletal muscle fibers are chronically stimulated (Péréon et al., 1997c).

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