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MODULATION OF CALCIUM CURRENT GATING IN FROG SKELETAL MUSCLE BY CONDITIONING DEPOLARIZATION

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SUMMARY

1. Ca^{2+} inward currents were measured by voltage clamping cut skeletal muscle fibres of the frog (*Rana esculenta*) in a double-Vaseline-gap system.

2. In order to study the basis of the previously described fast gating mode induced in the Ca^{2+} inward current by a conditioning depolarization we quantitatively analysed the response to differing features of the conditioning prepulse.

3. The faster activation seen during the second of two depolarizations was confined to the component of the inward current which could be blocked by 5 to 10 μM nifedipine.

4. By applying depolarizing conditioning pulses of gradually increasing length the time course of the transition to the fast gating mode could be determined.

5. Both the transition to the fast gating mode (point 4) caused by a depolarization and the slow inward current activated during the same depolarization showed similar voltage-dependent kinetics.

6. The kinetic change of the test current appeared to be equal when the same fractional activation was achieved at the end of the conditioning pulse independent of its duration or amplitude.

7. Flash photolysis of nifedipine in the interval between conditioning and test pulse showed that the predepolarization causes a rate-enhancing effect even though the slow channels were blocked by nifedipine during the conditioning pulse.

8. We conclude that the transition of the calcium channel from its slow to its fast gating mode is determined by the slow voltage-dependent reaction which limits the rate of channel opening under control conditions. This reaction is apparently not prevented by the binding of nifedipine and the block of current flow through the channel.

INTRODUCTION

L-type calcium channels of different vertebrate tissues (for references see Glossmann & Striessnig, 1988, 1990; Porzig, 1990) share a high binding affinity for

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organic Ca^{2+} agonists and antagonists (in particular dihydropyridines (DHPs)), but vary in terms of their voltage-dependent gating behaviour.

The L-type Ca^{2+} channel in skeletal muscle turns on considerably slower when activated by a depolarization than the structurally very similar channel in the heart (Sanchez & Stefani, 1983; Tanabe, Beam, Adams, Niidome & Numa, 1990). However, it has been shown that the activation kinetics of the skeletal muscle channel is accelerated following a conditioning prepulse (Feldmeyer, Melzer, Pohl & Zöllner, 1990; Garcia, Avila-Sakar & Stefani, 1990; Ma, Hosey & Rios, 1992). After activation and subsequent deactivation of the current this channel can remain in a condition that allows more rapid opening showing that the channel exhibits at least two closed states with very different kinetics of voltage-dependent transition to the open state, i.e. a slow and a fast gating mode (Feldmeyer *et al.* 1990).

In the present study we analysed the extent of accelerated activation when changing different parameters of the conditioning depolarization and show that the degree of pre-activation of the nifedipine-sensitive Ca^{2+} channels appears to be the principle determinant of the subsequent test response while the size of the Ca^{2+} current is of negligible importance. Furthermore, we demonstrate that the transformation to a fast gating mode of the channel caused by conditioning depolarization takes place even when the channels are blocked by the dihydropyridine nifedipine.

Some of the results have been published in abstracts (Zöllner, Feldmeyer, Melzer & Pohl, 1991; Melzer, Feldmeyer, Pohl & Zöllner 1991*a*, *b*).

METHODS

Current recording

The experimental approach and data analysis followed procedures described in a previous paper (Feldmeyer *et al.* 1990). Briefly, cut segments of single fibres of the m. semitendinosus dissected from frogs (*Rana esculenta*), which had been killed by decapitation, were voltage clamped at slack length in a double-Vaseline-gap system (Kovacs, Rios & Schneider, 1983). Currents were corrected for linear leak and capacitive components by using hyperpolarizing control pulses of one-quarter test pulse amplitude ($-P/4$ procedure). The control records used for the correction were averages of four to ten sweeps applied at intervals of about 5 s while the corresponding test records were single sweeps. The rapid initial inward phase which was usually present in addition to the slow inward current was determined as described previously (Feldmeyer *et al.* 1990) by finding the point at which the rate of rise of the current had fallen to a certain fraction (generally 50%) of its initial maximal value. To quantify the altered speed of inward current activation we used the half-time of the slow current component. The experimental temperature ranged between 16 and 21 °C.

Solutions

With the exception of the nifedipine experiments the external and internal solutions had the following compositions (mM). Internal solution: caesium glutamate, 80; $MgCl_2$, 6·2; Cs_2-EGTA, 20; Na_2-ATP, 5; glucose, 5·6; Antipyrylazo III, 0·4; Cs-HEPES, 10 (pH 7·0). External solution: $Ca(CH_3SO_3)_2$, 10; TEA(CH_3SO_3)$_2$, 120; 4-aminopyridine, 1; TTX, $3{\cdot}1 \times 10^{-4}$; TEA-HEPES, 2 (pH 7·4). The methanesulphonate was replaced by glutamate in several experiments.

The light-sensitive dihydropyridine calcium channel antagonist nifedipine was applied externally (5 or 10 μM). It was added to the bathing solution from a 1 mM stock solution in dimethyl sulphoxide. In the nifedipine experiments, the experimental solutions were slightly modified: 30 mM of the internal Cs was replaced by TEA and 0·5 mM of the chloride channel blocker anthracene-9-carbonic acid was added to the external solution.

After each experiment using nifedipine, the experimental chamber was carefully cleaned with

ethanol. To destroy residual nifedipine possibly sticking to the Perspex, the chamber was exposed to UV light from a continuous mercury lamp (HBO 200).

Flash photolysis

In several experiments we applied a rapid photo-inactivation procedure to destroy nifedipine bound to the cut fibre.

As a source of light flashes we used a xenon arc lamp system (JML, Rapp). The arc lamp (XSA-80-355-30171, Advanced Radiation Corporation) was powered by the discharge current of a large capacitor, which caused a light spike exhibiting a decay half-time of less than a millisecond (see Rapp & Güth, 1988). After collimation by a quartz condenser the light of the flash lamp passed through an optical filter with a broad transmission band in the UV region (UG11, Schott) and finally a quartz objective with an effective focal length of 20 mm. The lamp housing was mounted close to the experimental set-up and flashes were applied under an angle of approximately 45 deg onto the fluid surface of the external pool of the experimental chamber after adjustment to minimize light reflection. As a focussing aid we used a guide beam delivered by a 0·5 mW helium–neon laser (Novette, Uniphase). The slightly expanded beam of the laser could be reflected into the back plane of the objective by means of a small mirror which replaced the UG11 filter. For visual observation of the fibre when adjusting the focal point of the flash system we used a long working distance objective (UD 16/0.17, Zeiss 462044) in the transmitted light microscope (ACM, Zeiss) to which the experimental chamber was mounted.

RESULTS

Slow and fast inward current

In almost every experiment the inward currents consisted of a slow and a (usually smaller) rapid component. The presence of a fast component even in the unconditioned current raised the question whether part of the channels exhibit the fast gating mode irrespective of previous depolarizations or whether the rapid turn-on is even caused by a prepulse potentiation of a separate channel population with fast kinetics. Since it has been reported that the fast calcium current (Cota & Stefani, 1986) was resistent to nifedipine (Arreola, Calvo, García & Sánchez, 1987; Avila-Skar, Garcia & Stefani, 1990), we used this drug to block the slow inward current selectively in order to decide which component the kinetic effect can be attributed to. Figure 1 summarizes data from a number of experiments which allowed a comparison of the fast component and the nifedipine-insensitive component and showed their size relative to the slow component.

In fact nifedipine reduced the slow phase much stronger than the fast phase. In some cases, the fast phase of the inward current was exactly matched by the nifedipine-insensitive component (Fig. 1*A*, trace *b*1 and *b*2), but in most experiments the nifedipine-insensitive component was smaller in amplitude (Fig. 1*B*, trace *c* and 1*C*).

Figure 2*A* shows the double-pulse protocol which was applied in most experiments of this study: a conditioning pulse to a potential well above the threshold for activation, just long enough to reach the peak of the slow inward current under control conditions (Fig. 2*B*), was followed by a short period of repolarization to a potential somewhat below the threshold and a subsequent depolarization (test pulse) which initiates a considerably faster inward current activation.

Figure 2*C* shows the remaining current 17 min after application of 10 μM nifedipine to the external pool of the chamber. Since this nifedipine-insensitive

residual current (Fig. 2*C*) showed only a small increase in amplitude during both the conditioning pulse and the test pulse, a potentiation of a fast dihydropyridine-insensitive current can be ruled out as the cause of the faster rise time. Apparently the conditioning effect of prepulse activation originates from the dihydropyridine-

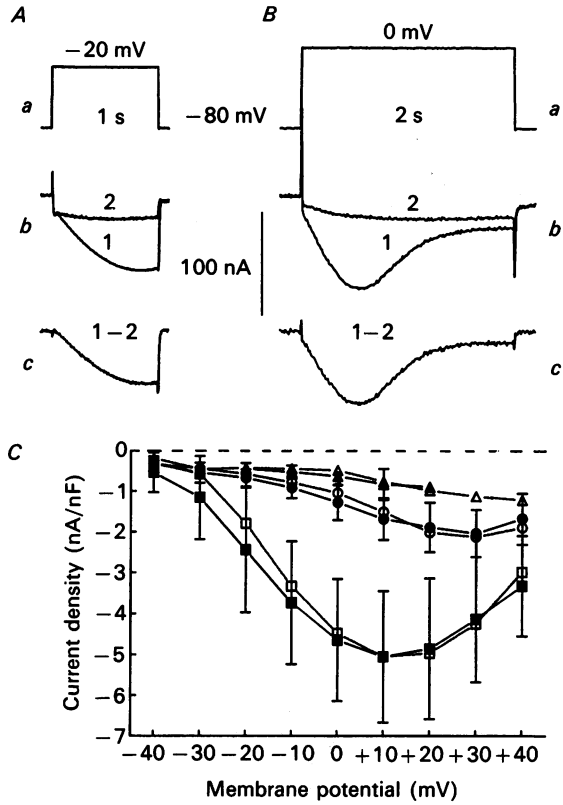

Fig. 1. The relation between fast inward current component and nifedipine-insensitive current. *A*, example of an experiment in which the fast inward component and the nifedipine-insensitive component were identical. *B*, example (more typical than *A*) where the nifedipine-insensitive component was smaller in amplitude than the fast component. Nifedipine (10 μM) was applied in both cases. *C*, current–voltage relation of total inward current, fast component, and nifedipine-resistent component. Results obtained from twenty-six fibres (filled symbols) in which the current was measured at various potentials prior to and at least at one after the application of nifedipine (5 or 10 μM). The mean current–voltage relation (■) of this subgroup of fibres showed no significant differences to that obtained from forty other fibres. The maximum current density was 5 μA/μF in both cases. The circles represent the voltage dependence of the fast inward phase and the triangles show the amplitude of the nifedipine-insensitive component (averages of the current at 30 ms after pulse-on compiled from the same group of fibres). Superimposed (open symbols) are the corresponding current–voltage relations obtained in a single representative fibre in which the current was measured at all voltages in the control solution and after complete block of the slow component by 10 μM nifedipine. *A*, fibre 290, effective capacitance 35·8 nF, temperature 17–18 °C. *B*, fibre 320, effective capacitance 16·9 nF, temperature 16–17 °C. *C*, open symbols: fibre 317, effective capacitance 16·5 nF, temperature 17 °C.

sensitive component (shown in Fig. 2*D* after subtracting the residual current from the original total current).

Even though the fast component resembles the fast calcium inward current described previously (Cota & Stefani, 1986; Garcia & Stefani, 1987; Avila-Sakar *et al.* 1990) our results did not allow a decision as to whether it in fact originates from a Ca^{2+} current, or from an inwardly rectifying background or leak current which was incompletely compensated by the $-P/4$ procedure. Regardless of its origin the fast component is not likely to contribute to the conditioning effect, which was investigated here.

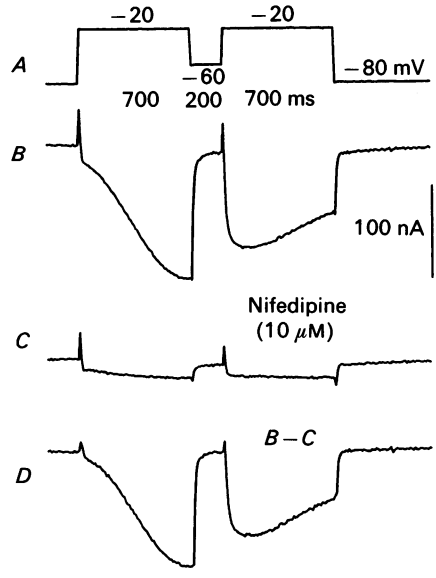

Fig. 2. Slow and fast turn-on of the inward current in subsequent depolarizations to the same potential and block of both responses by nifedipine. *A*, voltage clamp protocol; *B*, control current; *C*, residual current after application of 10 μM nifedipine to the external solution; *D*, result of subtracting trace *C* from trace *B*. Fibre 281, effective capacitance 23 nF, temperature 19 °C.

It can be noted that the amplitude of the nifedipine-sensitive current was smaller at the second pulse than during the conditioning prepulse. This was frequently found in the present series of experiments while in our previous study (Feldmeyer *et al.* 1990), the amplitude of the accelerated test current was usually close to the amplitude of the unconditioned current. The explanation for the decline in amplitude may be voltage-dependent inactivation at the subthreshold potential in the pulse interval or calcium-dependent inactivation caused by a rise of internal calcium.

Graded response of test current kinetics after variable conditioning pulses

Our main goal was to explore how the gating speed of the test current depends on the conditioning pulse parameters. In the experiment of Fig. 3, the duration of the conditioning pulse was gradually increased while the amplitude of the pulse was maintained at a constant value of 0 mV. During this experimental sequence the test depolarization (to −10 mV) was always separated from the end of the conditioning depolarization by a constant time interval of 50 ms. The potential in the interval (prepulse potential) was −60 mV, which was about 20 mV below the activation

threshold in this fibre. Pairs of measurements were carried out, one using the described pulse protocol and the other without the conditioning pulse, but including a subthreshold prepulse of the same duration (t_P) and amplitude (V_P) as the interval between a conditioning pulse (V_C) and a test pulse (V_T). The fast current component

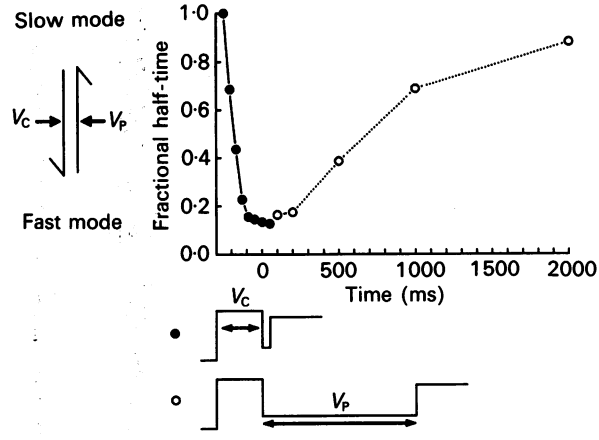

Fig. 3. Onset of the fast and return to the slow gating condition. Time course of the transition to the fast gating mode (●) determined by measuring the speed of activation at a test voltage step to −10 mV after conditioning pulses (V_C = 0 mV) of increasing durations and time course of return to the slow gating mode at V_P = −60 mV (○) determined in the same fibre by increasing the interval between a 300 ms conditioning pulse to 0 mV and a test step to −10 mV. Time 0 on the horizontal axis marks the end of the 300 ms conditioning pulse used in the recovery experiment. The time axis for the durations of the different conditioning pulses starts at −300 ms. In both experimental sequences each data value was plotted at the time of the onset of the test pulse. Fibre 237, effective capacitance 6·9 nF, temperature 19 °C.

and the half-time of the slow component were determined from this control record. The value for the fast component was then subtracted from the conditioned test current and its half-time of activation was determined and normalized by the control half-time. The fractional half-time of the current activated by the test depolarization after the conditioning pulse is plotted on the ordinate in Fig. 3 (●). It can be seen that it decreases with increasing length of the conditioning pulse and reaches a minimal asymptotic value of about one tenth of the normal value.

The longest conditioning pulse in the sequence was 300 ms, which corresponds to the time necessary to reach the peak of the current. This duration was then maintained in a second sequence during the same experiment, in which the interval between conditioning pulse and test pulse was gradually increased (○).

The filled symbols in Fig. 3 show the time course of the transition to the fast gating mode caused by the depolarization V_C, while the open symbols demonstrate the slow fading of the conditioning effect at one subthreshold potential (V_P), i.e. the return to the slow gating mode, which has previously been described in greater detail (Feldmeyer *et al.* 1990). The return to the normal slow gating occurred with a half-time of about 1 s at the chosen interpulse potential of −60 mV and was about 90% complete after 2 s. In the experiments described in our previous paper, we found a

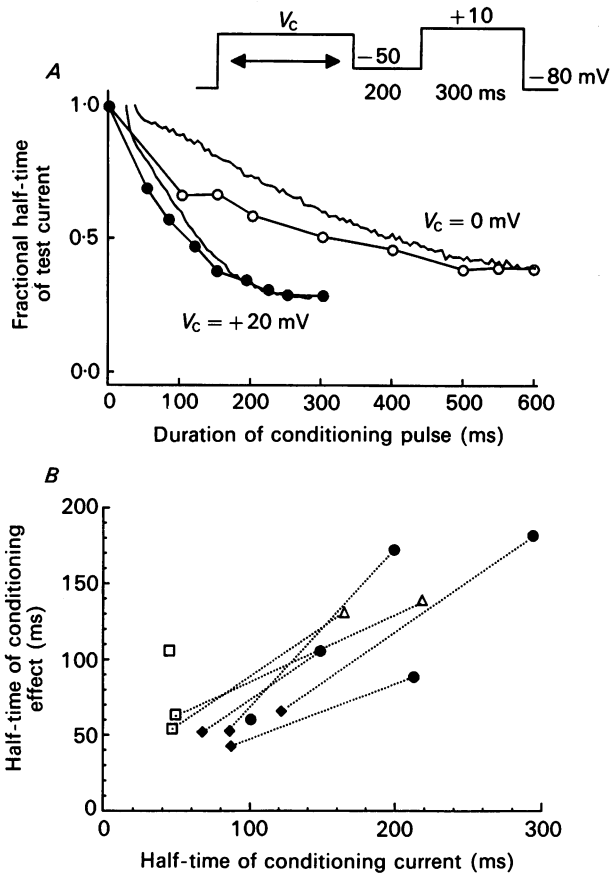

Fig. 4. Correlation between the time course of slow inward current activation and the transition to the fast gating mode. *A*, time course of transition to the fast gating mode as indicated by the half-time of the inward current activation at the test step to +10 mV when applying conditioning pulses of various durations at two different potentials (0 mV, ○; +20 mV, ●). Superimposed are the normalized inward currents measured during the 600 ms conditioning pulse at 0 mV and during the 300 ms conditioning pulse at +20 mV. *B*, comparison of the half-times of the slow inward currents measured at long conditioning pulses of different potentials (0 mV, circles; +10 mV triangles; +20 mV, diamonds; +30 mV, squares) with the half-times of transition to the fast gating mode determined by using the pulse protocol shown in *A*. Data from eight experiments; dotted lines connect values obtained in the same fibre. The open symbols represent experiments in which the conditioning pulses were not long enough to attain the final asymptotic value of the half-time, which is necessary to quantify the time course in this way. In these cases the average value of the minimum fractional half-time obtained in the other five experiments was used. *A*, fibre 230, effective capacitance 18·7 nF, temperature 18–19 °C. *B*, fibres 220, 227–230, 232, 234, 237, temperature range 17–20 °C.

very slow recovery phase evidenced by an incomplete restoration of the test current time course 1 to 3 s after the conditioning pulse (Feldmeyer *et al.* 1990). This slow phase was much less pronounced in the present experimental series which is probably due to the higher experimental temperature (16–21 °C compared with 12–16 °C in the previous experiments).

Figure 4*A* compares the onset of the conditioning effect at two different potentials (V_C, 0 mV (○) and +20 mV (●). As in the experiment of Fig. 3, the length of the conditioning pulse was gradually increased while keeping both the interval and the potential between the two superthreshold pulses constant.

The continuous curves superimposed on the temporal development of the half-times show the inward current records for the longest of the conditioning pulses at each potential. The curves were normalized so that the current baseline corresponds to the value 1·0 on the ordinate, the beginning of the depolarization corresponds to 0 on the time axis and the current values at 300 (+20 mV) and 600 ms (0 mV) were made to match the corresponding fractional half-times. Since the fast component was small in this experiment it was not subtracted. The development of the conditioning effect and the unconditioned inward current clearly follow a similar voltage-dependent time course.

This comparison of the temporal development of the test current acceleration at increasing conditioning pulse duration with the activation time course of the conditioning current was carried out in eight experiments. The result is summarized in Fig. 4*B*. On the abscissa, the half-time of the unconditioned slow inward current is plotted while the ordinate shows the half-time of the development of the conditioning effect determined from plots similar to the one in Fig. 4*A* using linear interpolation in between points.

In Fig. 4*B* the dotted lines connect values which were obtained at two different potentials in the same fibre (see the figure legend for further information). Even though there is a considerable degree of scatter it becomes apparent that there is a correlation between the time course of the slow inward current and the temporal development of the conditioning effect, suggesting that the effect of the conditioning pulse on the kinetic behaviour is causally related to the degree of slow inward current activation.

Fast gating as a function of the degree of channel activation during the conditioning pulse

In Fig. 5 the same pulse protocol as in Figs 3 and 4 was applied, i.e. the duration of the conditioning pulse was varied and the half-time of the test current activation was determined for each duration. Two conditioning potentials V_1 (−10 mV) and V_2 (+10 mV) were used. The current at the end of each conditioning pulse was converted to the degree of channel activation in arbitrary units by using the procedure described in detail in the figure legend, which involved a correction for the fast component as well as for the changes in driving force on the calcium ions due to the different conditioning potentials. The difference in driving force was determined at regular intervals during the experiment by activating the current with a step to V_2 long enough to reach the peak and observing the instantaneous change in current when stepping back to V_1.

The plot in Fig. 5*B* indicates that the acceleration of the Ca^{2+} current kinetics (decrease in half-time) shows a saturating dependence on the degree of channel activation attained during the conditioning depolarization but is otherwise voltage independent.

Conditioning by prepulse depolarization occurs in the nifedipine-blocked state

The result of Fig. 5 is consistent with a close causal relation between reaching the activated state of the channel during the conditioning pulse and the observed rapid channel reactivation during the test pulse. However, it must be noted that the

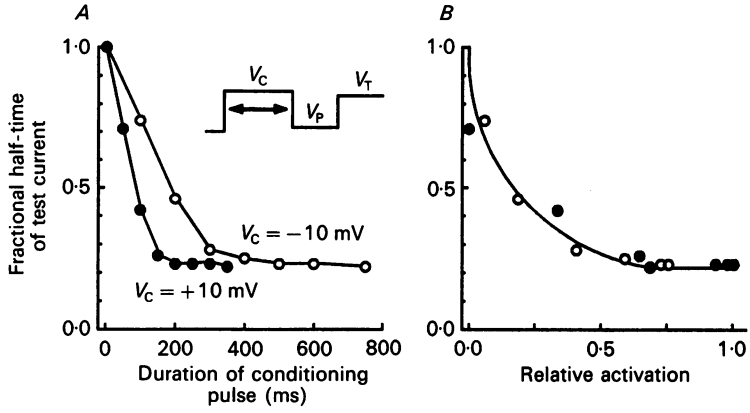

Fig. 5. Comparison of fast gating mode and degree of channel activation during the conditioning pulse. *A*, acceleration of test current activation with increasing length of conditioning pulses (−10 mV, ○; +10 mV, ●). Same experimental procedure as in Fig. 4*A*. V_P was −70 mV, V_T 0 mV, and the interval between conditioning pulse and test pulse was 200 ms. *B*, fractional half-time at the test step as a function of the normalized channel activation at the end of the conditioning pulse. To determine the relative activation of the L-type current, the total currents were corrected for the fast component and the differences in driving force at −10 and +10 mV were determined by applying a stepped-off pulse protocol at regular intervals during the experiment. The fractional instantaneous increase in current when stepping from +10 (350 ms pulse) to −10 mV was used to scale up the current records obtained at +10 mV. Bracketing measurements were linearly interpolated. A run-down of the amplitude of the slow inward current to about 50% within the 2 h of recording has also been corrected for in the graph. The value of 1 on the abscissa arbitrarily marks the largest degree of activation obtained in this experiment. Fibre 226, effective capacitance 12·6 nF, temperature 18–19 °C.

corrections involved make this result sensitive to errors when determining the fast current component and the relative change in the driving force. A more direct demonstration that the kinetic effect depends on a state of the channel molecule induced by the conditioning depolarization and not on the amount of ions carried into the cell during the conditioning pulse is given in Fig. 6. The current was blocked by 5 μM nifedipine. Nifedipine can be destroyed by UV radiation (Morad, Goldman & Trentham, 1983; Gurney, Nerbonne & Lester, 1985). This offers the possibility to unblock the channels at defined moments of time by applying a flash of strong UV light to the fibre.

In the experiment of Fig. 6 the pulse sequence applied to the fibre consisted of a conditioning and a test pulse (both to +10 mV and of 500 ms duration) separated by a 200 ms interval at −50 mV. Figure 6*A* and *B* shows the responses prior to and 13 min after the application of nifedipine. Nifedipine blocked the slow inward current and only a small inwardly directed component remained (compare Figs 1 and 2).

During the records shown in Fig. 6*B–D*, the fibre was exposed to UV flashes at different points during the pulse protocol, thus restoring the voltage-dependent calcium influx. In these experiments, the irradiation destroyed nifedipine only in a small portion of the external pool of the chamber. The interval between the

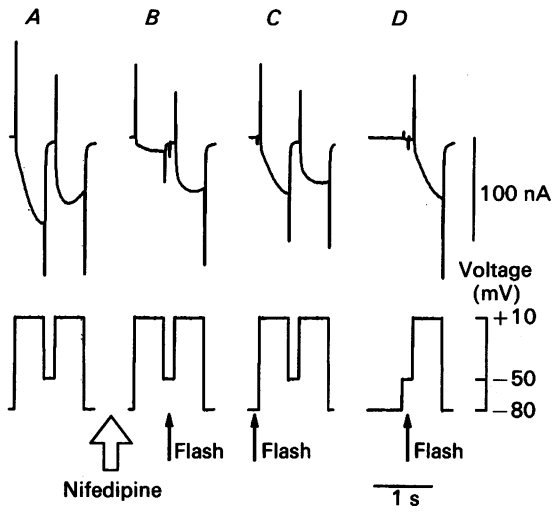

Fig. 6. Combination of double-pulse stimulation and flash photolysis of nifedipine. *A*, control; *B*, 13 min after application of 5 μM nifedipine, flash application 100 ms before the test pulse; *C*, 20 min after application of nifedipine and after restoration of block from previous photolysis by diffusional exchange with the large pool of non-irradiated nifedipine in the external compartment of the chamber; flash application 50 ms before the control pulse; *D*, 26 min after application of nifedipine, conditioning pulse omitted, flash 100 ms before the test pulse. Fibre 279, effective capacitance 17·3 nF, temperature 17–18 °C.

individual measurements was sufficiently long to allow restoration of the block by diffusion of intact nifedipine from neighbouring regions of the pool (not shown). Therefore, the flash-induced release of the block could be repeated several times during the experiment. When the flash was applied immediately before a depolarization to +10 mV that was not preceded by a conditioning pulse (i.e. either before the conditioning depolarization itself in Fig. 6*C* or during the prepulse in Fig. 6*D*), the current activation by the subsequent voltage step showed the normal slow time course. However, when the test pulse was preceded by a conditioning pulse the current activated by the test depolarization was always fast regardless of whether the flash was applied before the conditioning pulse or in the interval between conditioning pulse and test pulse (Fig. 6*B* and *C*), i.e. the test current was fast regardless of whether slow current was activated during the conditioning pulse or not.

This result indicates that the change at the channel molecule caused by the conditioning depolarization and leading to its modified gating behaviour takes place in the nifedipine-blocked state.

The results of Figs 5 and 6 suggest that the amount of calcium carried by the

current is not important for the conditioning effect but rather the percentage of channels which have been transformed into a particular state during the conditioning pulse. This is consistent with the finding (Garcia *et al.* 1990 and own unpublished observations) that the effect also occurs when the charge carriers are Ba^{2+} ions, which are generally less effective in supporting Ca^{2+}-mediated processes.

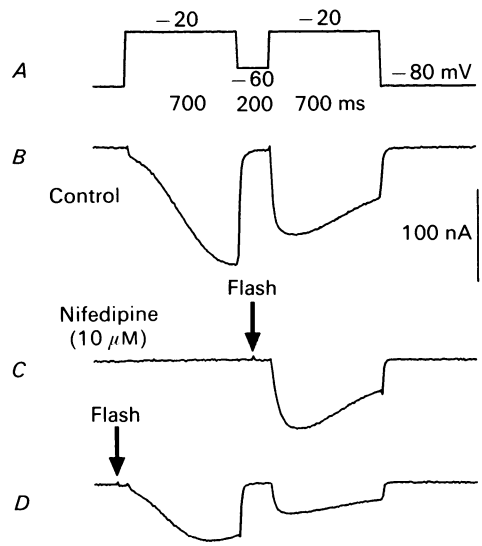

Fig. 7. Effect of slow inward current during the conditioning pulse on the test current kinetics and amplitude. *A*, pulse protocol; *B–D*, nifedipine-sensitive current; the total current response to the pulses shown in *A*, which could be recorded in 10 μM nifedipine, was subtracted in all current traces shown in this figure, i.e. no leak correction using hyperpolarizing control pulses was involved; *B*, total nifedipine-sensitive current; *C* and *D*, nifedipine-sensitive current restored by flash photolysis at different instants during the pulse protocol: 100 ms prior to the test pulse (*C*) and 50 ms prior to the conditioning pulse (*D*). Same fibre as in Fig. 2.

A similar procedure and pulse protocol to that in Fig. 6 was applied in the experiment shown in Fig. 7. However, this figure shows only the voltage and flash response of the nifedipine-sensitive current. It was obtained by subtracting the total nifedipine-resistant current elicited by the pulse protocol given in panel *A* (no leak correction) from the total current measured either before (*B*) or after the application of 10 μM nifedipine (*C* and *D*). During records *C* and *D*, UV light flashes were applied as indicated. As in Fig. 6, it is evident that the test current activates considerably faster at the second depolarization regardless of whether the slow conditioning current is blocked by nifedipine or not. A certain difference in the speed of activation is, however, notable: the half-time of rise to the peak is 26·8 ms in the test current of Fig. 7*B* and 84·7 ms in *C*, which corresponds to 8% and 25%, respectively, of the conditioning current half-time in *B*. The value is down to 28·4 ms again in Fig. 7*D*, where the flash had been applied 50 ms prior to the conditioning pulse and a sizable slow inward current became activated during this pulse. This observation may either

indicate a certain enhancement of the voltage-dependent transition to the fast gating mode by the inward current itself or slower kinetics of this transition in the nifedipine-bound state of the channel.

The amplitude ratio of control and flash-induced slow current (Fig. 7*B* and *D*) is close to the average value observed in twenty-three fibres, i.e. the smaller amplitude of the conditioning current in Fig. 7*D* compared to *B* is probably not due to a run-down of the channels but to incomplete flash-induced recovery from the block caused by nifedipine. Therefore, when comparing the records in Fig. 7*C* and *D* it seems that blocking the inward current during the conditioning pulse causes a potentiation of the current during the test pulse. This is also notable, though to a smaller extent, in Fig. 6, when comparing panels *B* and *C*. A possible explanation for this difference is the current-dependent decline of the L-type current due to a depletion of Ca^{2+} in the T-tubular system, which has been documented by other investigators (Almers, Fink & Palade, 1981) and which could take place under our conditions since the external free calcium had not been buffered by a chelator system.

DISCUSSION

The objective of this investigation was to analyse which events during a conditioning depolarization determine the transition of the L-type calcium channel to a mode with accelerated gating kinetics (Feldmeyer *et al.* 1990; Garcia *et al.* 1990; Ma *et al.* 1992).

We found that the transition proceeds with a time course very similar to the activation of the current during the conditioning step. Furthermore, the enhancement of the activation rate takes place even when the conditioning current is blocked by the dihyropyridine drug nifedipine.

The following conclusions can be drawn from these observations.

(1) The acceleration is not caused by the Ca^{2+} entering the cell. This is consistent with the finding that the same phenomenon could be observed after replacing Ca^{2+} by Ba^{2+} (Garcia *et al.* 1990; Ma *et al.* 1992 and authors' own unpublished observations).

(2) The transition to the fast mode is closely correlated with the transition from the closed to the open conformation of the channel, which is consistent with the kinetic schemes proposed by us as possible explanations of the conditioning effect (Feldmeyer *et al.* 1990) and with recent single channel recordings from rabbit transverse tubular membrane vesicles which were fused into lipid bilayers (Ma *et al.* 1992).

(3) Although it affects channel opening, dihydropyridine binding does not prevent the transition to the fast gating mode.

Figure 8 shows a molecular interpretation of our previously suggested four-state model (Feldmeyer *et al.* 1990) inspired by recent data of Tanabe, Adams, Numa & Beam (1991) who demonstrated that the slow gating of the skeletal muscle DHP receptor resides exclusively in the first internal repeat of its $\alpha 1$ subunit while the other repeats may exhibit fast reactions. Thus the $\alpha 1$ subunit alone seems to contain the structural basis for the postulated dual gating mechanism even though kinetic changes might also result from subunit interactions (see for example Lacerda, Kim,

Ruth, Perez-Reyes, Flockerzi, Hofmann, Birnbaumer & Brown, 1991 and Varadi, Lory, Schultz, Varadi & Schwartz, 1991).

In the model, the slow voltage-dependent onset and decay of the fast gating condition shown in Fig. 3 is due to a slow reaction of repeat I of the $\alpha 1$ subunit. Based

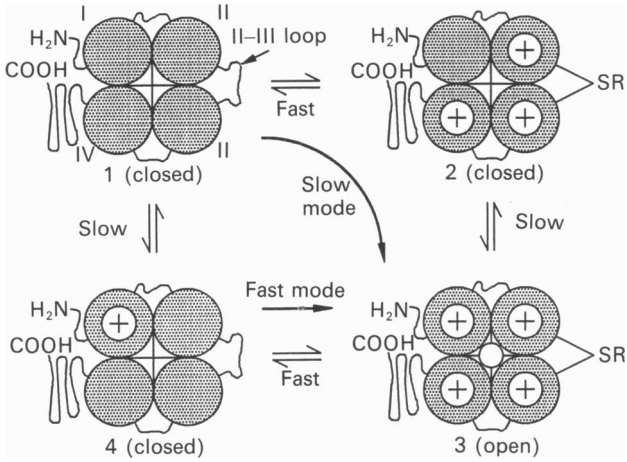

Fig. 8. Schematic model of possible voltage-dependent gating in the $\alpha 1$ subunit of the skeletal muscle dihydropyridine receptor (face-on views seen from the transverse tubular lumen). The movement of positive gating charge (perpendicular to the plain of the drawing) within the four homologous repeats (I–IV) is indicated by the plus signs. Assigning the same amount of gating charge to each domain has been chosen for simplicity and is not necessarily compatible with physiological results. The fast transitions of the repeats II, III, and IV have been lumped together and represent the proposed fast gate of the channel (Feldmeyer *et al.* 1990). In state 3 the opening of the pore is indicated by the circle while the shape change of the loop connecting domains II and III indicates the excitation–contraction coupling function (inducing Ca^{2+} release from the sarcoplasmic reticulum (SR)) proposed for the DHP receptor (Tanabe *et al.* 1990), which may be associated with the movement of the fast gate.

on our nifedipine results which show that the transition to the fast gating mode is not prevented by the drug we would suggest that there is little communication between the site of the slow reaction (i.e. repeat I in the model) and the DHP binding site. This seems consistent with the localization of the binding regions on or near repeats III and IV (Nakayama, Taki, Striessnig, Glossmann, Catterall & Kanaoka, 1991; Regulla, Schneider, Nastainczyk, Meyer & Hofmann, 1991; Striessnig, Murphy & Catterall, 1991).

That voltage-dependent state changes are possible in the presence of bound nifedipine has also been demonstrated by Gurney *et al.* (1985), who used flash photolysis of nifedipine in ventricular myocytes. They concluded that a large fraction of the nifedipine-blocked channels performs a transition from 'closed' to 'inactivated' after step depolarization.

In the case of skeletal muscle a possible mechanism of block by nifedipine is selective inhibition of the fast gate which may be associated with repeats II–IV (Fig. 8). This would fit with the reported reduction of rapid intramembrane charge

movement caused by nifedipine (Lamb, 1986; Lamb & Walsh, 1987; Rios & Brum, 1987; Pizarro, Brum, Fill, Fitts, Rodriguez, Uribe & Rios, 1988). In future experiments it should therefore be studied whether any of the intramembrane charge movement which is blocked by nifedipine becomes rapidly available after flash irradiation.

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