Pflügers Arch (1990) 416:98–105

Pflügers Archiv
European Journal of Physiology

The blockade of excitation/contraction coupling by nifedipine in patch-clamped rat skeletal muscle cells in culture

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Received May 4 / Received after revision October 4 / Accepted October 25, 1989

Abstract. The effects of the dihydropyridine derivative, nifedipine, well known as a blocker of calcium channels, were tested on cultured rat myoballs. Membrane currents and contractions were simultaneously recorded by means of the patch-clamp technique and a photoelectric transducing method. High concentrations of nifedipine (5 μM) inhibited the contractile responses and inward calcium current (I_{Ca}) elicited by long depolarizations. In the absence of I_{Ca} (1.5 mM cadmium in the bath), nifedipine inhibited both the I_{Ca}-independent contractile component and the outward current, supposed to depend on the intracellular calcium released during contraction. At low concentrations (0.5 μM) the blocking effects of nifedipine could be strongly enhanced by shifting the membrane potential towards less negative values (−60 mV) for 50 s prior to the test pulse. A blocking effect of nifedipine, at a usually ineffective concentration (0.1 μM), could also be observed when long-lasting (3 min) prepulses to 0 mV were applied from a reference membrane potential of −60 mV. This effect could be relieved by long-lasting cell hyperpolarizations (−90 mV). The blocking effects of nifedipine unrelated to I_{Ca} could be interpreted as an action on a molecule (voltage sensor) in the T-tubule membrane involved in the excitation/contraction coupling process and as a preferential binding of the dihydropyridine derivative on the inactivated form of this molecule, favored by the weak negative potentials or long-lasting depolarizations. The results provide data in favor of the existence of strong similarities between the calcium channels and voltage sensors since their operation was inhibited in a voltage-dependent manner by nifedipine.

Key words: Excitation/contraction coupling – Nifedipine – Calcium channels – Patch clamp – Cell culture – Rat skeletal muscle

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Introduction

In spite of numerous and extensive studies the nature of the link between excitation and contraction of skeletal muscle cells remains unclear. Three transducing mechanisms have been proposed to explain the coupling between the skeletal muscle membrane depolarization and the sarcoplasmic reticulum calcium release: a chemical transmission with inositol 1, 4, 5-trisphosphate as a messenger, a mediation in which calcium ions trigger the calcium release from intracellular stores and an electromechanical transduction via a voltage-sensing protein. Recent experiments on the excitation/contraction coupling (ECC) process have brought forth important data in favor of the two latter mechanisms working simultaneously during long-lasting depolarizations.

It has been demonstrated that the sarcoplasmic reticulum calcium-release channels are activated by less than micromolar concentrations of calcium (Smith et al. 1986; Hymel et al. 1988; Lai et al. 1988). This property could explain the Ca^{2+}-induced Ca^{2+}-release mechanism observed in skinned fibres (Endo 1977; Volpe and Stephenson 1986) and supposed to play a role in ECC during long-lasting depolarizations (Rios and Pizarro 1988) and in the Ca-dependent contractions recorded in voltage-clamped skeletal muscle cells (Potreau and Raymond 1980; Ildefonse et al. 1985; Rivet et al. 1989).

On the other hand, Rios and Brum (1987) have demonstrated that the so-called calcium-channel blocker nifedipine inhibits a portion of charge movements attributed to the voltage-sensing molecules involved in the ECC process (Schneider and Chandler 1973) as well as the calcium-release flux.

Dihydropyridines, which are supposed to act on both calcium entry and ECC voltage-sensors (see Agnew 1987), could be useful for tentatively dissecting the role of each of these two mechanisms in ECC. Nifedipine has, therefore, been tested on patch-clamped rat skeletal muscle cells in primary culture, the contraction of which was controlled by both voltage and calcium entry via calcium channels (Cognard et al. 1988; Rivet et al. 1989).

The present work demonstrates that nifedipine inhibits contractile responses induced by depolarizing steps independently of its antagonistic action on calcium currents. This inhibitory effect exhibits some features that agree with the effects of phenylalkylamine derivatives (D600 and D888) observed on adult muscles (Eisenberg et al. 1983; Lüttgau et al. 1986; Berwe et al. 1987; Erdmann and Lüttgau 1989) and are compatible with ideas of Lamb and Walsh (1987), Lüttgau et al. (1987), Rios and Brum (1987), Tanabe et al. (1987), who hypothesized that the voltage-sensing molecules involved in ECC are calcium-channel-like proteins.

Materials and methods

Cell culture. Satellite cells from hindlimbs of 1–3-day-old rats were obtained as described elsewhere (Rivet et al. 1989). The myoblast suspension (0.2×10^6 – 1×10^6 cells/ml) was plated (2 ml/dish) in 35-mm-gelatin-coated (0.5%) dishes and incubated for 3 days at 37°C (95% air, 5% CO_2) in the growth medium (HAM F12 with 10% fetal calf serum and 10% heat-inactivated horse serum). Then the medium was exchanged with a Dulbecco's modified eagle medium (+5% heat-inactivated horse serum) to induce fusion of myoblasts. Every 3 days the culture medium was renewed. Colchicine (10–30 nM) was added to obtain myoballs, which were used during 15 days (exceptionally up to 3 weeks) after the culture started.

Recording membrane currents. The whole-cell configuration of the patch-clamp technique (Hamill et al. 1981) was used to record ionic membrane currents. Pipettes (2–5 MΩ) were connected to the headstage of the patch-clamp amplifier (RK300, Biologic, Grenoble, France) driven by a PC-AT-compatible microcomputer equipped with an A/D–D/A conversion board (Labmaster TM40, Scientific Solutions, Solon, USA). Data acquisition and analysis were performed by means of a software package (pClamp, Axon Instruments, Foster City, USA). Unless stated otherwise in the figures legends, data were sampled at 285 Hz and corrected for leakage currents from linear extrapolation of membrane current magnitudes (assumed to be ohmic) for small depolarizations, which induced no (or negligible) dynamic currents.

Mechanical recording. Contractile activity was recorded, simultaneously with the membrane currents (Rivet et al. 1989), by means of an IP28 photomultiplier tube (Hamamatsu, Japan), as light transmission variations induced during the cell contraction. Throughout the paper, the terms contraction, contractile response or contractile activity are used interchangeably and refer to the mechanical activity recorded as indicated. The photomultiplier was mounted on an auxiliary light-path exit of the inverted microscope (IMT-2, Olympus, Japan). The patch-clamped myoball was held between two fixed points: the membrane sticking at the bottom of the gelatin-coated culture dish and the pipette tip. In this way it was generally possible to avoid cell detachment from the dish, the cell shifting out of the photomultiplier beam or biphasic contraction recording, otherwise the cell was discarded. The photoelectric transducer was connected to a current/voltage converter (low-pass filter cut-off frequency = 150 Hz) before digital acquisition.

Solutions and experimental conditions. Currents and contractions were recorded at room temperature from myoballs (20–50 μm in diameter). The culture medium was exchanged before each experiment with the saline bath solution (in mM : 135 tetraethylammonium chloride; 2.5 $CaCl_2$; 0.8 $MgCl_2$; 5.6 glucose; 10 HEPES; pH=7.4, adjusted with tetraethylammonium hydroxide). The pipette was filled with (in mM) : 145 CsCl; 1 $MgCl_2$; 0.005 $CaCl_2$; 1 [ethylenebis(oxonitrilo)]tetraacetic acid (EGTA); 5.6 glucose; pH=7.2 with *Tris* base. Nifedipine (Sigma, USA) was added to the saline medium from a concentrated stock solution (10 mM in dimethylsulfoxide) kept in the dark. During the experiments, between two recording sequences, the light beam was interrupted by means of a screen to prevent a possible inactivation of nifedipine during the prolonged exposure to light. However, during preliminary studies, no significant effect related to nifedipine inactivation by light was observed even without the above precautions. The maximum concentration of the solvant in the experimental solutions was 0.05%. Dimethylsulfoxide was added to all control solutions at a final concentration corresponding to that of the nifedipine-containing salines.

Results

The effects of a high concentration of nifedipine (5 μM) on membrane currents and contractions of rat myoballs are illustrated in Fig. 1A. Depolarizing steps (450 ms in duration) above −30 mV from a holding potential of −90 mV elicited slow inward calcium currents and contractions as already described (Cognard et al. 1988; Rivet et al. 1989).

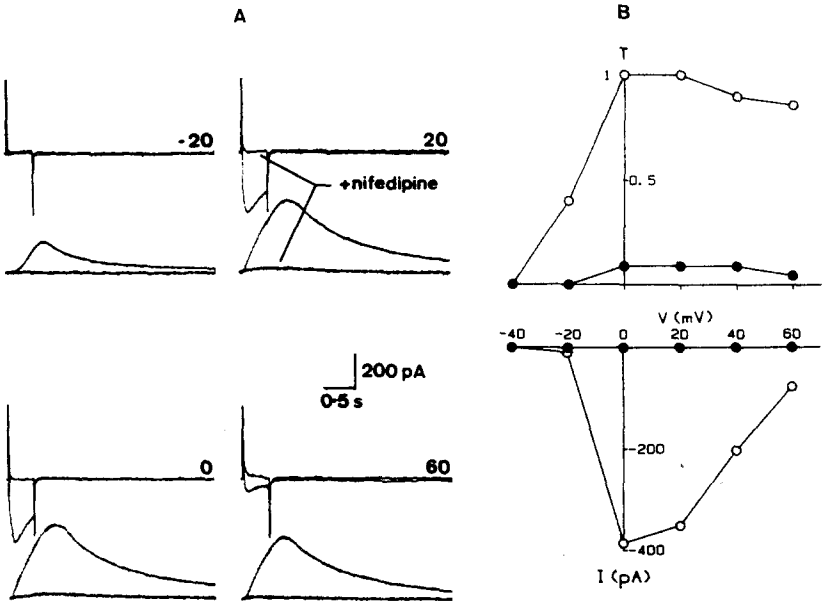
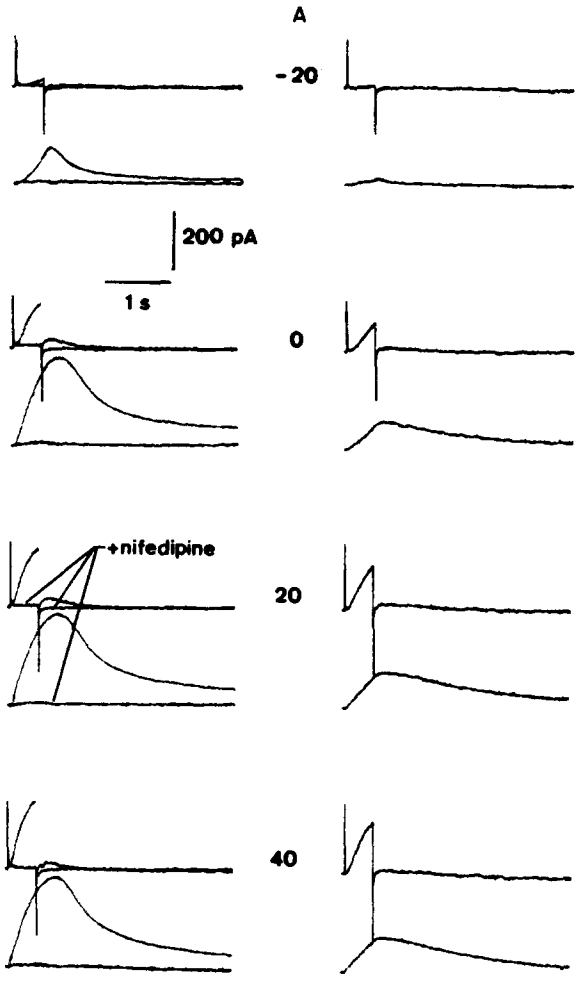


Fig. 1A, B. Effect of nifedipine on currents and contractions of a patch-clamped rat myoball in culture. **A** Superimposed traces of currents (*top traces* of each set of recordings) and of contractions (*bottom traces* of each set) elicited by depolarizing pulses (450 ms in duration) of various levels (indicated in mV), from a holding potential of −90 mV, in the absence (*lower traces of current* and *upper traces of contraction*) or in the presence of 5 μM nifedipine (*ubber traces of current* and *lower traces of contraction*). **B** Current/voltage (inward current peak amplitude, *I*, vs membrane potential value, *V*) and contraction/voltage (contration peak amplitude, *T*, vs membrane potential value, *V*) relationship in the absence (○) and the presence (●) of nifedipine for the experiment illustrated in **A**



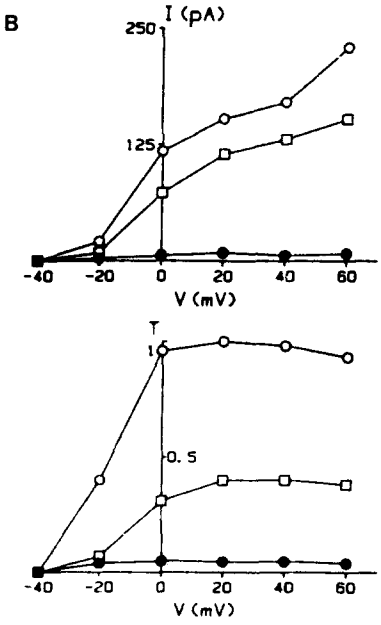


Fig. 2A, B. Effect of nifedipine on currents and contractions in the presence of 1.5 mM external $CdCl_2$. **A** Superimposed traces (*left column*) of currents and contractions elicited by depolarizing steps (450 ms) of different levels (indicated in mV near each recording) from a HP of −90 mV in the absence (*upper traces* of currents and contractions in each set of recordings) or in the presence (*lower traces*) of nifedipine (5 μM), and after 3 min of washing (*right column of recordings sets*). **B** Current-voltage (outward current amplitude, I, measured at the end of the depolarizing pulse vs membrane potential value, V) and contraction/voltage (contraction peak amplitude, T, vs membrane potential value, V) relationships for the experiment (illustrated in **A**) performed in the absence (○) presence (●), and after washing out nifedipine (□)

In the presence of nifedipine, inward calcium currents and contractions were drastically inhibited for all depolarizations. The *I/V* curves of Fig. 1B show that the Ca current was fully inhibited by nifedipine. The *T/V* curve which, in control solution, exhibits the complex shape previously analysed (Cognard et al. 1988; Rivet et al. 1989) reveals that, in the presence of 5 μM nifedipine, the maximum contraction never reached 10% of control.

Similar inhibitory effects of a dihydropyridine derivative and of other calcium-channel antagonists have been already reported on mouse myotubes in culture (Romey et al. 1988). Because, in cultured rat skeletal muscle cells (Cognard et al. 1988; Rivet et al. 1989), the contraction partly depends on the slow inward calcium current (I_{Ca}), it was difficult to assess whether the nifedipine blockade of the mechanical activity was a consequence of the I_{Ca} inhibition or the result of a more direct alteration of a voltage-sensing mechanism that governs the ECC processes.

Therefore the following experiments were performed in the presence of cadmium, a well-known inorganic blocker of I_{Ca}. This experimental approach (cadmium blockade of I_{Ca}), instead of reducing the extracellular calcium concentration or substituting another divalent cation, allowed us to maintain external [Ca] to its control value since calcium ions are required for a normal operation of some processes involved in ECC: potential-sensing molecules (Brum et al. 1988b), intracellular calcium movements (Brum et al. 1988a), and contractions or contractures of batrachian (Lüttgau and Spiecker 1979; Cota and Stéfani 1981; Lüttgau et al. 1986; Huerta et al. 1986) or of mammalian (Graf and Schatzmann 1984; Léoty and Noireaud 1987; Dulhunty and Gage 1988) skeletal muscle fibres. Although some displacement of calcium by cadmium from critical sites for ECC cannot be discarded, a similar ionic manipulation has been used by Berwe et al. (1987) without noticeable alteration of the induced contractures.

Figure 2A shows that, in the presence of 1.5 mM cadmium, whatever the depolarization, the inward calcium current was not present whereas large mechanical responses were generated (left column, upper traces in each set of superimposed current and contraction traces). The presence of 5 μM nifedipine in the bath (Fig. 2A, left column, lower traces in each set of superimposed traces) led to a nearly complete inhibition of contraction. In addition, nifedipine blocked the development of an outward current that could be also observed as an outward tail current after the end of the depolarization. It appears worthwhile to describe this current here, although it is not the central topic of the present investigation. It seems that it might be attributed to a K^+-type permeability (Cs^+ as charge carrier in the present conditions) depending on the intracellular calcium as briefly outlined in the Discussion. Its relative importance exhibited

Table 1. Dose-dependent effect of nifedipine on peak contraction of rat myoballs in presence of 1.5 mM $CdCl_2$. Depolarizing pulse from −90 mV to 0 mV (450 ms in duration); 34 different cells

Nifedipine concentration (μM)	Decrease of the control peak contraction amplitude (%) mean ± SEM	n
0.1	17.5 ± 11.4	8
0.5	26.1 ± 10.2	6
1.0	59.7 ± 9.3	10
5.0	91.5 ± 1.8	10

a great variability from cell to cell: not easy to observe in Fig. 1A, and obviously present in the tail traces of Fig. 5a, b, c, and f. Washing out the dihydropyridine derivative (right column of traces in Fig. 2A) led to a rapid reappearance of both the contractile response and the outward current. Between −20 mV and 40 mV, a bump can be seen on the *I/V* curves (Fig. 2B) in control experiments (open circles) and after washing out nifedipine (open squares), but not in the presence of nifedipine (filled circles). The *T/V* curve of Fig. 2B, drawn from data obtained in the presence of 1.5 mM cadmium (e.g. in the absence of I_{Ca}), exhibits a classical S-shape (no marked decrease for high depolarizations) already described by Rivet et al. (1989). In the presence of nifedipine *I/V* and *T/V* curves are flat as the result of drastic inhibition of contractions and outward currents. The inhibitory effect of nifedipine was dose-dependent as shown in Table 1, which gives the percentage inhibition of the contractile response obtained with different concentrations of nifedipine for a depolarization to 0 mV. The large variability of the responses precludes drawing up an accurate dose-response curve. Nevertheless an ED_{50} between 0.5 μM and 1 μM could be a reasonable estimation.

Since Rios and Brum (1987) have shown, in frog skeletal muscle fibres, that inhibition by nifedipine of charge movements and of calcium release from sarcoplasmic reticulum was dependent on holding potential, a voltage dependence of the nifedipine effect has been qualitatively investigated.

In the experiment illustrated in Fig. 3 the membrane potential was held at two different values (−90 mV and −60 mV) for 50 s before a test pulse (500 ms) at 0 mV. In control conditions (1.5 mM cadmium in the bath solution) the level of the holding potential (HP) had little or no effect on contractions and currents (Fig. 3A) as clearly seen in the displays of traces with an expanded time base (inset). When the HP was held at −90 mV prior to the test pulse, a low concentration of nifedipine (0.5μM) had little effect on contraction and current (Fig. 3Ba). In contrast, setting the HP to −60 mV during the prepulse led to a nearly complete block of the mechanical response and the outward current induced by the test pulse (Fig. 3Bb). These effects were partly reversible by washing out nifedipine (Fig. 3C).

A similar experiment (Fig. 4) was performed on another cell in the absence of external cadmium. Compared to the previous experiment, the control recordings (Fig. 4A) displayed slow inward calcium currents in addition to the mechanical events elicited by depolarizing steps to 0 mV. Adding 0.5 μM nifedipine to the external medium fully blocked I_{Ca} whatever the HP. The contractile response was partly

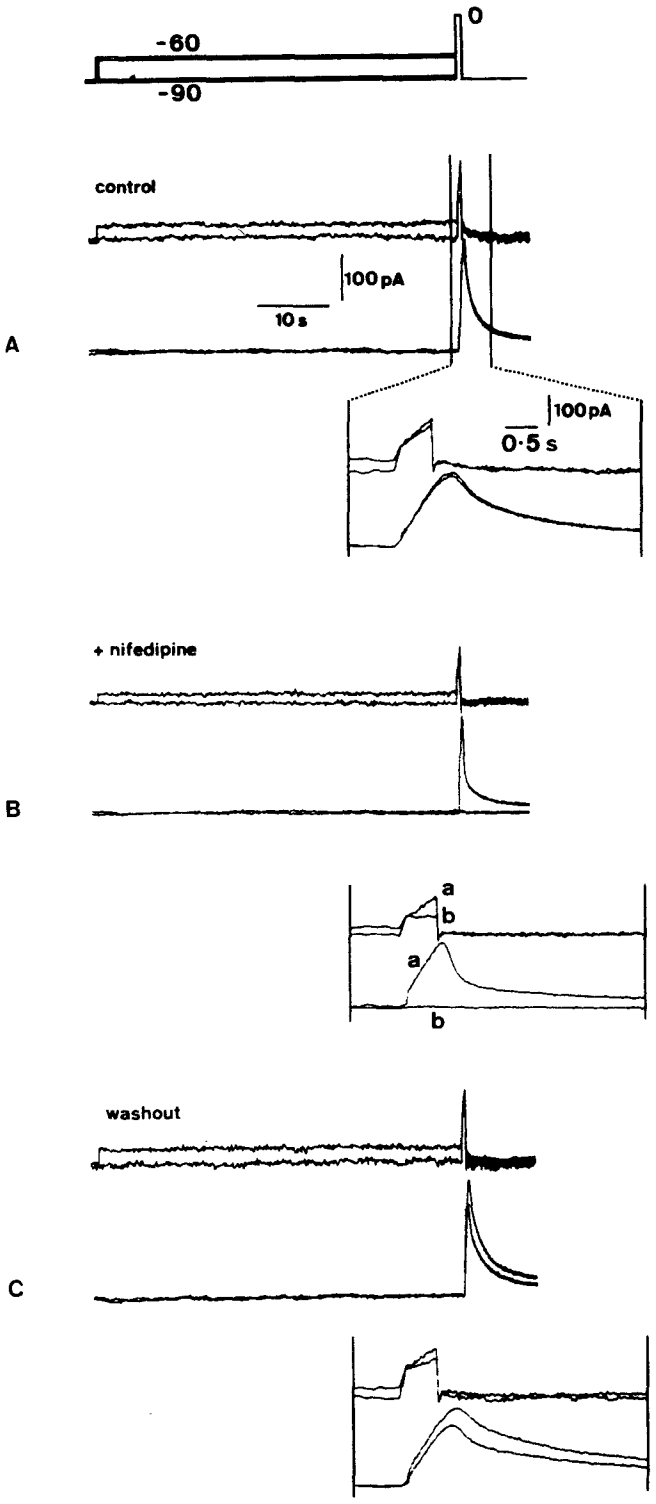


Fig. 3A–C. In the presence of 1.5 mM $CdCl_2$, influence of the holding potential on the effect of 0.5 μM nifedipine on membrane currents and contractions. Currents and contractions were elicited according to the protocol schematized at the top with membrane protential values indicated in mV. Two sample frequencies were used: 20 Hz during the conditioning potential and 100 Hz during the test pulse (500 ms in duration) and the end of recordings. For each set of superimposed recordings in the absence (**A**), the presence (**B**), and after washing out (**C**) nifedipine, the *insets* correspond to the expanded-time-base displays of the indicated part of the original recordings. In **B** traces marked *a* and *b* correspond to the current and contraction records obtained with a preconditioning potential of −90 mV and −60 mV respectively

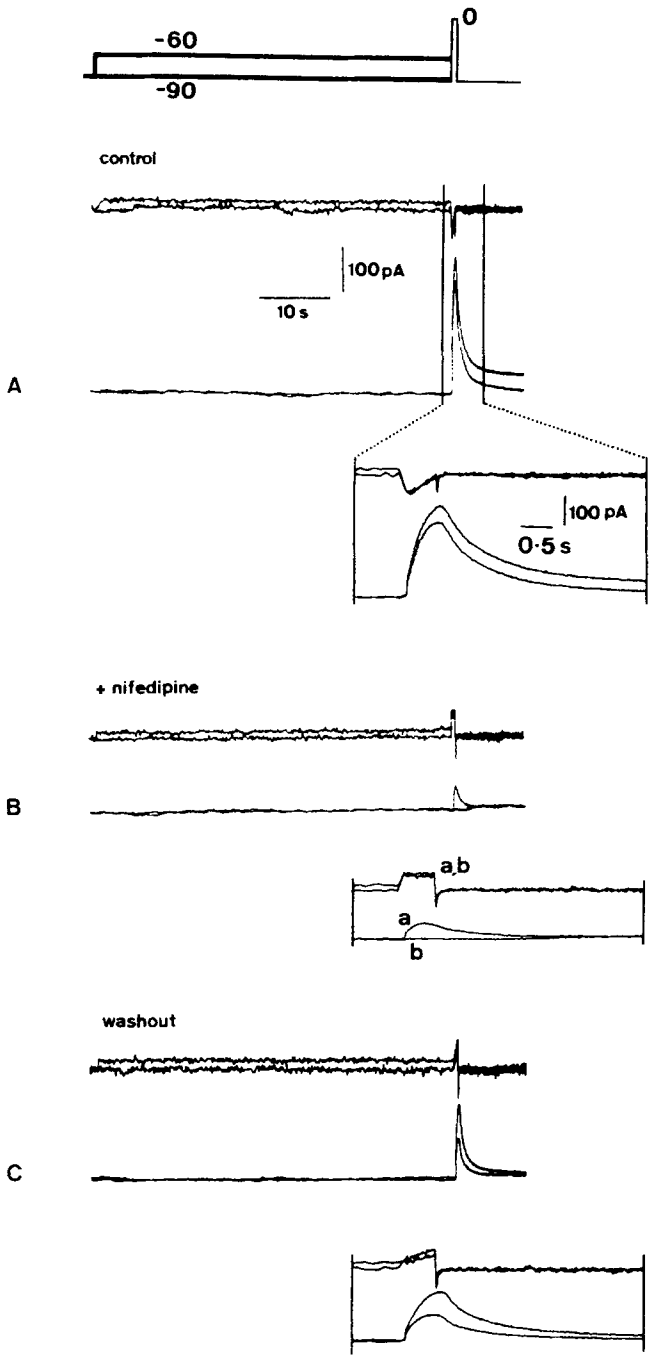


Fig. 4. Same as Fig. 3 but in the absence of $CdCl_2$

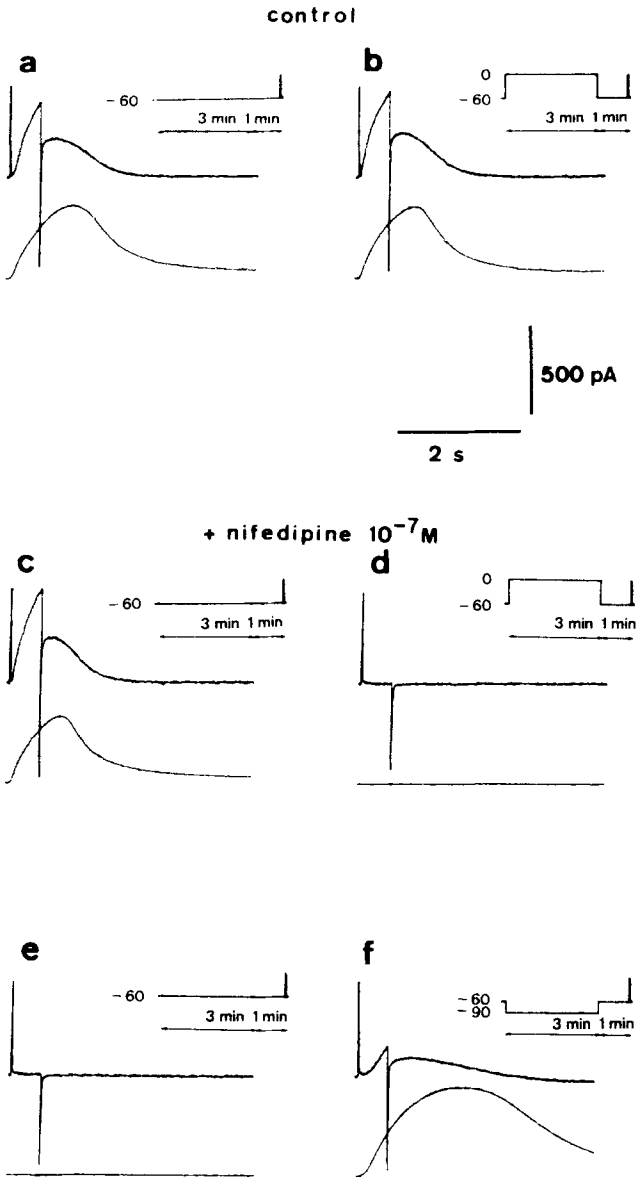


Fig. 5. The effect of long-lasting conditioning potentials, in the presence of $CdCl_2$, on the action of nifedipine at very low concentration (0.1 μM). The six sets of recordings (**a–f**) have been successively obtained from **a** to **f** according to pulse protocols indicated in *inset*. From a reference holding potential of −60 mV, long-lasting (3 min) conditioning prepulses (from −60 to 0 mV, **b** and **d**, or from −60 to −90 mV, **f**) preceded a 1-min resting period at −60 mV and then a test pulse (450 ms in duration) to 0 mV. Currents and contractions have been recorded only during the test pulse (*thick vertical bar* in the diagrams). **a, b** Sets of control recordings in the absence of nifedipine. **c–f** Obtained successively in the presence of 0.1 μM nifedipine with 1 min between each pulse sequence.

inhibited with a HP at −90 mV (Fig. 4B, contraction trace a) and suppressed at HP = −60 mV (Fig. 4B, contraction trace b). After 3 min of washing (Fig. 4C), a partial reappearance of contractile activity clearly occurred (33% of the control value at HP = −60 mV and 55% at −90 mV) whereas I_{Ca} recovered poorly (never more than 20%), which demasked the outward current restoration. This experiment is difficult to analyse because of overlapping between the calcium and outward currents and because of the dual nifedipine effect on the two components of contraction (this explains the strong effect on contraction even with a HP = −90 mV, Fig. 4Ba). Nevertheless the present results reinforce the previous observations of a potential-dependent inhibitory effect of nifedipine on a current-independent component of contraction and of a link between contraction and the outward current. In addition, they suggest the existence of some differences between the recovery mechanisms of I_{Ca} and contraction.

The voltage dependence of the nifedipine effect was then tested (Fig. 5), with the pulse protocol described below,

for a smaller nifedipine concentration (0.1 μM), which was usually ineffective at a HP of −90 mV as well as at a HP of −60 mV. From a reference value of −60 mV, the HP was shifted to different levels (0 or −90 mV) for 3 min and then returned to −60 mV for 1 min, to allow a full repriming of contractile activity, before a test pulse at 0 mV was applied. Compared to Fig. 5a, Fig. 5b shows that in control conditions, a prepulse at 0 mV for 3 min did not noticeably alter either the contractile response or the outward current markedly present during the pulse and the tail current. A nifedipide concentration as low as 0.1 μM had no effect on the membrane current and the contraction induced by depolarizing pulses from −60 mV to 0 mV (Fig. 5c). In contrast, the shift of HP from −60 to 0 mV for 3 min led to the suppression of the contractile response and of the outward current elicited by the test pulse (Fig. 5d). In the presence of nifedipine, holding the membrane potential at −60 mV for 5 min did not allow any repriming of contraction and outward current (Fig. 5e). By contrast a conditioning prepulse at −90 mV for 3 min allowed the recovery of a large part of the mechanical and electrical responses (Fig. 5f). The contraction was even often increased; this was probably accounted for by the slowing down of the relaxation mechanism rather than by a specific increase in the mechanical activation process, since the activation rate was always decreased.

Discussion

The experimental results reported in the present article can be summarized as follows: (a) independently of its effects on I_{Ca} and on the associated contraction component, nifedipine inhibited in a dose-dependent manner the contractile responses of patch-clamped rat myoballs in culture; (b) this blocking effect was accompanied by the inhibition of an outward current and (c) the effect of nifedipine at low concentration was strongly voltage-dependent.

Eisenberg et al. (1983) first described the inhibiting effects of an organic antagonist of calcium channels (D600) on contraction of skeletal muscle fibres. These and other results reactivated the idea that, in skeletal muscle, the excitation/contraction coupling process was a Ca-induced Ca-release mechanism. Such a mechanism could be triggered by calcium ions entering the cell through voltage-dependent calcium channels since the simplest explanation for the blockade of contraction by the calcium channels' organic antagonists is a primary inhibition of calcium current. The results presented in Fig. 1 could be interpreted in this way. This hypothesis is supported by the existence, in voltage-clamped skeletal muscle cells of the frog (Potreau and Raymond 1980; Ildefonse et al. 1985) and of the rat (Cognard et al. 1988; Rivet et al. 1989), of a component of contraction that depends on calcium entry through calcium channels.

In fact the situation is more complicated. If the calcium-current-dependent mechanism can be involved in long-lasting events, such as potassium contractures or contractions induced by sustained depolarizing steps, the kinetics of I_{Ca} are too slow for I_{Ca} involvement in the fast physiological coupling processes.

Furthermore, in calcium-free solutions buffered with EGTA, twitches can still be elicited for some time (see for example Armstrong et al. 1972). In addition, I_{Ca} blockade by inorganic antagonists like Mn^{2+} or Cd^{2+} leads to the block of only a part of the mechanical response (Potreau and Raymond 1980; Rivet et al. 1989). The results obtained here, in rat myoballs (Fig. 2), obviously show that the inhibitory effect of nifedipine on contraction is independent of its effect on I_{Ca} since it was observed after blockade of this current by cadmium. The data obtained in various experimental conditions, by Berwe et al. (1987), Léoty and Noireaud (1987), Dulhunty and Gage (1988), Gamboa-Aldeco et al. (1988), Jacquemond and Rougier (1988), and Fill and Best (1989), led to an identical conclusion. In spite of (or because of) numerous studies, the reports on the actions of dihydropyridine derivatives and other organic blockers of I_{Ca} on contraction seem sometimes conflicting: these compounds have been shown to depress (Eisenberg et al. 1983; Ildefonse et al. 1985; Berwe et al. 1987; Caputo and Bolaños 1987; Léoty and Noireaud 1987; Frank et al. 1988; Gamboa-Aldeco et al. 1988; Jacquemond and Rougier 1988; Fill and Best 1989), to potentiate (Gonzalez-Serratos et al. 1982; Gallant and Goettl 1985; Dulhunty and Gage 1988) or to have no effects (McCleskey 1985) on mechanical events in muscle cells. In our experimental conditions, the inhibitory effect of nifedipine on contraction was beyond doubt: it was observed in the presence or absence of I_{Ca}, was concentration-dependent (Table 1) and was relieved by washing or membrane potential manipulations.

The outward current, which is simultaneously blocked by nifedipine, seems to depend on intracellular calcium release during contraction: particularly (Fig. 5f) where the contractile response developed more slowly than before application of nifedipine, the outward currents exhibited a similar slowing down. The *I/V* curve of this current (Fig. 2B) displayed a bump, indicating that its amplitude is not controlled by the sole membrane potential. Since this current was only observed when contraction was present, we think, as stated above (see Results), that it is due to a cesium efflux through a K-type permeability and, since it could still be recorded after I_{Ca} blockade by cadmium, that it is more likely dependent on intracellular calcium ions released during contraction than on calcium ions entering the cell through calcium channels. Unless nifedipine exerts an independent but kinetically similar inhibiting effect on the outward current, it seems reasonable to hypothesize that this current is, at least partially, controlled by the calcium released from sarcoplasmic reticulum during contraction, since alterations of contractile response always led to parallel outward current modifications. This observation includes the voltage-dependent effects discussed below. In cultured rat skeletal muscle cells, different types of calcium-dependent K^+ channels have been demonstrated. One with a large conductance (BK channels) is blocked by tetraethylammonium ions and is not permeable to Cs^+ (Pallotta et al. 1981; Blatz and Magleby 1984; Romey and Lazdunski 1984), therefore it could not be involved in the present experiment. A second one is sensitive to the neurotoxin apamin; it has been reported (Cognard et al. 1986) to contribute to the apparent inactivation of I_{Ca} and has been studied at the

single-channel level by Blatz and Magleby (1986). In addition, a third type, with a very small conductance, has been observed (Blatz and Magleby 1986) after blockade of the other ones. As a matter of fact, further experiments are necessary to determine the nature, the properties and the physiological role of this current. This could be important in the view of possible membrane current modulations by the calcium released during contraction.

Despite the fact that I_{Ca} cannot play a major role in the coupling process, the molecules involved in the ECC mechanism exhibit calcium-channel-like properties since they require external calcium ions (see above) and are sensitive to calcium-channel blockers in a voltage-dependent manner (Lüttgau et al. 1986; Berwe et al. 1987; Erdmann and Lüttgau 1989). This latter property has been tested with procedures similar to those used by Lüttgau and co-workers. Their results are interpreted (from the modulated receptor hypothesis of Hille 1984) as a preferential binding of the antagonistic drug to the inactivated state of the target molecule, turning it into a stabilized form ("paralysis" state), from which it dissociates only if the membrane potential is returned to very negative levels. Our results, reported in Figs. 3 – 5, probably relate to these mechanisms.

The qualitative nature of the present experiment obviously precludes any detailed interpretation and determination of a multiple-states model for the coupling molecules. However, the results clearly demonstrated that typical characteristics of the ECC mechanism described elsewhere for frog or rat adult muscle fibres are present in rat embryonic skeletal muscle cells in culture, particularly the voltage-dependence of the inhibition of contration by calcium-channel organic blockers. Since I_{Ca} cannot account for a large part of the ECC mechanism, alternative processes have been proposed. The most documented hypothesis, originally proposed by Schneider and Chandler (1973), is the control of ECC by a voltage-sensing molecule, the activation of which is reflected by a part of the charge movements recorded in the skeletal musle membrane. Since the organic blockers of calcium channels affect both the calcium release flux and the charge motions in a voltage-dependent manner (Rios and Brum 1987) it was proposed that the "voltage sensors" are calcium channel molecules with or without calcium ion permeation or are calcium-channel-like proteins (Rios and Brum 1987; Rios and Pizarro 1988) responsible for the ECC mechanism. In the experiments of Fig. 4 the recovery of I_{Ca} and of contraction seems to be different. Does this indicate that calcium channels and voltage sensors are different molecules? Even if these proteins (or this complex of proteins) are biochemically identical, it would not be surprising that the voltage sensor controlling ECC, because of its possible association with sarcoplasmic reticulum Ca-release channels and/or the "feet structures" (Block et al. 1988), exhibits some different properties from those of functional calcium channels.

Whatever the exact mechanisms that operate in ECC processes, it is important to note that the ECC of embryonic (non-innervated) mammalian skeletal muscle cells in culture, used in the present study, exhibits some features observed in adult frog or mammalian muscle fibres, indicating that elaborate links between the membrane depolarization and the contraction are established early during the cell development.

Acknowledgement. The authors are indebted to J. Alix and J.-P. Poindessault for their skilful assistance. This work was supported by grants from INSERM (CRE no. 875013), Association Française contre les Myopathies, Fondation pour la Recherche Medicale, Fondation Langlois.

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