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CALCIUM ACTION POTENTIALS IN RAT FAST-TWITCH AND SLOW-TWITCH MUSCLE FIBRES

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SUMMARY

1. Slow action potentials were evoked in twitch fibres of rat extensor digitorum longus (e.d.l.) and soleus muscles after drastically reducing the Cl and K conductances of the muscle fibres.

2. Cl conductance was eliminated by exposing the muscles to a Cl-free saline in which methanesulphonate replaced Cl. K conductance was reduced by adding tetraethylammonium (TEA) and 3,4-diaminopyridine (3,4-DAP) to the Cl-free saline or by overnight incubation of the muscles in a saline containing Cs and TEA.

3. The delayed rectifier was markedly blocked by TEA and 3,4-DAP. In contrast, the inward rectifier was blocked only by TEA.

4. Depolarization with pulses of increasing amplitude triggered slow responses which had a threshold of -30 to -10 mV and a peak amplitude of 50–60 mV. In e.d.l. muscles the time course of the response was sustained for the duration of the pulses and was not affected by repeated stimulation. In soleus muscles the first evoked response was sustained in about 60 % of the fibres and transient in the rest. Transient responses reached a peak amplitude and were followed by a hyperpolarization. Repeated stimulation irreversibly transformed the sustained responses of soleus fibres into transient ones.

5. The responses were blocked when the Ca in saline was replaced by Mg (10 mM) or Co (5 mM) or by the addition of Cd (0·1–1·0 mM) or nifedipine (5–6 μM). Tetrodotoxin did not affect the responses. These results strongly suggest that Ca is the main carrier of current during the response.

6. Nifedipine blocked both the Ca response and the subsequent hyperpolarization, suggesting that the latter is due to the activation of a Ca-dependent K conductance.

INTRODUCTION

Some years ago it was demonstrated that in frog skeletal muscle fibres it was possible to evoke slow, Ca-dependent action potentials after drastically reducing both K and Cl conductances (Beaty & Stefani, 1976). More recent studies with voltage clamp have shown the presence of a slow inward Ca current in frog muscle (Stanfield,

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1977; Sanchez & Stefani, 1978) which is located mainly in the transverse tubular system (Nicola Siri, Sanchez & Stefani, 1980; Almers & Palade, 1981). The present paper reports the existence of similar slow action potentials in mammalian skeletal muscle fibres, with some differences between fast- and slow-twitch fibres. Preliminary results have been reported (Chiarandini & Stefani, 1982).

METHODS

Experiments were performed at room temperature on extensor digitorum longus (e.d.l.) and soleus muscles of Wistar rats. Muscles were removed from the animals in a few minutes and placed in a chamber with fully oxygenated normal saline. Conventional techniques for intracellular recording of voltage were used. Recording micropipettes were filled with 3 M-KCl and had resistances of 40–60 MΩ. Square pulses of current were applied intracellularly with a micropipette filled with 2 M-K citrate, positioned 20–40 μm away from the recording micropipette. A current-clamp circuit was used to deliver constant pulses of the desired amplitude. The membrane potential of the fibres was held at −80 mV by applying constant current. The effective resistance (R_{eff}) of the fibres was obtained by applying pulses of current and dividing the evoked voltage deflexions by the intensity of the injected currents at the end of 2–4 s pulses.

Table 1. Composition of experimental solutions (mM)

	Na^+	K^+	Ca^{2+}	Mg^{2+}	TEA	Cs^+	Cl^-	$MeSO_3^-$	SO_4^{2-}
Normal saline	136	5	10	1·2	—	—	161	—	1·2
Cl-free saline	136	5	10	1·2	—	—	—	161	1·2
TEA saline	—	5	10	1·2	136	—	—	161	1·2
K-free TEA saline	—	—	10	1·2	141	—	—	161	1·2
Cs–TEA saline	—	—	10	1·2	70·5	70·5	—	161	1·2

The composition of the solutions is listed in Table 1. Solutions also contained 2 mM-imidazole sulphate buffer (pH 7·4), 11 mM-glucose and, occasionally, 5×10^{-7} M-tetrodotoxin (TTX). The saline in the muscle chamber was bubbled constantly with O_2. To avoid mechanical artifacts muscles were stretched by about 25–30% of their slack length and in some experiments 350 mM-sucrose was added. 3,4-Diaminopyridine (3,4-DAP) (Fluka AG) was used at a concentration of 5 mM. Nifedipine, a gift from Pfizer Pharmaceuticals, was added to the saline from an alcohol-concentrated solution as described by Almers, Fink & Palade (1981). The intracellular K concentration was estimated by flame spectrophotometry. Results are expressed as mean ± standard error. Number of observations is given in parentheses.

RESULTS

To demonstrate the existence of a voltage signal generated by a voltage-dependent increase in the cell membrane conductance to Ca it is necessary to reduce or block other shunting ionic conductances. In rat muscle fibres Cl and K conductances are the predominant conductances (Palade & Barchi, 1977). They were blocked as follows. Cl conductance was eliminated by incubating the muscle in salines in which the impermeant anion methanesulphonate substituted for Cl. K conductance was reduced by: (*a*) exposing the muscle to tetraethylammonium (TEA) or 3,4-diaminopyridine (3,4-DAP) or both, or (*b*) overnight incubation of the preparation in Cs-TEA saline (see Table 1). The effects of these treatments are detailed below.

Effects of TEA, 3,4-DAP and Cs-TEA saline on K conductances in e.d.l. and soleus muscles

In both e.d.l. and soleus muscle the inward rectifier is blocked by TEA, as previously described in frog muscle (Stanfield, 1970) but not by 3,4-DAP. Fig. 1 shows voltage deflexions produced by square pulses of current in e.d.l. muscles, in Cl-free saline (*A*), in TEA saline (*B*) and in Cl-free saline with 5 mM-3,4-DAP added (*C*). Fig.

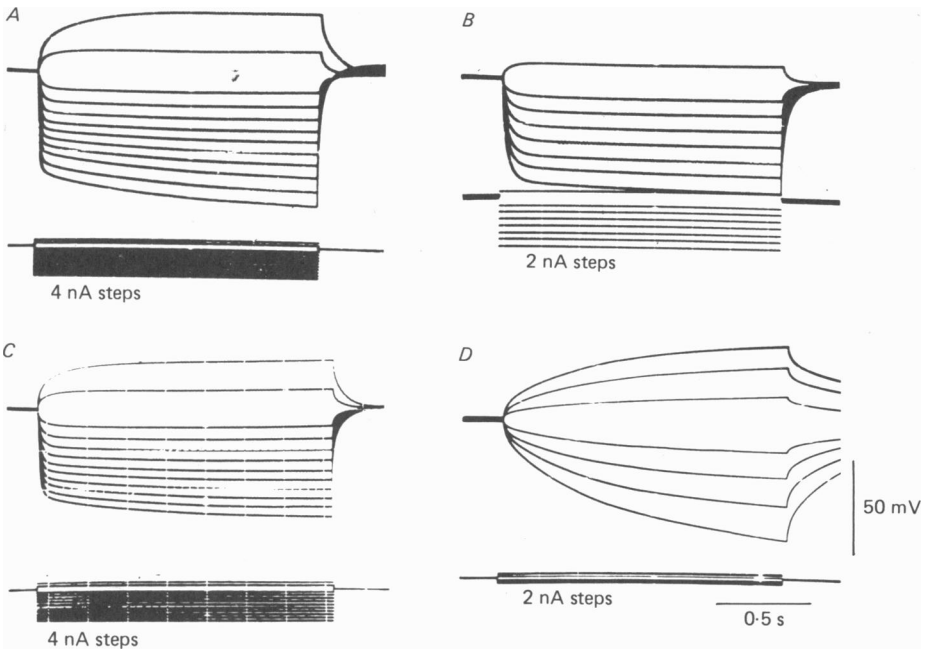

Fig. 1. Effects of various treatments on the inward rectifier. Records obtained in four different e.d.l. muscles. Muscle fibres were held at −80 mV. *A*, in Cl-free saline the membrane resistance is smaller for inward current than for outward current, denoting the presence of the inward rectifier. *B*, in TEA saline the resistances to inward and outward current are very similar, indicating blockade of the inward rectifier. *C*, in Cl-free saline with 5 mM-3,4-DAP added the inward rectifier is unmodified. *D*, after overnight incubation in Cs–TEA saline the R_{eff} is considerably increased and the inward rectifier is blocked. See text for further details of the treatments.

2 shows voltage–current (*V–I*) relationships obtained from these types of experiments. It can be seen that the *V–I* curves obtained in Cl-free saline with or without 5 mM-3,4-DAP are indistinguishable. In contrast, the inward rectification disappears when the muscle is exposed to TEA.

In e.d.l. muscle the delayed rectification, which is clearly observed when the preparation is exposed to Cl-free saline (Fig. 2; 3*A*), was reduced by exposing the muscle to TEA saline with 3,4-DAP added (Fig. 2; Fig. 3*B*). With this combination of compounds most of the delayed rectification in the e.d.l. muscle was blocked but the resting R_{eff} measured with small pulses at −80 mV was not affected. R_{eff} was

2·49 ± 0·31 MΩ ($n = 4$) in Cl-free saline and 3·16 ± 0·33 MΩ ($n = 8$) in TEA saline with 3,4-DAP added ($P > 0·1$).

In soleus muscle, in contrast to e.d.l. muscle, pulses of outward current revealed the existence of rectification despite the treatment of the preparations with TEA and 3,4-DAP combined (Fig. 6*A* and *B*). To eliminate this rectification, possibly due to the prominent slow K-channels recently described in soleus muscle (Duval & Léoty,

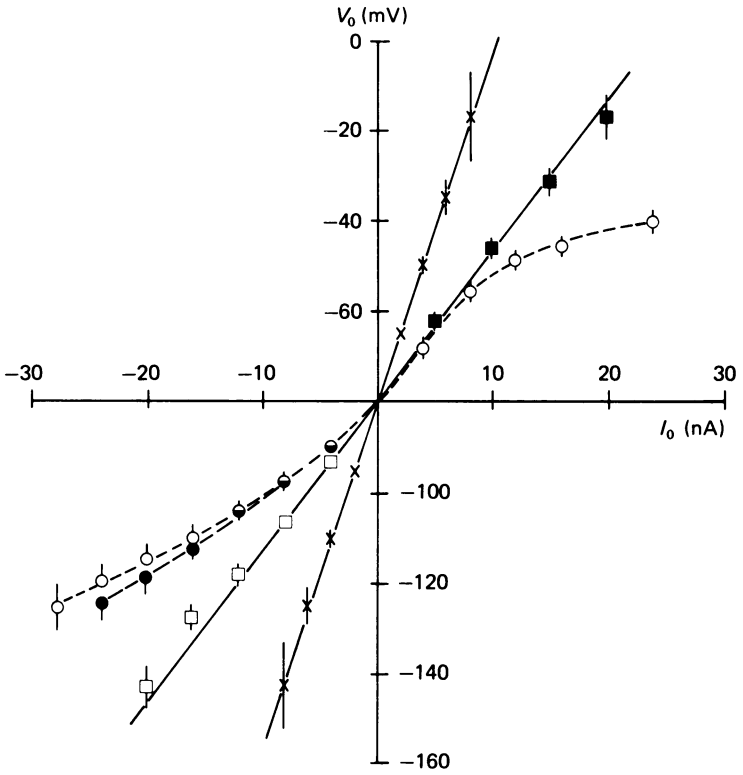

Fig. 2. Effects of various treatments on the *V–I* relationship in e.d.l. muscles. Each symbol is the mean ± standard error of six to ten fibres. Muscles were incubated as follows: Cl-free saline with TTX (○); Cl-free saline with TTX and 5 mM-3,4-DAP added (●); TEA saline (□); TEA saline with 5 mM-3,4-DAP added (■); and overnight incubation in Cs-TEA saline (×). See text for further details of the treatments.

1980*a*), the muscles were first incubated for 12–18 h in Cs–TEA saline at 4 °C and then transferred to K-free TEA saline for the experiment. With this treatment the intracellular K concentration was reduced from a control value of 86 and 104 mequiv/kg of wet tissue (measured in two fresh soleus muscles) to 17·9 ± 5·8 mequiv/kg of wet tissue ($n = 4$). The resting potential (E_{rp}) ranged from −40 to −70 mV, the R_{eff} markedly increased from a control of 2·06 ± 0·19 MΩ ($n = 6$) measured in Cl-free saline to 4·23 ± 0·35 MΩ ($n = 6$) ($P < 0·001$), and the *V–I* relationship became linear for membrane potentials more negative than −20 mV. In e.d.l. muscles which were similarly treated, comparable changes in the electrical characteristics were observed (Fig. 1*D*; Fig. 2).

Responses evoked in e.d.l. muscles

Ca responses were routinely evoked in fibres of e.d.l. muscles incubated for at least 30 min in TEA saline with 3,4-DAP. In this solution most of the muscle fibres had a E_{rp} ranging from −70 to −78 mV. When the fibres were depolarized stepwise with long pulses of current, a rather sudden depolarization appeared, during the course of the electrotonic pulses (Fig. 3*B*). The threshold for this response ranged from −27

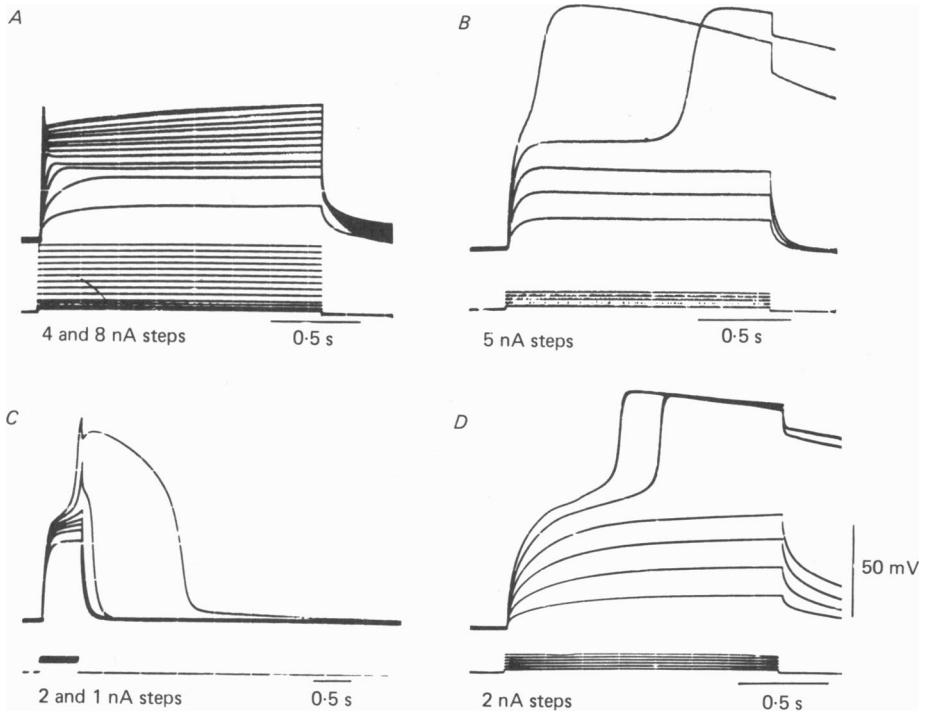

Fig. 3. Ca responses in e.d.l. muscles. *A*, in Cl-free saline delayed rectification is clearly present. *B*, in TEA saline with 3,4-DAP added, delayed rectification is greatly reduced and when the applied depolarization drives the membrane potential to about −20 mV a spontaneous and sudden depolarization is observed. A larger depolarizing pulse evokes another response with a similar rising phase and with a sustained time course. *C*, a similar response at a slower sweep speed. The response continues for about 1·5 s after the end of the pulse. Note that the intensity of the first pulse was 14 nA, then it was increased by 2 and 1 nA steps. *D*, muscle incubated overnight in Cs-TEA saline; the Ca response is similar to that illustrated in *B*.

to −6 mV. The response reached a peak membrane potential of about 50–60 mV in about 150–200 ms and thereafter it decayed slowly. Fig. 3*C* shows the response in another fibre at a slower sweep speed. Most of the response was over in 1·5 s after the end of the stimulating pulse but a tail of depolarization remained for another 2·0 s.

Responses nearly identical to that illustrated in Fig. 3*B* were observed in more than thirty fibres from many e.d.l. muscles. These responses were very similar to those described previously in frog twitch fibres (Beaty & Stefani, 1976; Nicola Siri *et al.* 1980). In a very few e.d.l. fibres, however, the responses were markedly briefer and

had a time course similar to that of the responses recorded in soleus muscles under identical experimental conditions (Fig. 6*A* and *B*).

In several other muscles K conductance was blocked with either TEA or 3,4-DAP. For this purpose a TEA saline or a Cl-free saline containing 5 mM-3,4-DAP and TTX was used. Ca responses were also obtained but, usually, they had a smaller amplitude and slower rise time than the responses evoked in fibres treated with TEA and 3,4-DAP combined.

The participation of Ca ions in the responses described above was established in another group of experiments. It was found that the normal responses obtained in

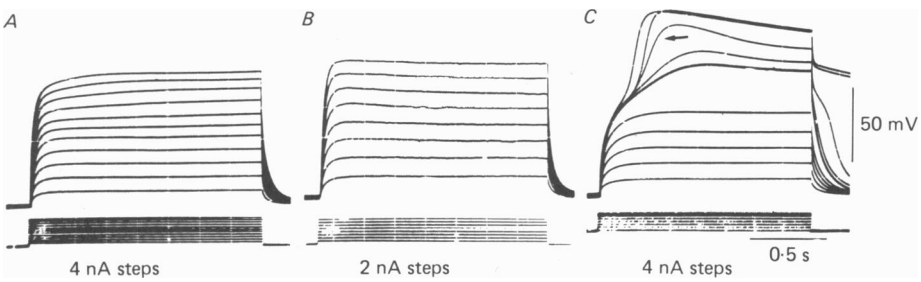

Fig. 4. Blockade of Ca responses in e.d.l. muscles. Records obtained in three different preparations. Muscles incubated in a TEA saline with 3,4-DAP which was modified as follows: *A*, Ca omitted and 10 mM-Mg added; *B*, 1 mM-Cd added; *C*, 5 μM-nifedipine added.

TEA saline with 3,4-DAP were abolished by replacing the Ca of the medium by 10 mM-Mg or by adding Cd (0·1–1·0 mM) (Fig. 4*A* and *B*). Moreover, after the addition of 5 μM-nifedipine, a specific Ca-channel blocker (Almers *et al.* 1981), the control response (Fig. 4*C*, two top records) was reduced in a few minutes (Fig. 4*C*, third (arrow), fourth and fifth records from the top). Furthermore, the responses were not affected by TTX.

To determine the magnitude of the conductances underlying the Ca response we studied the *V–I* relationship at rest and during the response. Fig. 5*A* shows eight superimposed voltage pulses obtained in a muscle fibre equilibrated in TEA saline with 3,4-DAP, which were evoked by current steps with an intensity increasing by 5 nA (bottom four pulses) or 10 nA (top four pulses). The first three pulses evoked only electrotonic responses which, after about 250 ms, gave rise to a Ca response. The slope of the R_{eff} of the fibre measured at membrane potentials below or above the threshold for the Ca response is clearly different. This is indicated by the difference in the *IR* drops induced by pulses at those two different levels of cell polarization (Fig. 5*A*) and by the difference in the slopes of the *V–I* relationships shown in Fig. 5*B*. In seven fibres the slopes of the R_{eff} measured at rest and during the Ca response were $3{\cdot}5 \pm 0{\cdot}8$ MΩ and $0{\cdot}75 \pm 0{\cdot}15$ MΩ, respectively. The membrane potential at which the two straight lines intersect represents the reversal potential of the response (E_{rev}). The mean value of E_{rev} was $56{\cdot}9 \pm 2{\cdot}5$ mV ($n = 7$). The value of E_{rev} is much smaller than the equilibrium potential for Ca (E_{Ca}) which can be assumed to be about 150 mV. This could be explained by the activation of a K conductance during the response.

The Ca response can be analysed, as was done in the squid presynaptic terminal (Katz & Miledi, 1969), in terms of an electric circuit comprising three conductances in parallel: a resting leak conductance (g_L), a K and a Ca conductance (g_K, g_{Ca}), with the corresponding batteries (E_L, E_K and E_{Ca}). The slope of R_{eff} at rest corresponds to $1/g_L$ and during the response to $1/(g_L+g_K+g_{Ca})$. Assuming that E_{Ca} is 150 mV and that E_L and E_K are -80 mV, the calculated values for g_K and g_{Ca} are: $0{\cdot}40 \times 10^{-6}$ S and $0{\cdot}64 \times 10^{-6}$ S, respectively. Furthermore, by applying the infinite cable equations and assuming a fibre diameter of 50 μm and a R_i of 200 Ω cm with a Q_{10} of 1·37 (Adrian & Marshall, 1977) a g_{Ca} of about 0·1 mS/cm^2 is obtained.

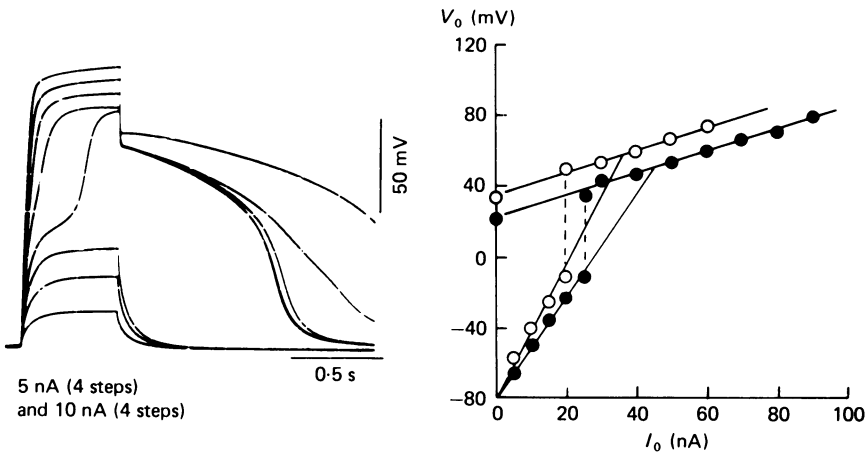

Fig. 5. Voltage–current relationship at rest and during the Ca response in e.d.l. muscle. *A*, muscle incubated in TEA saline with 3,4-DAP added. Records correspond to eight superimposed voltage deflexions evoked by current steps of 5 nA (four bottom pulses) and 10 nA (four top pulses). The first three pulses were subthreshold. The fourth and subsequent pulses gave rise to Ca responses. *B*, plot of the maximal amplitude of the pulses or responses as a function of the intensity of the applied current. ○, data from records shown in *A*; ●, another fibre. The intercepts of the slope resistance during the response with the ordinate correspond to the amplitude of the Ca response immediately after the end of the pulse. The intersections between the two straight lines correspond to the reversal potential of the response.

Responses evoked in soleus muscles

The responses obtained in soleus muscles incubated in TEA saline with 3,4-DAP (Fig. 6*A* and *B*) were not sustained as in e.d.l. muscles (Fig. 3*B* and *C*). They consisted of a peak component that reached a maximum value in 150–200 ms after the pulse onset and that then subsided in the following 400–600 ms to a plateau component which lasted until the termination of the pulse. Frequently, the membrane potential reached a more hyperpolarized level at the beginning of the plateau than at the end. Rectification was clearly evident during the plateau, suggesting that some channels, possibly the slow K-channels recently described in soleus muscle (Duval & Léoty, 1980*a*), had not been blocked by TEA and 3,4-DAP. The peak component of these responses disappeared when the Ca in the saline was replaced by 5 mM-$CoCl_2$. This effect cannot be attributed to a shunting action of the added Cl because in control experiments in which 10 mM-NaCl was added to the saline the peak component was not reduced.

To block the K-channels further, muscles were incubated overnight in Cs–TEA saline at 4 °C before performing the experiment in K-free TEA saline. With this treatment the response of depolarizing pulses became prominent although some degree of rectification was still present during the plateau (Fig. 6*C* and *D*, 8*A* and *B*). This treatment was used in all the experiments described below.

In thirty-four fibres the first response was sustained and had a time course identical to that of e.d.l. muscles (compare Fig. 6*C* with Fig. 3*B*). In contrast, in twenty-one

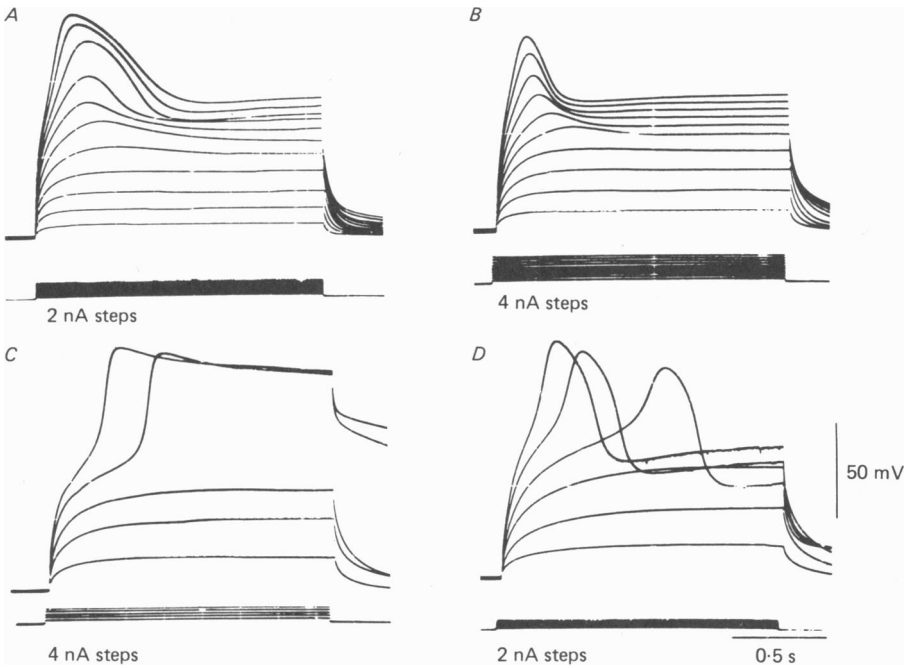

Fig. 6. Ca responses in soleus muscles. Records in four different preparations. *A* and *B*, in TEA saline with 3,4-DAP soleus muscles showed brief Ca responses and marked rectification. *C* and *D*, experiments performed in K-free TEA saline after overnight exposure of the muscle to Cs–TEA saline. Under these conditions the responses were sustained (*C*) or transient (*D*).

other fibres, the first response was transient (Fig. 6*D*) and was consistently followed by a well-defined hyperpolarization (Fig. 6*D*). Nineteen out of the thirty-four fibres which gave an initial sustained response were repeatedly stimulated at intervals ranging from 20 s to 2 min. In sixteen of those fibres, a transformation from a sustained to a transient response was observed after evoking seven or less responses. In the three other fibres, however, repeated stimulation with 10, 16 and 17 pulses failed to modify the response. Once the transformation occurred, the response did not return to its original, sustained time course, despite resting the fibres for 10–15 min between stimulations. Fig. 8 shows the transformation of the response. In this muscle fibre the first response was clearly sustained (Fig. 8*A*, second trace from top). It displayed a faster decay when evoked for a second time (Fig. 8*A*, top record) and became clearly transient when elicited with a third pulse (Fig. 8*B*).

The R_{eff} values at rest and during the response were $4{\cdot}7 \pm 1{\cdot}0$ and $0{\cdot}96 \pm 0{\cdot}10$ MΩ, respectively ($n = 7$). The mean value of the E_{rev} was $43{\cdot}3 \pm 3{\cdot}5$ mV. These values are similar to those found in e.d.l. muscles except for the E_{rev}, which is significantly lower ($P < 0{\cdot}02$). For comparison, e.d.l. muscles were also incubated overnight in Cs–TEA saline before performing the experiments in K-free TEA saline. The E_{rp} of the muscle

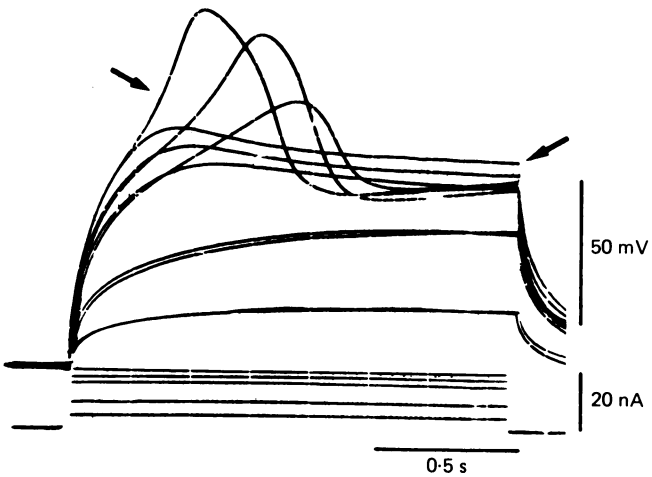

Fig. 7. Effect of nifedipine on Ca responses in soleus muscles. Depolarizing pulses of increasing intensity evoked three transient responses (left-hand arrow) which were followed by transient hyperpolarizations. After treating the preparation with 6 μM-nifedipine both the responses and transient hyperpolarizations disappeared (right-hand arrow). The similarity in the amplitude of subthreshold pulses before and after adding the Ca blocker indicates that nifedipine does not affect the resting R_{eff} of the fibres.

fibres ranged from −50 to −25 mV, the R_{eff} increased to $7{\cdot}5 \pm 0{\cdot}5$ MΩ ($n = 6$) and the *V–I* relationship became linear (Fig. 2). The time course of the response was similar to that of the responses evoked in TEA saline with 3,4-DAP added (Fig. 3*D*) and it did not change even when as many as ten responses were successively evoked at 20–60 s intervals.

The Ca responses in soleus muscles were abolished by 0·2 mM- and 1 mM-Cd and by 6 μM-nifedipine. The effect of nifedipine is illustrated in Fig. 7 which shows several superimposed records obtained in the same fibre before and after adding the Ca blocker. The top three traces show (Fig. 7, left-hand arrow) typical transient responses followed by a transient hyperpolarization. After treatment with nifedipine for several minutes both the Ca response and the transient hyperpolarization were abolished (Fig. 7, right-hand arrow). The similarity between the amplitude of the electrotonic pulses evoked with two subthreshold intensities in the absence and presence of nifedipine indicates that the drug does not affect the resting R_{eff} of the fibres.

The effects of replacing the extracellular Ca by Ba are shown in Fig. 8. Records *A* and *B* were obtained with the muscle exposed to K-free TEA saline and show the transformation of a sustained Ca response into a transient one, as previously described. After recording the third response (*B*), the saline in the chamber was aspirated and replaced by K-free TEA saline with no Ca and with 10 mM-Ba added.

About 6 min later it was observed that the rectification had disappeared (compare *A* with *C* in Fig. 8). The time course of the response remained unchanged. Immediately after, a second wash was done with the Ca-free, K-free TEA saline with Ba, to reduce the level of Ca contamination in the chamber. Approximately 9 min later the response became sustained (Fig. 8*D*). The change in the time course of the response was not

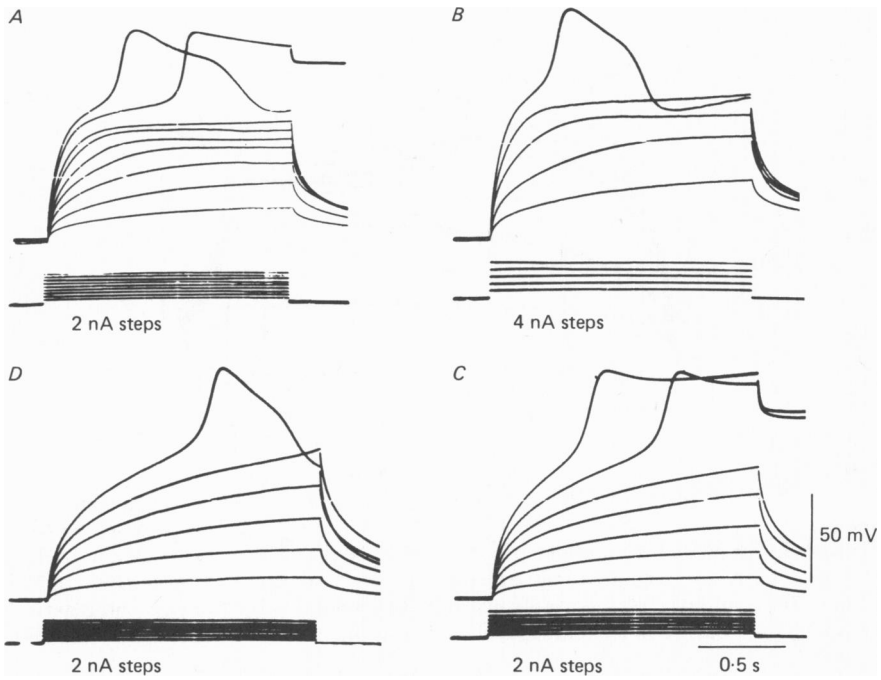

Fig. 8. Effects of replacing Ca by Ba on the Ca responses in soleus muscle. *A*, control response evoked in K-free TEA saline after overnight incubation in Cs–TEA saline. Note that the first response (second record from top) is sustained and that the second response (first record from top) has a shorter duration. *B*, the third response is clearly transient. *C*, records obtained 6 min after substituting the bathing saline with a similar one in which Ba replaced Ca. Rectification disappeared completely and the response remained transient. *D*, after a second wash with the Ba-containing saline to reduce Ca contamination, the response became sustained and was unmodified by repeated stimulation.

reversed by returning the muscle to K-free TEA saline for 30 min and after evoking seventeen additional responses. The effects of substituting Ba for Ca on the responses were studied in ten other fibres. All of them exhibited sustained responses which remained unmodified despite repeated stimulation with 10 pulses

DISCUSSION

The present study indicates that in rat skeletal muscle there is a voltage-dependent Ca conductance which gives rise to slow action potentials when the shunting K and Cl membrane conductances are drastically reduced. These Ca responses are similar to those previously observed in frog twitch muscle fibres (Beaty & Stefani, 1976).

Moreover, an inward Ca current has been recently described in rat skeletal muscle (Donaldson & Beam, 1982). As in other tissues, the Ca responses recorded in mammalian twitch fibres are not affected by TTX and are blocked by well-known Ca blockers, such as Cd, Co and nifedipine (Hagiwara & Byerly, 1981; Almers *et al.* 1981).

There are marked differences in the time course of the Ca responses evoked in soleus and e.d.l. muscle fibres treated with TEA and 3,4-DAP combined. In soleus, which contains mostly slow-twitch fibres (Close, 1972), the responses were brief (Fig. 6*A* and *B*), most probably because they were shunted by the slow K conductance recently described in this muscle by Duval & Léoty (1980*a*), which, presumably, is TEA-resistant. In frog twitch fibres slow K-channels are not blocked by TEA (Stanfield, 1970). In contrast, in e.d.l. muscle, which contains mostly fast-twitch fibres (Close, 1972), the Ca responses were long-lasting, most probably because of their reported lack of a slow K conductance (Duval & Léoty, 1980*b*). The very few e.d.l. muscle fibres which showed responses similar to those of soleus fibres most probably were slow-twitch fibres, of which a small percentage is known to exist in rat e.d.l. muscle (Edgerton & Simpson, 1971).

After blocking the slow K conductance of soleus muscle fibres by depleting the fibres of their intracellular K, which, presumably, was replaced by the external Cs or TEA or both, the rising phase and peak amplitude of the Ca responses were always identical to those of the e.d.l. fibres. However, there was still a striking difference between the two muscles with respect to the duration of the response. While all responses in e.d.l. fibres were consistently sustained, in soleus the first-evoked response was sustained in about 60 % of the fibres and transient in the rest. Furthermore, in most of the soleus muscle fibres that displayed a sustained first response, repetitive stimulation irreversibly transformed that response into a transient one.

There are at least three explanations for the difference in the time course of the sustained and transient responses. First, it is conceivable that despite the prolonged incubation in the Cs–TEA saline, the degree of blockade of the shunting K conductance is not uniform and that in the fibres in which some K conductance remains the response is transient. Secondly, it is possible that the degree of activation of the voltage-dependent Ca-channels varies from fibre to fibre and that a sustained response is only observed when the channels are fully activated. Thirdly, the presence of a functional Ca-dependent K conductance (Meech & Standen, 1975; Gorman & Thomas, 1978) could give rise to transient responses. In most soleus fibres such a conductance would be present and the entry of Ca during the response would be followed by the opening of K-channels which would tend to hyperpolarize the cell and, thus, shorten the duration of the response. The observation that in soleus muscle the transient responses are consistently followed by a hyperpolarization that disappears when the response is eliminated with nifedipine, suggests the existence of a Ca-dependent K conductance in these fibres. Furthermore, such a conductance has been described very recently in rat myotubes (Pallota, Magleby & Barrett, 1981). Whether the Ca-dependent K conductance is present in some or all fibres is unclear.

The irreversible transformation of sustained responses into transient ones observed exclusively in soleus muscle fibres upon repeated stimulation is a puzzling phenomenon. It could be due to a progressive inactivation of Ca-channels brought about by

repeated stimulation. A comparable shortening of Ca responses observed in squid synapse and frog motoneurones (Katz & Miledi, 1969; Alvarez-Leefmans & Miledi, 1980) has been explained in these terms. However, the observation that in the presence of Ba, a powerful blocker of K conductance (Werman & Grundfest, 1961), the responses consistently have a sustained time course and are unmodified by repeated stimulation suggests that stimulation shortens the response by facilitating the activation of a shunting K conductance.

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