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VOLTAGE DEPENDENT ACTIVATION OF TONIC CONTRACTION IN CARDIAC MYOCYTES

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Contractions of isolated single myocytes of guinea pig heart stimulated by rectangular depolarizing pulses consist of a phasic component and a voltage dependent tonic component. In this study we analyzed the mechanism of activation of the graded, sustained contractions elicited by slow ramp depolarization and their relation to the components of contractions elicited by rectangular depolarizing pulses. Experiments were performed at 37°C in ventricular myocytes of guinea pig heart. Voltage-clamped myocytes were stimulated by the pulses from the holding potential of -40 to +5 mV or by ramp depolarization shifting voltage within this range within 6 s. $[Ca^{2+}]_i$ was monitored as fluorescence of Indo 1-AM and contractions were recorded with the TV edge-tracking system. Myocytes responded to the ramp depolarization between -25 and -6 mV by the slow, sustained increase in $[Ca^{2+}]_i$ and shortening, the maximal amplitude of which was in each cell similar to that of the tonic component of Ca^{2+} transient and contraction. The contractile responses to ramp depolarization were blocked by 200 μM ryanodine and Ca^{2+}-free solution, but were not blocked by 20 μM nifedipine or 100 - 200 μM Cd^{2+} and potentiated by 5 mM Ni^{2+}. The responses to ramp depolarization were with this respect similar to the tonic but not to the phasic component of contraction: both components were blocked by 200 μM ryanodine, and were not blocked by Cd^{2+} or Ni^{2+} despite complete inhibition of the phasic Ca^{2+} current. However, the phasic component but not the tonic component of contraction in cells superfused with Ni^{2+} was inhibited by nifedipine. Both components of contraction were inhibited by Ca^{2+}-free solution superfused 15 s prior to stimulation. Conclusions: In myocytes of guinea pig heart the contractile response to ramp depolarization is equivalent to the tonic component of contraction. It is activated by Ca^{2+} released from the sarcoplasmic reticulum by the ryanodine receptors. Their activation and inactivation is voltage dependent and it does not depend on the Ca^{2+} influx by the Ca^{2+} channels or reverse mode Na^+/Ca^{2+} exchange, however, it may depend on Ca^{2+} influx by some other, not yet defined route.

Key words: *heart; myocytes; tonic contraction; voltage relation*

INTRODUCTION

Contraction of cardiac myocytes of many mammalian species, humans included, consists of phasic component, relaxation of which is independent on repolarization and of a tonic component lasting as long as a cell is depolarized. It is generally agreed that the phasic component is mostly activated by Ca^{2+} released from the sarcoplasmic reticulum (SR) upon activation of its ryanodine receptors (RyRs). RyRs are activated by increase in $[Ca^{2+}]_i$ brought about by activation of dihydropyridine receptors (DHPRs) (1) or, under some conditions, by DHPRs acting like voltage sensors (2). Mechanism of activation of the tonic component is, however, controversial. It has been proposed that it might be activated directly or indirectly by Ca^{2+} current (3-8), by the reversed Na^+/Ca^{2+} exchange (9-11) or by depolarization (12-14). Recently two groups found that the tonic component is not blocked by inhibitors of the Ca^{2+} current or Na^+/Ca^{2+} exchange but is blocked by ryanodine (Ry) (13, 14). Hence these authors proposed that the tonic component is activated by Ca^{2+} released from the SR. The mechanism of the release is, however, not clear. Ferrier et al. (14) proposed that release is directly dependent on membrane voltage. If this is true, the release should follow the slowly changing membrane potential independently on the phasic Ca^{2+} current. In order to check this hypothesis we used the ramp depolarizing pulses changing membrane potential from -40 mV to 5 mV within 6 s without activation of the phasic Ca^{2+} current. Voltage clamped myocytes of guinea pig hearts responded to the ramp depolarization with graded contraction and increase in $[Ca^{2+}]_i$ of the maximal amplitude equal to the amplitude of the tonic component of contraction activated by rectangular pulses. They were negligible in cells in which the tonic component of contraction was very small. Moreover, the responses to ramp depolarization were blocked by 200 μM Ry and were not blocked by Cd^{2+} or Ni^{2+} likewise tonic component of contraction. However, they were inhibited by removal of Ca^{2+} from the extracellular solution Thus we conclude that responses to ramp depolarization are equivalent to the tonic component of contraction isolated from the phasic component. They are iniciated by Ca^{2+} release from the SR through the RyRs. These RyRs are not activated by Ca^{2+} influx by sarcolemmal Ca^{2+} channels or reverse mode Na^+/Ca^{2+} exchange. However, they cannot be activated without the presence of Ca^{2+} in extracellular solution.

MATERIAL AND METHODS

Cells' isolation

Experiments were performed in the enzymatically isolated ventricular myocytes of guinea pig hearts at 37°C. Guinea pigs of both sexes weighing 250-300 g were injected i.p. with 2,500 U heparin followed 30 min later by an overdose of pentobarbital sodium. After the heart was rapidly excised and washed in cold Tyrode solution, the aorta was cannulated and retrogradely perfused for 3 min with nominally Ca^{2+} free solution containing 100 μM EGTA (for composition of

solutions see below). Initial washout period was followed by 10-15 min of perfusion with Ca^{2+} free Tyrode solution containing 15 mg collagenase B (Boehringer) and 3 mg protease (Sigma) per 50 ml. Thereafter the ventricles were cut from the atria and placed in a 50 ml beaker containing the same solution, disrupted with pincettes into small strands and agitated. The cell suspension was filtered through the nylon mesh, and allowed to sediment. The supernatant was discarded and cells were washed twice with Tyrode solution, the Ca^{2+} concentration being increased gradually to 1 mM.

Cells' superfusion and recording of contractions

Cells were placed in the 0.5 ml superfusion chamber mounted on the stage of an inverted microscope (Nikon Diaphot) and allowed to attach to its glass bottom. The chamber was perfused at a rate of ~2 ml/min. Three lines of perfusion solution heated up to the inlet enabled to change its composition within ~ 30 s. Temperature within the chamber was kept at ~37°C. The TV camera was mounted in the side port of the microscope and the cell length monitored by video edge-tracking system designed and built by John Parker (Cardiovascular Laboratories, School of Medicine, UCLA).

Recording of Indo 1-AM fluorescence

A Nikon mercury lamp was used as a source of illumination for epifluorescence. A concentric diaphragm enabled illumination of a small fragment of a cell. The fluorescent light was passed to the 405-nm DE35 and 495 DE20 photomultipliers mounted in the holders attached to the side port of the microscope. The ratio of 405 to 495 nm fluorescence was obtained from the output of Dual Channel Ratio Fluorometer (Biomedicel Instrumentation Group, University of Pennsylvania). Cells were loaded with Indo 1-AM form as described by Spurgeon et al. (15): 12.5 µl of a solution containing 50 µl of 1 mM Indo 1-AM dissolved in dimethyl sulfoxide, 2.5 µl of 25% Pluronic, and 75 µl of bovine calf serum was added to 500 µl of cell suspension. Cells were incubated 5 - 15 min at room temperature, washed in Tyrode solution and placed in superfusion chamber. No attempt was made to convert the fluorescence ratio to Ca^{2+} concentration.

We were not able to record Ca^{2+} transients and contractions simultaneously in one cell.

Recording of ionic currents

The currents were recorded using whole cell clamp method. Pipettes of 1.8 - 2.2 MΩ resistance were pulled by the programmed Flaming/Brown Puller model 97 from the borosilicate glass capillaries. Rectangular or ramp pulses from a holding potential of -40 mV to +5 mV were applied at 1 Hz. Currents were recorded using a Patch Clamp L/M-EP7 (List Electronic) amplifier controlled by ISO2 Multitask-Patch-Clamp Software and computer interface designed by M. Friedrich and K. Benndorf.

Solutions

For cells isolation and throughout the experiments we used Tyrode solution of the following composition (in mM): 144 NaCl, 5 KCl, 1 $MgCl_2$, 0.43 NaH_2PO_4, 10 N-2-hydroxyethylpiperazine-N'-2-etanesulfonic acid (HEPES), 11 glucose and 5 sodium pyruvate. The pH of the solution was adjusted with NaOH to 7.3 for cells isolation and to 7.4 for experiments. In the experiments $CaCl_2$ was added to concentration of 2 -3 mmol/L. The patch pipettes were filled with a solution containing (in mM): 135 KCl, 7.5 NaCl , 1 $MgCl_2$, 10 HEPES, 4 Mg ATP, 0.05 8-Br-cAM. Experimental protocols will be for clarity described in detail in RESULTS section.

Statistical evaluation

The quantitative results are presented as means ±SD. Student's t test for paired samples was used in order to check statistical significance of differences between the means.

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the USA National Institutes of Health (NIH Publications No. 85-23, revised 1996).

RESULTS

Cells' responses to rectangular and ramp depolarizing pulses

Myocytes of guinea pig hearts responded to rectangular pulses with inward, presumably Ca^{2+} current and contractions or Ca^{2+} transients consisting of phasic and tonic components (*Fig. 1A, E*). The ratio of the amplitude of the tonic component to the amplitude of the phasic component is shown in *Table 1*.

The ramp pulses introduced after the rectangular pulses elicited the slow contractile response or increase in fluorescence (*Fig. 1B, F*), the maximal amplitude of which was similar to that of the preceding tonic component elicited by rectangular pulse (differences not statistically significant). The phasic current and phasic component of contraction were absent. The ratios of the amplitudes of contractile responses to ramp depolarizations and phasic components of

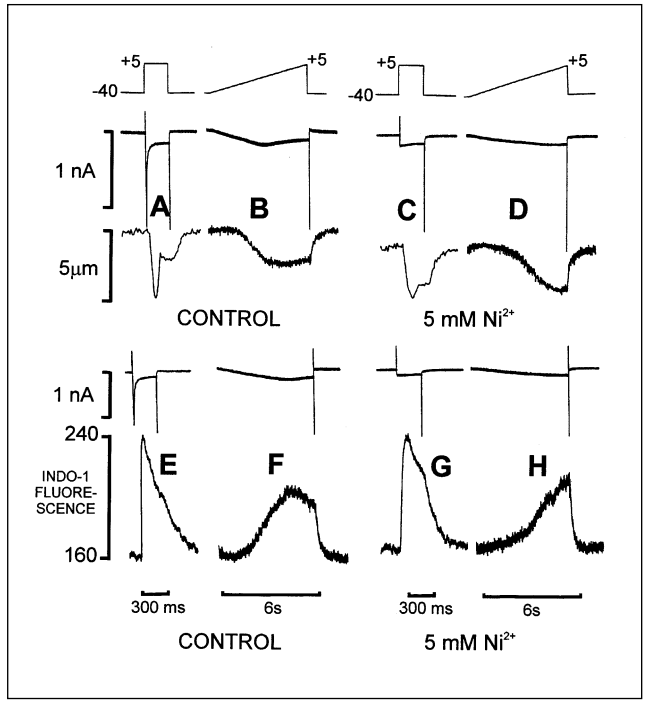

Fig. 1. Membrane currents (top records), changes in cell length (A through D, contraction downwards), and Ca^{2+} transients (E through H) recorded as fluorescence of Indo-1 elicited in two myocytes of guinea pig heart by voltage protocols shown above the records. C, D and G, H: the effects of 5 mM Ni^{2+} superfused from the beginning of 30 s break in stimulation preceding recording. Please, notice that in C and G the phasic Ca^{2+} current is completely blocked and in D and H the current elicited by ramp depolarization (gain doubled) is partly blocked. Stimulation rate 60/min. Ramp pulses intercalated between the rectangular pulses. Time scale pertains to all panels.

preceding contractions are shown in *Table 1*. The contraction or fluorescence - voltage relation is shown in *Fig. 2B* and *2C*. Contraction or increase in fluorescence began at ~-25 mV, reached its maximum at ~-6 mV and slightly declined when voltage approached +5 mV.

The contractile and fluorescence responses to the ramp pulses were accompanied by the inward current partly decaying at the less negative potential (*Fig. 1B, F*).

The effect of 5 mM Ni^{2+} or 100 μM Cd^{2+} on responses to rectangular and ramp pulses

In order to investigate the effect of Ni^{2+} we used the experimental protocol enabling to stimulate nearly normal contractions or Ca^{2+} transients without

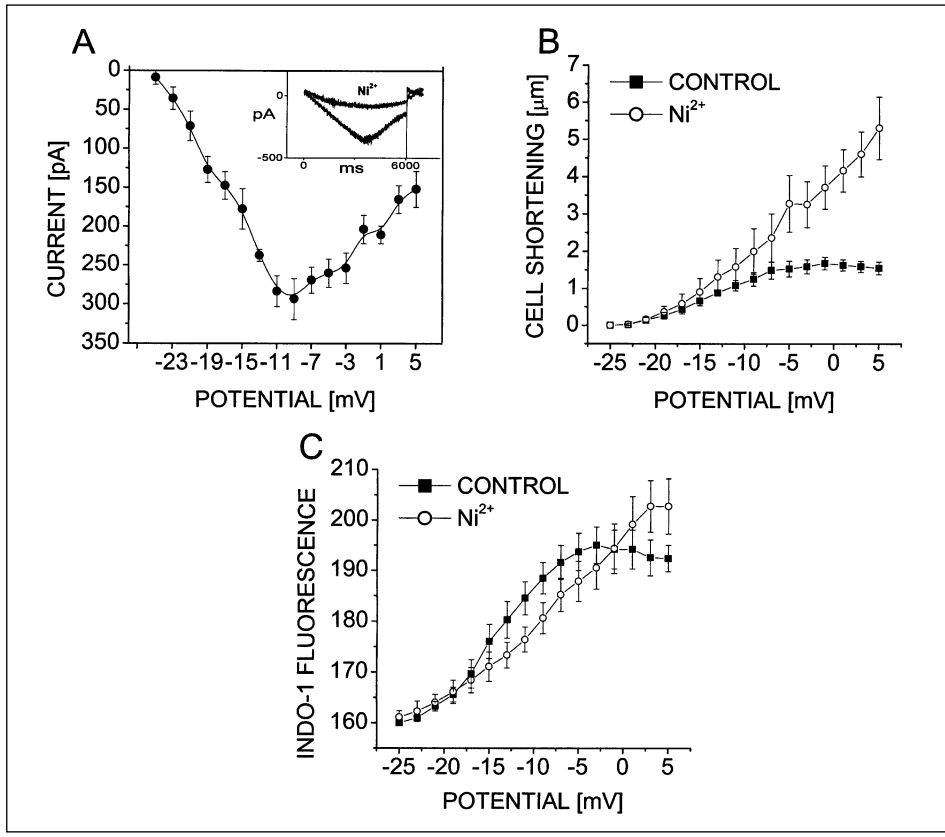

Fig. 2. Voltage relation of the responses of single myocytes of guinea pig heart (n= 14) to ramp depolarization (shown in *Fig. 1*) measured at 100 ms intervals. A: Ni^{2+}- sensitive current-voltage relation; B: cell shortening - voltage relation; C: Indo-1 fluorescence-voltage relation. Ni^{2+}-sensitive current calculated by subtraction of the residual current in a cell superfused with 5 mM Ni^{2+} from the control current (*Fig. 1B, D* and *inset in A*).

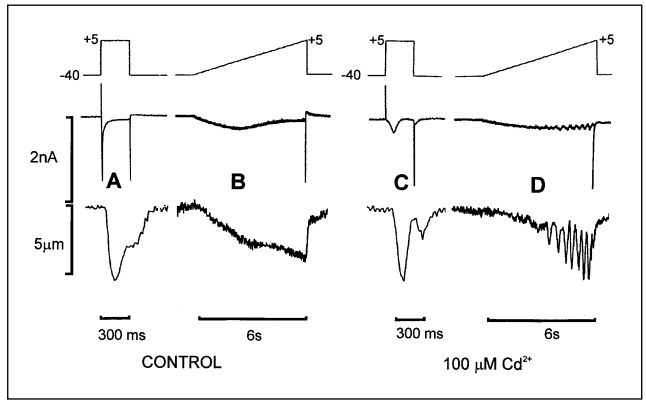

Fig. 3. The effect of 100 μM Cd^{2+} on membrane currents (top records) and cell shortening (contraction downwards) in single myocyte of guinea pig heart.

apparent activation of Ca^{2+} current, described in detail elsewhere (2). Briefly, myocytes of guinea pigs were stimulated for few minutes by the rectangular pulses at 1 Hz. The intercalated ramp pulses were also applied. Thereafter stimulation was stopped and 5 mM Ni^{2+} immediately superfused. Since Ni^{2+} blocks Ca^{2+} currents and Na^{+}/Ca^{2+} exchange, the intracellular Ca^{2+} was trapped by this maneuver. After 30 s stimulation with rectangular pulses was resumed and response to the ramp pulses tested. As reported previously (2, 13) the rectangular pulses elicited nearly normal biphasic contractions or Ca^{2+} transients despite apparent inhibition of the Ca^{2+} current. The amplitude of the tonic component increased (*Fig. 1C, G* and *Table 1*). The contractile responses to the ramp pulses were potentiated at all potential levels (*Fig. 1D, Fig. 2B* and *Table 1*). Their voltage relation was modified so, that it was more monotonous and the plateau was no longer present (*Fig. 2B*). The pattern of responses of fluorescence to ramp depolarization was changed similarly as that of contractile responses. However, they were only slightly potentiated at the positive voltages (*Fig. 1H* and *Fig. 2C*). The difference in the effect of Ni^{2+} on the contractile and fluorescence responses to ramp depolarization is difficult to explain. It may only partly depend on nonlinear relation between fluorescence and $[Ca^{2+}]_i$. Nevertheless these responses were modified but not inhibited by Ni^{2+} which blocks the Ca^{2+} currents and both modes of Na^{+}/Ca^{2+} exchange (13, 16).

Table 1. Ratio of the amplitude of the tonic component of contraction or contractile response to ramp depolarization to the amplitude of the phasic component of contraction. Mean ± SD; n=12

TONIC COMPONENT		RESPONSE TO RAMP DEPOLARIZATION	
CONTROL	Ni^{2+}	CONTROL	Ni^{2+}
0.22 ± 0.07	0.36 ± 0.14	0.22 ± 0.08	0.48 ± 0.17

The inward currents accompanying responses to the ramp pulses were partly blocked. The difference between the control current and the residual current (*Fig. 2A, inset*) in cells superfused with Ni^{2+} will be called the Ni^{2+}-sensitive current. Its voltage relation is shown in *Fig. 2A*. The nature of Ni^{2+}-sensitive current accompanying responses to the ramp depolarization is important for interpretation of mechanism of their activation. It could be the Na^{+}/Ca^{2+} exchange current or sustained and graded Ca^{2+} current. In order to differentiate between these possibilities we repeated the above experimental protocol, however, 100 μM Cd^{2+} instead of Ni^{2+} was superfused (*Fig. 3*). This resulted in inhibition of phasic Ca^{2+} current activated by the rectangular pulses, and partial inhibition of the current activated by the ramp pulses (Ni^{2+}-sensitive current). Despite inhibition of the phasic Ca^{2+} current rectangular depolarizing pulses still elicited biphasic contractions consistent with results of Hobai et al. (17) (*Fig. 3C*). The contractile responses to ramp depolarization became more monotonous, as in experiments with Ni^{2+}. They were not, however, potentiated. Small phasic contractions superposed on the slow decrease in length appeared at voltages above -10 mV (*Fig. 3D*).

Cd^{2+} at concentrations used in these experiments blocks the Ca^{2+} currents but it doesn't inhibit the Na^{+}/Ca^{2+} exchange (16, 18). Thus the experiments with Cd^{2+} suggest that slow, inward, Ni^{2+} sensitive current elicited by ramp depolarization was mostly a Ca^{2+} current rather, then Na^{+}/Ca^{2+} exchange current. Its inhibition did not, however block the contractile response to the ramp depolarization. Potentiation of contractile response to ramp depolarization and lack of potentiation by Cd^{2+} suggest important role of the Na^{+}/Ca^{2+} exchange in

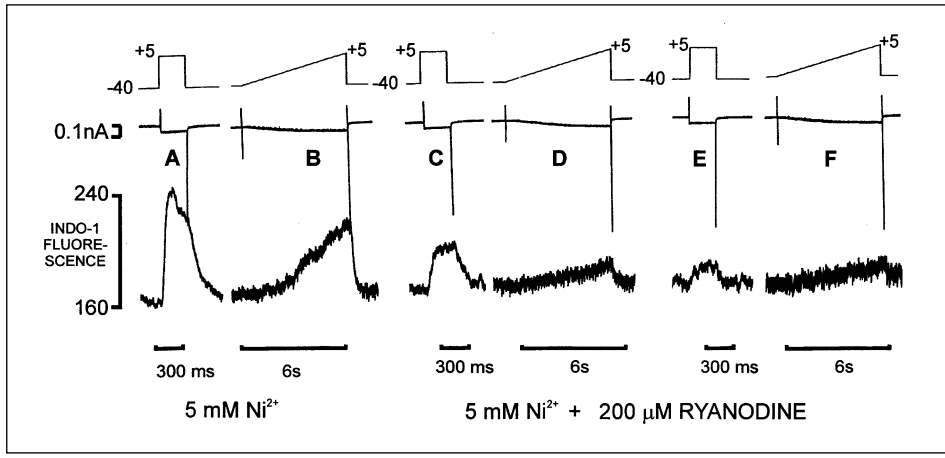

Fig. 4. The effects of 200 μM ryanodine on membrane currents (top records) and Ca^{2+} transients measured as fluorescence of Indo-1 in single myocyte of guinea pig heart superfused with 5 mM Ni^{2+} (continuation of experiment shown in *Fig. 1E through H*). The phasic Ca^{2+} current and sustained Ni^{2+} sensitive current are blocked.

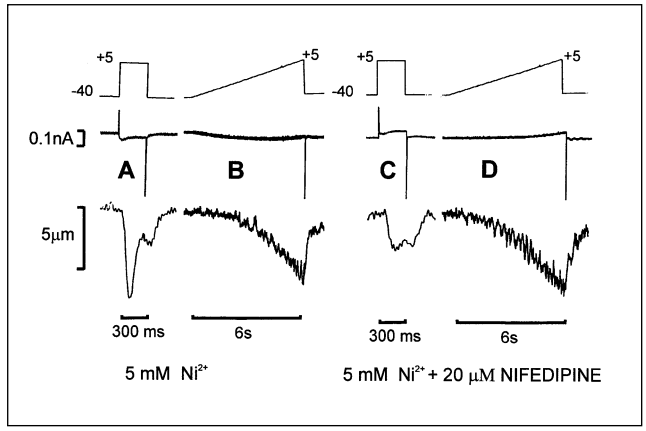

Fig. 5. The effects of 20 μM nifedipine on membrane currents (top records) and cell length (contraction downwards) in a single myocyte of guinea pig heart superfused with 5 mM Ni^{2+}. The phasic Ca^{2+} current and sustained Ni^{2+} sensitive current are blocked.

maintaining steady or even decreasing $[Ca^{2+}]i$ at voltages positive to -6 mV in normal cells.

Experiments with Ni^{2+} and Cd^{2+} strongly suggest that the responses to ramp depolarization did not depend on the Ca^{2+} influx by sarcolemmal Ca^{2+} channels or Na^{+}/Ca^{2+} exchange. In order to determine the source of Ca^{2+} activating these responses we tested the effect of 200 μM Ry, which blocks the RyRs of the SR (19, 20).

The effect of 200 μM Ry on responses to rectangular or ramp depolarizing pulses

The effect of Ry was tested in cells superfused with Ni^{2+} in order to eliminate the direct activation of contraction or Ca^{2+} transient by the Ca^{2+} influx. Ry inhibited both phases of contractions or Ca^{2+} transients within 2 - 4 min (*Fig. 4C through F*). As already reported elsewhere (13), inhibition of the phasic component preceded that of the tonic component (*Fig. 4C, E*). Contractile and fluorescence responses to ramp depolarization were almost completely inhibited. The amplitude of the residual response did not exceed 10% of the control one (*Fig. 4D, F*).

These results suggest that the responses to ramp depolarization were due to graded, voltage dependent activation of the RyRs of the SR. In the next experiments we tested the possible mechanism of their activation. In the previous study (2) we found that the phasic component of contraction elicited by rectangular depolarizing pulses in cells superfused with Ni^{2+} is inhibited by nifedipine, which block conformational changes of DHPRs. This suggested that when the Ca^{2+} current is blocked by Ni^{2+}, the RyRs responsible for activation of phasic contraction are activated by DHPRs acting like the voltage sensors. The tonic component of contraction was not, however, affected by nifedipine. Therefore in the present experiments we applied 20 mM nifedipine in order to

check whether the RyRs responsible for the responses to ramp depolarization are activated by the DHPRs acting as the voltage sensors.

The effect of 20 μM nifedipine on responses to rectangular or ramp depolarizing pulses

As reported previously (2), in cells superfused with Ni^{2+} nifedipine inhibited strongly the phasic component of contraction whereas the tonic component was not changed or even increased (*Fig. 5C*). The responses to ramp depolarization were not inhibited (*Fig. 5D*). Nifedipine did not affect the slow inward current in cells superfused with Ni^{2+}.

The effect of Ca^{2+}-free solution on responses to ramp depolarization

Superfusion of nominally Ca^{2+}-free solution 15 s prior to stimulation completely abolished contractile responses to the rectangular or ramp depolarizing pulses (not shown). This effect was observed both in normal myocytes and in myocytes superfused with 5 mM Ni^{2+}.

DISCUSSION

Cardiomyocytes of guinea-pigs responded to ramp depolarization with sustained rise in $[Ca^{2+}]_i$ and contraction gradually increasing when the voltage changed within the window between ~-25 mV and ~-6 mV. Above this voltage both events reached a plateau or slightly declined. A number of results suggest that responses to ramp depolarization are equivalent to the tonic component of contraction elicited by the rectangular depolarizing pulses: i. the ratio of their amplitude to the amplitude of the phasic component of contraction is the same as the ratio of amplitude of the tonic to phasic component, ii.both ratios change in the same way under the effect of Ni^{2+} (*Table 1*), iii. the response to ramp is negligible in some cells of guinea pig in which the tonic component is very small (not shown). Thus the ramp depolarization provides the method of activation of the tonic component in isolated form. This is important for at least two reasons: i. it opens new possibilities of its analysis; ii. it has been proposed that Ca^{2+} activating the phasic component releases Ca^{2+} activating the tonic component (21) which apparently is not the case.

The responses to ramp depolarization and both components of contraction are inhibited by 200 μM Ry, which blocks the RyRs (19, 20). This means that there are two fractions in the total population of RyRs in the SR of myocytes of guinea pig heart. A fraction, which is responsible for activation of the phasic component, inactivates spontaneously independently on the instantaneous membrane potential. The other fraction responsible for activation of the tonic component is activated as long as a cell is depolarized and inactivates only upon

its repolarization. This fraction of RyRs shows the graded, sustained activation in response to the membrane voltage slowly changing within the window between -25 mV and -6 mV. The further analysis suggests that their activation does not depend on Ca^{2+} influx by the sarcolemmal Ca^{2+} channels or by the reverse mode $Na+/Ca^{2+}$ exchange, however it needs the presence of extracellular Ca^{2+}.

Contractile and $[Ca^{2+}]_i$ responses to ramp depolarization were accompanied by slow, sustained inward current. Superfusion of 5 mM Ni^{2+} according to the protocol of Mackiewicz *et al.* (2) blocked the phasic L-type Ca^{2+} current, did not block the phasic component of contraction consistent with the previous results of these authors and those of Hobai *et al.* (17), and increased the amplitude of the tonic component. The contractile responses to ramp depolarization were greatly potentiated and their voltage relation rendered more monotonous (in most cells no plateau was seen). The inward current elicited by the ramp pulses was partly blocked. The voltage relation of the Ni^{2+} sensitive current (*Fig. 2A*) might suggest that it was the current of Na^{+}/Ca^{2+} exchange working in the Ca^{2+} out mode and stimulated by increase in $[Ca^{2+}]_i$. However, the Ni^{2+} sensitive current was also blocked by Cd^{2+} which in the concentrations used in these experiments does inhibit the L-type Ca^{2+} current but does not block the Na^{+}/Ca^{2+} exchange (16, 18) which is also apparent in *Fig. 3*. This result suggests that Ni^{2+} sensitive current is a Ca^{2+} current, rather then the Na^{+}/Ca^{2+} exchange current. Cd^{2+} did not block the phasic or tonic component of contraction elicited by rectangular pulses consistent with the results of Hobai *et al.* (17) and did not affect the amplitude of contractile responses to the ramp pulses. However, the contraction-voltage relation became more monotonous (linear) as under the effect of Ni^{2+} (*Fig. 3*). Comparison of the effects of Ni^{2+} and Cd^{2+} with the control records suggests that only a slight fraction of contractile response to ramp depolarization might depend on the Ca^{2+} influx by the Ca^{2+} current. Since we have proved that 5 mM Ni^{2+} blocks effectively reverse mode Na^{+}/Ca^{2+} exchange (13), activation of the RyRs responsible for the responses to ramp depolarization by Ca^{2+} influx by this route also seems very unlikely.

In cells superfused with Ni^{2+}, 20 μM nifedipine did not further affect the inward current or the contractile response to ramp depolarization although the phasic component (but not the tonic component) of contractions elicited by rectangular depolarizing pulses was almost completely inhibited (*Fig. 5*). This result supports the conclusion that the sustained, Ni^{2+} sensitive current is a Ca^{2+} current and that it does not initiate the bulk of the contractile response to the ramp depolarization or the tonic component of contraction. Since dihydropyridines block the voltage-dependent conformational changes of the Ca^{2+} channels (22), inhibition of the phasic component of contraction by nifedipine motivated Mackiewicz *et al.* (2) to hypothesize that in cells superfused with Ni^{2+} RyRs responsible for activation of the phasic component

of contraction are activated by DHPRs acting like voltage sensors in skeletal muscle. Apparently this is not true in the case of the tonic component of contraction and response to the ramp depolarization.

Talo *et al.* (8) investigated the effect of the small (3 - 5 mV) steps of depolarization from the holding potential of -50 - -40 mV on the length and $[Ca^{2+}]_i$ of the single rat myocytes. They found that these steps were subthreshold for the phasic Ca^{2+} current or contraction, but elicited small sustained inward current and sustained increase in $[Ca^{2+}]_i$ and sustained decrease in cell length. The contractile response was inhibited by Ry. Nitrendipine (and other Ca^{2+} current blockers) blocked the contractile responses to depolarization below -20 mV. However, a fraction of response to depolarization above -20 mV was nitrendipine resistant. The authors concluded that RyRs responsible for contractile responses were activated by the steady Ca^{2+} current. Since responses to the small, subthreshold depolarizing steps seem to be equivalent to the responses to the ramp depolarization, these results seem to be at variance with ours. However, we also performed similar experiments with small steps of depolarization in myocytes of guinea pig and rat heart (unpublished). We found that contractile responses to these steps are not blocked by 5 mM Ni^{2+} in guinea pigs but are blocked in rat myocytes. Thus it seems that there are species differences in the mechanism of activation of the contractile responses to the subthreshold depolarizations: in rat they depend mostly on steady Ca^{2+} current whereas in guinea pig they do not depend on Ca^{2+} influx by sarcolemmal Ca^{2+} channels.

In conclusion, we propose that single myocytes of guinea pig heart respond to the slow ramp depolarization with activation of a fraction of their RyRs, which results in slow, graded, sustained increase in $[Ca^{2+}]_i$ and contraction. Activation of this fraction of RyRs does not depend on the Ca^{2+} influx by Ca^{2+} current or reverse mode Na^+/Ca^{2+} exchange. They are not activated by DHPRs acting like Ca^{2+} channels or voltage sensors. These results suggest two possible mechanism of activation of the tonic component of contraction: i. a fraction of RyRs is directly or indirectly sensitive to the membrane voltage. In the second case they might be coupled to some protein(s) other then DHPRs acting as their voltage sensors. For some reason this mechanism needs the presence of extracellular Ca^{2+}. ii. RyRs responsible for initiation of the tonic component of contraction are activated by Ca^{2+} influx by the yet not defined route other than sarcolemmal Ca^{2+} channels or reversed mode Na^+/Ca^{2+} exchange.

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