

## CALCIUM CURRENTS, CHARGE MOVEMENT AND DIHYDROPYRIDINE BINDING IN FAST- AND SLOW-TWITCH MUSCLES OF RAT AND RABBIT

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### SUMMARY

1. The Vaseline-gap technique was used to record slow calcium currents and asymmetric charge movement in single fibres of fast-twitch muscles (extensor digitorum longus (e.d.l.) and sternomastoid) and slow-twitch muscles (soleus) from rat and rabbit, at a holding potential of  $-90$  mV.

2. The slow calcium current in soleus fibres was about one-third of the size of the current in e.d.l. fibres, but was very similar otherwise. In both e.d.l. and soleus fibres, the dihydropyridine (DHP), nifedipine, suppressed the calcium current entirely.

3. In these normally polarized fibres, nifedipine suppressed only part ( $q_{ns}$ ) of the asymmetric charge movement. The proportion of  $q_{ns}$  suppressed by various concentrations of nifedipine was linearly related to the associated reduction of the calcium current. Half-maximal suppression of both parameters was obtained with about  $0.5 \mu\text{M}$ -nifedipine. The calcium current and the  $q_{ns}$  component of the charge movement also were suppressed over the same time course by nifedipine. Another DHP calcium antagonist, (+)PN200/110, was indistinguishable from nifedipine in its effects of suppressing calcium currents and  $q_{ns}$ .

4. In all muscle types, the total amount of  $q_{ns}$  in each fibre was linearly related to the size of the calcium current (in the absence of DHP). On average,  $q_{ns}$  was 3.3 times larger in e.d.l. fibres than in soleus fibres.

5. In contrast to the other dihydropyridines, (–)bay K8644, a calcium channel agonist, did not suppress any asymmetric charge movement.

6. The potential dependence of the slow calcium current implied a minimum gating charge of about five or six electronic charges. The movement of  $q_{ns}$  occurred over a more negative potential range than the change in calcium conductance.

7. Experiments on the binding of (+)PN200/110 indicated that e.d.l. muscles had between about 2 and 3 times more specific DHP binding sites than did soleus muscle.

8. These results point to a close relationship between slow calcium channels, the  $q_{ns}$  component of the charge movement and DHP binding sites, in both fast- and slow-twitch mammalian muscle.  $q_{ns}$  appears to be part of the gating current of the T-system calcium channels.

## INTRODUCTION

Contraction in a skeletal muscle fibre occurs when depolarization of the transverse-tubular system (T-system) produces a rise in the myoplasmic calcium concentration. Although such depolarization opens slow voltage-dependent calcium channels in the T-system (Sanchez & Stefani, 1978), it seems that the inflow of calcium ions through such channels is not necessary for the initiation of contraction, as muscle fibres (in vertebrates) can still contract when the extracellular calcium concentration is very low (Armstrong, Bezanilla & Horowicz, 1972), and when specific blockers of the slow calcium channel are present (e.g. nifedipine; McCleskey, 1985; Gallant & Goettl, 1985). Instead, the primary source of the calcium involved in contraction appears to be the sarcoplasmic reticulum (s.r.). However, the mechanism by which the membrane potential in the T-system controls calcium release from the s.r. is unknown.

In both nerve fibres and skeletal muscle cells, appropriate potential changes produce small non-linear capacitive currents, which reflect the movement of charged particles within the surface or T-tubular membranes (Armstrong & Bezanilla, 1973; Schneider & Chandler, 1973). In fact, the existence of such charge movements had been proposed many years earlier as the most straightforward way in which a potential change could open ion channels (Hodgkin & Huxley, 1952); the electric field across the membrane induces the (net) movement of some charged part(s) of the ion channel molecule, eventually revealing a path for ion movements through the membrane. The charge movement associated with the opening of an ion channel is referred to as the 'gating' current. The existence and properties of  $\text{Na}^+$  gating currents in nerve fibres (for review see Armstrong, 1981) and muscle fibres (Collins, Rojas & Suarez-Isla, 1982) have been firmly established. A calcium gating current has also been described in molluscan neurones (Adams & Gage, 1976, 1979b).

It has been proposed that in vertebrate skeletal muscle, the great majority of the large non-linear (or asymmetric) charge movement is involved in controlling calcium release from the s.r. (Schneider & Chandler, 1973; Adrian, Chandler & Rakowski, 1976; Chandler, Rakowski & Schneider, 1976*a, b*; Hollingworth & Marshall, 1981; Horowicz & Schneider, 1981*a, b*; Dulhunty & Gage, 1983, 1985). However, it seems that charge movement consists of a number of different components, some of which may not be involved in calcium release from the s.r.; in particular one component (denoted as  $q_x$ ) is not affected by prolonged depolarization (Adrian & Peres, 1979; Lamb, 1987), a condition known to block contraction. Furthermore, in normally polarized fibres, nifedipine (a dihydropyridine (DHP)) suppresses both the slow calcium current in the T-system and another component of the asymmetric charge movement (denoted  $q_{ns}$ ), there being little suppression at concentrations below  $0.1 \mu\text{M}$  and maximal effect near  $10 \mu\text{M}$  (Lamb, 1986*b*), but the remaining charge (both  $q_x$  and another component denoted as  $q_{ni}$ ) is unaffected by nifedipine even at concentrations of  $120 \mu\text{M}$ . These observations suggest that the component suppressed by nifedipine under these conditions ( $q_{ns}$ ), may be part of the gating current of the slow calcium channels in the T-system.

This paper shows that the slow calcium current is much smaller in slow-twitch fibres than in fast-twitch fibres, and provides further evidence suggesting that  $q_{ns}$  is

involved in gating the slow calcium channels in the T-system. Furthermore, the amount of specific DHP-binding in fast- and slow-twitch muscle is measured, and the relationship between binding sites and calcium channels considered. Finally, in the light of this and other studies, it is questioned whether this proposed gating current,  $q_{ns}$ , plays any direct role in calcium release from the s.r.

#### METHODS

Most of the electrophysiological methods used here were the same as described in detail previously (Lamb, 1986*a, b*), although the internal solutions have been slightly modified. Single fibres from white sternomastoid or soleus muscles of the rabbit or extensor digitorum longus (e.d.l.) muscles of the rat, were dissected free and mounted in a triple Vaseline-gap chamber-clamp. The fibre was bathed internally with the following solution (mM): sodium glutamate, 140; Mg ATP, 3; Tris cyclic AMP, 1; Tris phosphocreatine, 5; TES buffer (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid), 10; with the pH adjusted to 7.0 (with NaOH) and the osmolarity adjusted to 290 mosmol l<sup>-1</sup>. As fibres bathed with this internal solution could contract vigorously, in most experiments 10 mM-EGTA was added to suppress contraction and the glutamate correspondingly reduced. The external solution contained (mM): tetraethylammonium bromide, 150; TES, 2; 2,4-dichlorophenoxyacetic acid, 2.5; sucrose, 60; tetrodotoxin, 10<sup>-3</sup>; with CaSO<sub>4</sub> between 0.15 and 8 mM and with MgSO<sub>4</sub> added to make a total of 8.15 mM. The pH of the external solution was 7.2. Stock solutions (10 mM) of nifedipine (Sigma, U.S.A.), (+)PN200/110 (Sandoz, Australia) and (-)bay K8644 (Bayer, Australia) were made using 96% ethanol, stored in light-resistant containers and added to the external solution to give the appropriate drug concentrations. All control solutions had a final concentration of ethanol equal to that of the corresponding DHP-containing solution.

The membrane potential of the fibre was clamped at -90 mV. Asymmetric charge movement was measured by appropriately scaling and subtracting the (supposedly) 'linear' capacitive currents generated by a 'control' potential step (-90 to -110 mV) from the total capacitive current generated by a depolarizing 'test' step (Lamb, 1986*a*). (See discussion of 'linear' capacitance in Lamb, 1987.) Test and control steps were both either 35 or 55 ms long and test steps always followed the corresponding control step by less than 100 ms. Charge movement records presented in this paper have been neither smoothed nor corrected for non-linear ionic currents. Records at each potential are the result of a single control-test sequence or of four sequences, 5 s apart. The voltage and current records were filtered at 1 kHz (corner frequency) by a 4-pole Bessel filter, sampled digitally at 5 kHz and stored by a PDP 11/23 computer. As linear ionic and capacitive currents were removed by the above procedures, and virtually all other non-linear currents were blocked, any calcium current activated during these short pulses could be clearly identified and quantified, other than where it overlapped the asymmetric charge movement.

The calcium current evoked by long (2 s) depolarizing pulses was recorded similarly, but with the control step (from -90 to -80 mV) preceding the test step by 2 s, and with consecutive sequences 30 s apart. Linear ionic and capacitive currents were subtracted as detailed above. Records were filtered at 250 Hz or 1 kHz and sampled at 100 Hz. All experiments were performed at 22 ± 1 °C.

*Dihydropyridine binding.* The e.d.l. and soleus muscles of rats were dissected free and divided by microdissection into bundles of intact fibres of between 20 and 40 mg weight. The fibre bundles were bathed in a high-potassium solution of composition (mM): KCH<sub>3</sub>SO<sub>4</sub>, 145; CaCl<sub>2</sub>, 2.5; MgCl<sub>2</sub>, 1; glucose, 10; TES, 5; pH 7.3. Weighed fibre bundles were covered with 400 μl of the high-potassium solution with varying concentrations of [<sup>3</sup>H](+)PN200/110 (New England Nuclear, DuPont, U.S.A.) in capped 1.5 ml microfuge tubes and incubated as described by Schwartz, McCleskey & Almers (1985), with minor modifications. Incubation solutions also contained 2 mg bovine serum albumin/ml (Armour Pharmaceutical, Eastbourne, U.K.) to prevent adsorption losses, and Diltiazem (10 or 100 μM) (Sigma, U.S.A.), as this drug has been reported to increase binding of dihydropyridines under some conditions (Jaimovich, Donoso, Liberona & Hildago, 1986). Occasionally the protease inhibitor phenylmethylsulphonyl fluoride (0.2 mM) was included, but was found to have no measurable effect. Incubations were mixed periodically by gentle stirring; solutions containing dihydropyridines were protected from the light, and were not exposed to fluorescent light or daylight during sampling. Duplicate 10 or 20 μl samples were withdrawn from the bathing solutions at two-hourly intervals until the difference between

successive samplings was  $\leq 10\%$  (usually 8–10 h); at this time unlabelled (+)PN200/110 was added (20–40  $\mu\text{M}$  final concentration) and sampling continued (at one or two hourly intervals) for a further 2–4 h by which time the concentration of labelled (+)PN200/110 was approximately constant. Fibre bundles were then blotted, weighed and digested with Protosol (New England Nuclear, Dupont, U.S.A.) according to the manufacturers recommendations. Uptakes measured in this manner agreed with those calculated from the change in bathing solution concentrations within 10–15%; control digestions using [ $^3\text{H}$ ](+)PN200/110 alone showed that the treatment causes a 5–15% loss of label. All binding experiments were carried out at 22 °C.

## RESULTS

### *Slow calcium current*

The slowly activating calcium current in mammalian muscle was investigated using 2 s depolarizing voltage steps. When the external solution contained at least 0.5 mM-free  $\text{Ca}^{2+}$ , depolarizing pulses to potentials between about  $-30$  and  $+40$  mV invariably elicited a slow inward current in both fast-twitch fibres (e.d.l. and sternomastoid) and slow-twitch fibres (soleus) (Fig. 1). This inward current was considerably smaller in soleus fibres (Fig. 1*D*) than in e.d.l. fibres (Fig. 1*A* and *B*). The time for this current to reach a peak decreased from about 1 s for depolarizations near  $-20$  mV, to a minimum time of between 90 and 100 ms (e.d.l.) or 170 and 240 ms (soleus) for depolarizations near  $+20$  mV. In both fibre types, the maximal inward current was produced by depolarizations to near  $+10$  mV, and in 8 mM- $\text{CaSO}_4$  had a mean value of  $14.8 \pm 1.3$  ( $\pm$  s.e. of mean)  $\mu\text{A}/\mu\text{F}$  in seven e.d.l. fibres and  $6.1 \pm 1.4$   $\mu\text{A}/\mu\text{F}$  in nine soleus fibres. The mean peak current in four sternomastoid fibres was  $8.6 \pm 0.8$   $\mu\text{A}/\mu\text{F}$  and was reached in 120–160 ms. The value of the current in each fibre was normalized by the fibre capacitance, rather than by the apparent surface area which is more affected by measurement errors (Lamb, 1986*a*). If, instead, the currents are normalized by apparent surface area (mean capacitance per unit apparent surface area: 9.0, 9.2 and 14.5  $\mu\text{F}/\text{cm}^2$ , for soleus, e.d.l. and sternomastoid fibres, respectively) the value of the mean maximum current in the two fast-twitch muscles is very similar, and considerably larger than in slow-twitch (soleus) muscle. This is the same as was found previously for asymmetric charge movement in these muscles (Lamb, 1986*a*).

In many e.d.l. fibres, the initial inward current produced by depolarizations between  $-20$  and  $+10$  mV, was followed by an outward current (Fig. 1*B*), which reached a peak approximately 1–1.5 s after the start of the depolarization. No such outward current was observed in any soleus fibre, or in the e.d.l. fibre with the smallest inward current (Fig. 1*A*). With larger depolarizations to between  $+20$  and  $+40$  mV, the peak inward current in all fibre types was progressively smaller, and was followed by a progressively larger outward current. Depolarizations to above about  $+50$  mV elicited only outward currents in all fibres.

The inward current was almost certainly carried by calcium ions, as it was totally abolished when (a) external  $\text{Ca}^{2+}$  was replaced with  $\text{Mg}^{2+}$  (Fig. 2), and (b) when 10  $\mu\text{M}$ -nifedipine was added to the external solution (Fig. 1*C* and *E*). The magnitude and general characteristics of this 'slow' calcium current in e.d.l. muscle are very similar to those described previously for other fast-twitch muscles in amphibia (Sanchez & Stefani, 1978; Almers & Palade, 1981; Almers, Fink & Palade, 1981) and

mammals (Donaldson & Beam, 1983; Walsh, Bryant & Schwartz, 1986), with the exception that Walsh *et al.* (1986) could not block the calcium current with the dihydropyridine, nitrendipine. As dihydropyridines block calcium currents at lower concentrations in depolarized fibres (Cognard, Romey, Galizzi, Fosset & Lazdunski, 1986), it was conceivable that a small difference in holding potential ( $-90$  mV in this

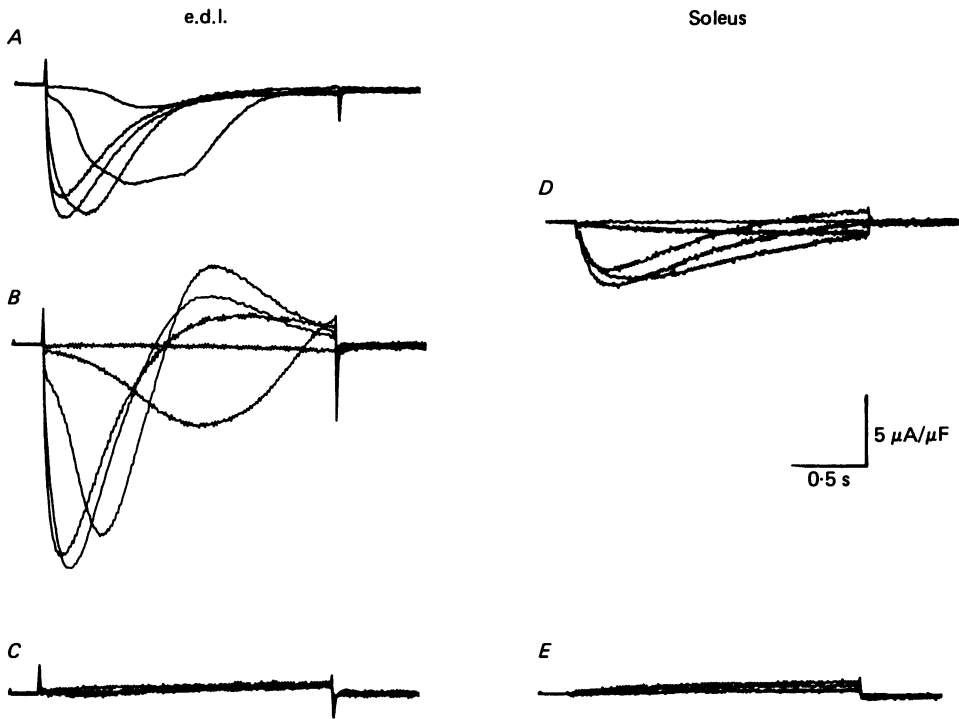


Fig. 1. Non-linear currents in two e.d.l. fibres (*A* and *B*) and a soleus fibre (*D*). In all cases, the currents were elicited by 2 s depolarizations to  $-30$ ,  $-20$ ,  $0$ ,  $+10$  and  $+20$  mV, the inward current being smallest at  $-30$  mV and becoming progressively larger and faster with increasing depolarizations, reaching a maximum at  $+10$  mV. The inward currents in *A* were the smallest for any e.d.l. fibre, and those in *D* were typical of soleus fibres. After addition of  $10 \mu\text{M}$ -nifedipine to the e.d.l. fibre in *B* and the soleus fibre in *D*, the same depolarizations produced only small outward currents in both fibres (*C* and *E*, respectively). The external solution contained  $8 \text{ mM-CaSO}_4$ , and each fibre was internally perfused with a high- $\text{Na}^+$  solution.

study,  $-100$  mV in Walsh *et al.* 1986) might account for the difference in results. To test this, the effect of nifedipine was examined at a holding potential of  $-110$  mV, and with  $\text{Na}^+$  as the predominant external cation (as in the experiments of Walsh *et al.* 1986), and it was found that  $10 \mu\text{M}$ -nifedipine still blocked the calcium current.

The peak and rate of decline of the calcium current may be affected by both depletion of calcium ions in the T-system (Almers *et al.* 1981) and inactivation of the calcium channels (Avila-Sakar, Cota, Gamboa-Aldeco, Garcia, Huerta, Muniz & Stefani, 1986). As larger calcium currents would be expected to deplete calcium in

the T-system more quickly, any calcium depletion effects may be proportionately greater in e.d.l. fibres than in soleus fibres, if the geometry of the T-system is similar in the two muscle types. This might explain why the calcium current reaches a peak more quickly in e.d.l. fibres than in soleus fibres. Furthermore, if the peak current is used as an indicator of the number of activated calcium channels, calcium depletion effects may result in an underestimation of the relative number of channels in e.d.l. muscle compared to soleus muscle.

Consequently, the relative sizes of the calcium currents in e.d.l. and soleus muscles were also compared at short times after the start of the depolarization, when any depletion or inactivation effects should be small. Figure 2 shows the calcium current produced by a 35 ms depolarization to 0 mV in both an e.d.l. and a soleus fibre, recorded with high (8 mM) and very low (0.15 mM) external calcium; the calcium current is indicated by the difference between the traces in both cases. For both fibres the asymmetric charge movement produced by the potential change is also apparent; when the depolarizing step was applied the non-linear capacitive current was outwards ('on' charge) and upon repolarization there was an equal inward current ('off' charge). This asymmetric charge was most clearly defined when the external  $[Ca^{2+}]$  was very low, that is, when there was virtually no calcium current at all. Nevertheless, the superposition of the 'on' charge for the two different calcium concentrations makes it clear that the asymmetric charge was unaffected by such differences in  $[Ca^{2+}]$ .

Because of the occurrence of the 'on' charge, it is difficult to precisely identify the early time course of the calcium current, though it obviously begins to rise within 2 or 3 ms after the start of the depolarization. In both the slow- and fast-twitch fibres, the current rises almost linearly for the remainder of the 35 ms depolarization, probably indicating that any effects due to calcium depletion or channel inactivation are quite small. The size of the calcium current at the end of a 35 ms step was quantified in every fibre for depolarizations to between  $-40$  and  $+50$  mV. The mean of the largest current in thirteen soleus fibres was  $2.8 \pm 0.5 \mu A/\mu F$ , and in fourteen e.d.l. fibres was  $10.2 \pm 0.8 \mu A/\mu F$ . Thus the maximal current, for a 35 ms depolarization, was 3.6 times larger in e.d.l. fibres than in soleus fibres, compared with 2.4 times larger when quantified at its peak using 2 s depolarizations.

The relationship between current and potential, for 35 ms pulses, is shown for both fibre types in Fig. 2C. For each fibre, the current elicited at each potential was normalized by the corresponding peak current; in this manner the potential dependence of the currents in the two fibre types can be easily compared. The two curves in Fig. 2C are very similar. The potential dependence of the current in soleus muscle appears to be positioned about 5 mV more negative than that for e.d.l. muscle, though this may be partly a consequence of the total current in the former case being affected proportionately more by the presence of small currents through other channels. Such currents were of similar absolute size in e.d.l. and soleus muscle (about 1 to  $1.5 \mu A/\mu F$  after 2 s at  $+10$  mV, Fig. 1C and E), and would be expected to lead to a more negative value for the apparent reversal potential in soleus compared to e.d.l. muscle.

Hence, both e.d.l. and soleus fibres have a 'slow' nifedipine-sensitive calcium current, which is about 3 times larger in e.d.l. fibres than in soleus fibres, but is very

similar otherwise. This could be explained simply if (a) the calcium channels in the two muscle types are identical and (b) the density of these channels is about 3 times higher in e.d.l. muscle.

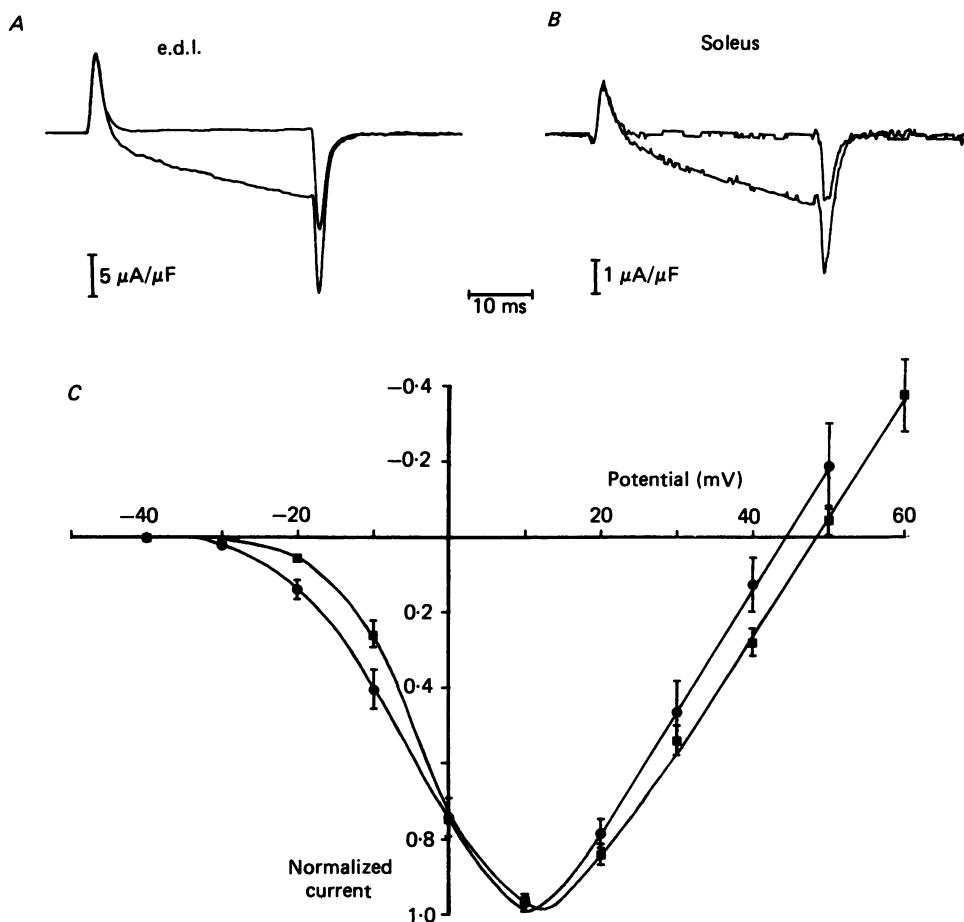


Fig. 2. Asymmetric charge movement and calcium currents in a fast- and a slow-twitch fibre. *A*, superposition of the non-linear currents produced in an e.d.l. fibre by a 35 ms depolarization to 0 mV when the external  $[Ca^{2+}]$  was high (8 mM- $CaSO_4$ , lower trace) or very low (0.15 mM- $CaSO_4$ , upper trace). The calcium current is given by the difference between the two traces. The 'on' and 'off' charge movement can be seen at the start and the end of the depolarization, respectively. The charge movement is unaffected by the  $[Ca^{2+}]$ . *B*, superposition of the currents in a soleus fibre for the same conditions as in *A*. Note the different current scales in *A* and *B*; both the asymmetric charge movement and the calcium currents in slow-twitch fibres were much smaller than those in fast-twitch fibres. *C*, relationship between mean normalized current and potential for fourteen e.d.l. fibres (■) and ten soleus fibres (●). Each symbol represents the mean current at the end of a 35 ms depolarization to the potential indicated; the currents in each fibre were normalized by the maximum current elicited at any potential in that fibre. The error bars indicate  $\pm$  s.e. of the mean, other than where this was smaller than the symbol. The external solution contained 8 mM- $CaSO_4$  in all cases. The curves for the two fibre types are very similar.

*Charge movement and dihydropyridine calcium antagonists*

The amount of asymmetric charge was quantified at a holding potential of  $-90$  mV using a 'control' step from  $-90$  to  $-110$  mV (see Methods). The mean of the

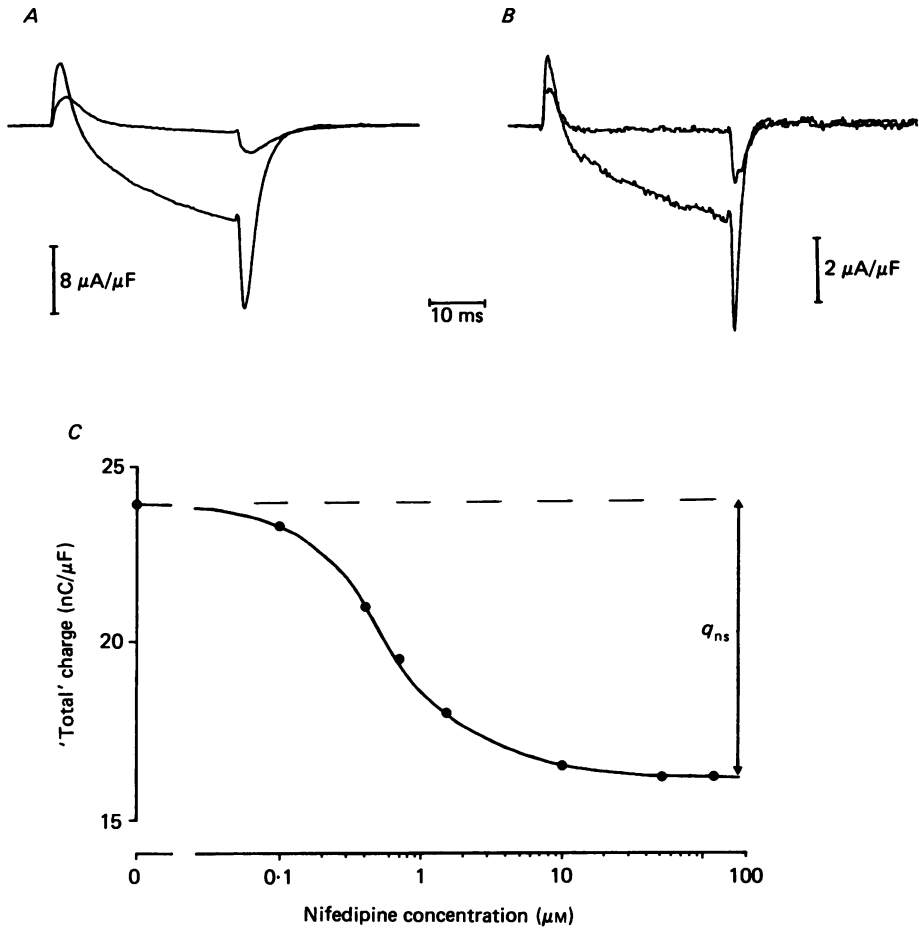


Fig. 3. Addition of  $10 \mu\text{M}$ -nifedipine to the external solution blocked virtually all the calcium current but only part of the charge movement in both e.d.l. (A) and soleus (B) fibres. Note the different current scales. Depolarizations to  $0$  mV,  $8 \text{ mM-Ca}^{2+}$  in all solutions. C, amount of charge movement remaining *versus* nifedipine concentration in an e.d.l. fibre. Most of the charge movement is unaffected by even extremely high concentrations of nifedipine. The component of charge which can be suppressed is denoted as  $q_{ns}$ .

maximum asymmetric charge found with this paradigm was  $25.4 \pm 0.8 \text{ nC}/\mu\text{F}$  in nineteen e.d.l. fibres,  $13.5 \pm 0.6 \text{ nC}/\mu\text{F}$  in ten sternomastoid fibres and  $5.1 \pm 0.9 \text{ nC}/\mu\text{F}$  in thirteen soleus fibres. (There are several different components of charge movement and the total amount measured depends on the exact experimental paradigm used (Lamb, 1987); consequently, to indicate this dependence, the



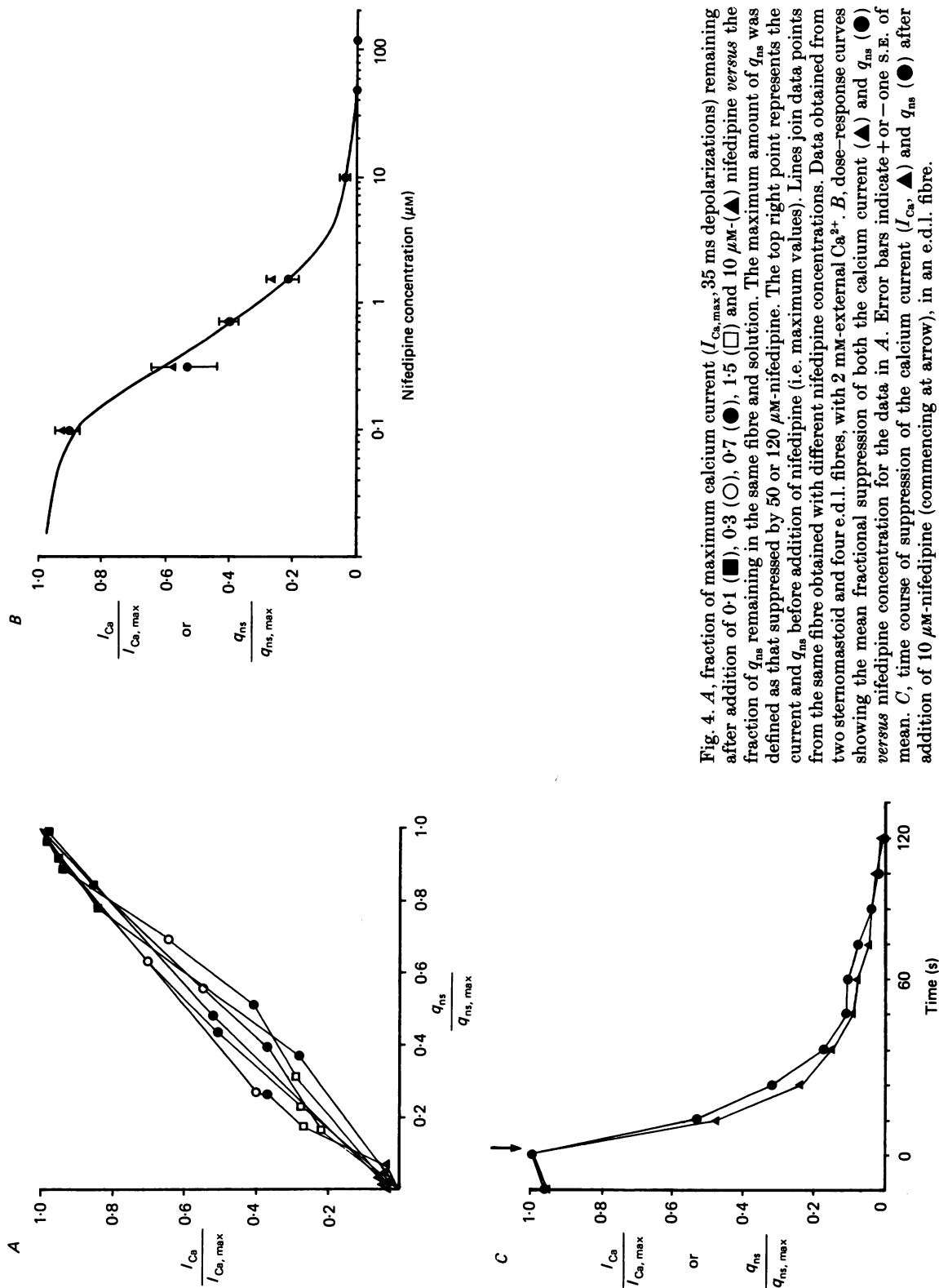


Fig. 4. *A*, fraction of maximum calcium current ( $I_{Ca, max}$ , 35 ms depolarizations) remaining after addition of 0.1 ( $\blacksquare$ ), 0.3 ( $\circ$ ), 0.7 ( $\bullet$ ), 1.5 ( $\square$ ) and 10  $\mu M$ -( $\blacktriangle$ ) nifedipine versus the fraction of  $q_{ns}$  remaining in the same fibre and solution. The maximum amount of  $q_{ns}$  was defined as that suppressed by 50 or 120  $\mu M$ -nifedipine. The top right point represents the current and  $q_{ns}$  before addition of nifedipine (i.e. maximum values). Lines join data points from the same fibre obtained with different nifedipine concentrations. Data obtained from two sternomastoid and four e.d.l. fibres, with 2 mM-external  $Ca^{2+}$ . *B*, dose-response curves showing the mean fractional suppression of both the calcium current ( $\blacktriangle$ ) and  $q_{ns}$  ( $\bullet$ ) versus nifedipine concentration for the data in *A*. Error bars indicate + or - one s.e. of mean. *C*, time course of suppression of the calcium current ( $I_{Ca}$ ,  $\blacktriangle$ ) and  $q_{ns}$  ( $\bullet$ ) after addition of 10  $\mu M$ -nifedipine (commencing at arrow), in an e.d.l. fibre.

maximum charge obtained with this particular paradigm will be referred to as 'total'.) The above values are close to those reported previously using similar paradigms (Dulhunty & Gage, 1983; Simon & Beam, 1985; Lamb, 1986b).

It has been briefly reported previously that the dihydropyridine, nifedipine, suppressed calcium currents and *part* of the charge movement in sternomastoid fibres (Lamb, 1986b). Figure 3 shows that this effect is very similar in both fast- and slow-twitch fibres. Addition of 10  $\mu\text{M}$ -nifedipine blocked virtually all of the calcium current, but suppressed only *some* of the charge movement; higher concentrations of nifedipine had almost no further effect (Fig. 3C), indicating that under these experimental conditions one component of the charge movement is sensitive to nifedipine ( $q_{\text{ns}}$ ) whereas the remainder is not. The mean amount of charge movement ( $q_{\text{ns}}$ ) that could be suppressed by nifedipine (up to concentrations of 120  $\mu\text{M}$ ) was  $8.0 \pm 0.9 \text{ nC}/\mu\text{F}$  in twelve e.d.l. fibres and  $2.4 \pm 0.8 \text{ nC}/\mu\text{F}$  in eight soleus fibres. The effect of increasing concentrations of nifedipine on both calcium currents and  $q_{\text{ns}}$  is illustrated in Fig. 4A. At any concentration of nifedipine, the fraction of  $q_{\text{ns}}$  suppressed appears to be linearly related to the fraction of the calcium current blocked. The amount of suppression of both parameters is plotted against the concentration of nifedipine in Fig. 4B, and as expected from the relationship in Fig. 4A, both sets of data are adequately fitted by the same curve. The concentration of nifedipine producing half-maximal suppression is approximately 0.5  $\mu\text{M}$ . The close relationship between  $q_{\text{ns}}$  and the calcium current is further illustrated in Fig. 4C, which shows that they are both suppressed over the same time course by nifedipine.

The effect of another dihydropyridine calcium antagonist, the (+) enantiomer of PN200/110, was also examined. In the four fast-twitch fibres examined, 10  $\mu\text{M}$ -(+)PN200/110 suppressed at least 95% of the calcium current and an amount of charge movement similar to that suppressed by 10  $\mu\text{M}$ -nifedipine in other fast-twitch fibres. When 10  $\mu\text{M}$ -nifedipine was added to the solution after the charge movement and calcium currents had been suppressed by 10  $\mu\text{M}$ -(+)PN200/110, no further suppression of charge movement or calcium current could be discerned. Therefore it seems that both dihydropyridine calcium antagonists affect the same components of charge movement ( $q_{\text{ns}}$ ).

Both the largest calcium current (for a 35 ms depolarization) and the maximum amount of  $q_{\text{ns}}$  were ascertained in each of eight e.d.l. fibres, four sternomastoid fibres and eight soleus fibres. (For each fibre, both measurements were normalized in the same way: by the linear capacitance of that fibre.) Figure 5A shows that there was a linear relationship between the size of the calcium current and the maximum amount of  $q_{\text{ns}}$ , both within and between the three fibre types. If the size of the calcium current is indicative of the relative number of activatable calcium channels, the above relationship suggests that the amount of  $q_{\text{ns}}$  per activatable calcium channel is approximately the same in every fibre, regardless of muscle type. (Comparison of the amount of charge movement in low-calcium solutions, with that estimated in high-calcium (8 mM) solutions by extrapolating the calcium current back to the zero-current level at the start of the depolarization (Fig. 2), indicated that the latter measurement often resulted in about a 5–10% overestimation of the amount of charge movement. About half of the values of  $q_{\text{ns}}$  in Fig. 5A may have been affected by this error; the other half were not affected as  $q_{\text{ns}}$  was ascertained in



a low-calcium solution after the size of the calcium current had been determined using the high-calcium solution.)

In contrast, there did not appear to be a direct linear relationship between the 'total' amount of charge movement and the calcium current, within each fibre type (Fig. 5*B*). Linear regression analysis of the data of each fibre type gave intercepts with the abscissa (i.e. zero-calcium current) of 18.3, 7.4 and 1.1 nC/ $\mu$ F for the e.d.l., sternomastoid and soleus fibres, respectively. The amount of charge movement remaining in the presence of nifedipine appeared to be unrelated to the size of the calcium current in each fibre, instead being almost the same in all fibres of the same muscle type, with mean values ( $\pm$  s.e. of mean) of  $16.1 \pm 0.8$ ,  $5.6 \pm 0.3$  and  $2.4 \pm 0.4$  nC/ $\mu$ F in twelve e.d.l., four sternomastoid and eight soleus fibres, respectively. The lack of correlation between the amount of nifedipine-insensitive charge and the size of the calcium current suggests that this charge may not be involved in opening calcium channels in the T-system.

#### *Potential dependence of calcium current and $q_{ns}$*

Figure 6*A* shows the relationship between peak inward current and potential in an e.d.l. fibre when using 2 s depolarizations; the threshold potential was near  $-30$  mV. The currents produced at more negative potentials are also plotted on a logarithmic scale in Fig. 6*B*. If the current through an open calcium channel does not vary greatly between  $-30$  and  $-20$  mV, the asymptote fitted to the data at such potentials (Fig. 6*B*) should give a reasonable approximation to the limiting logarithmic potential sensitivity (see Almers, 1978). The fitted asymptote displays an e-fold change in 4.8 mV, indicating a minimum gating charge of between five and six electronic charges per calcium channel.

The potential dependence of the mean amount of  $q_{ns}$  in five e.d.l. fibres is shown in Fig. 7, together with the relative calcium current in same fibres. As the driving force on the calcium ions must decrease at more positive potentials, at each potential the relative current must be greater than the relative calcium channel conductance. Thus, it is clear that the movement of  $q_{ns}$  occurs over a more negative potential range than does the conductance change of the calcium channels, rather than the reverse. This is consistent with the hypothesis that the movement of  $q_{ns}$  is necessary for the calcium channels to open, or in other words that  $q_{ns}$  is (or is part of) the calcium gating current. In such a case, the potential dependences indicated in Fig. 7 imply that a substantial amount of  $q_{ns}$  must move before there is appreciable opening of calcium channels (Armstrong, 1981).

#### *Effect of (-)bay K8644, a calcium channel agonist*

In contrast to nifedipine and (+)PN200/110, the (-) enantiomer of bay K8644 appears to be a calcium channel *agonist* in cardiac and smooth muscle (Frankowiak, Bechem, Schramm & Thomas, 1985). The data in Fig. 8 show that (-)bay K8644 is an agonist of the slow calcium channels in skeletal muscle also. The relative effect was most marked near the threshold potential for the calcium current, as has been previously reported for racemic bay K8644 (i.e. a mixture of both the (+) and (-) isomers) in cultured skeletal muscle (Cognard *et al.* 1986) and in cardiac muscle (Hess, Lansman & Tsien, 1984). (-)Bay K8644 also greatly increased and prolonged

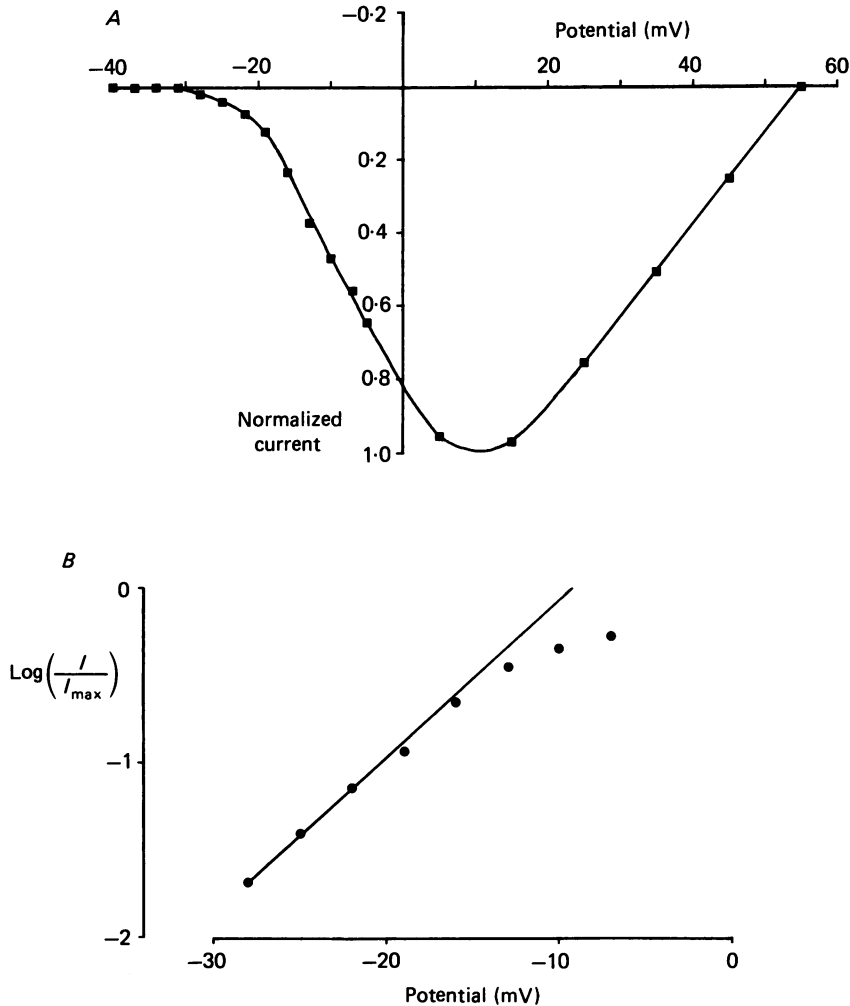


Fig. 6. *A*, the potential dependence of the peak current in an e.d.l. fibre subjected to 2 s depolarization from  $-90$  mV to the potential indicated. The peak current at each potential was normalized by the maximum current ( $\sim 18 \mu\text{A}/\mu\text{F}$  near  $+10$  mV, determined by interpolation of the data). *B*, current ( $I$ ) on logarithmic scale versus potential for data in *A*. The asymptote fitted to the data near threshold (continuous line) indicates an e-fold change in current in 4.8 mV, which implies that the minimum gating charge for the calcium channel is between five and six electronic charges.

the calcium tail current (Fig. 8*A*). This effect was even noticeable in low-calcium solutions (Fig. 8*B*, upper panel). Similar results were obtained with  $0.5 \mu\text{M}$ -(-)bay K8644.

Again in contrast to nifedipine and (+)PN200/110, (-)bay K8644 did not suppress any component of the asymmetric charge movement (Fig. 8), even at high concentration ( $10 \mu\text{M}$ ). Thus it seems that only those dihydropyridines which suppress a particular component of the charge movement ( $q_{\text{ns}}$ ) block the slow calcium

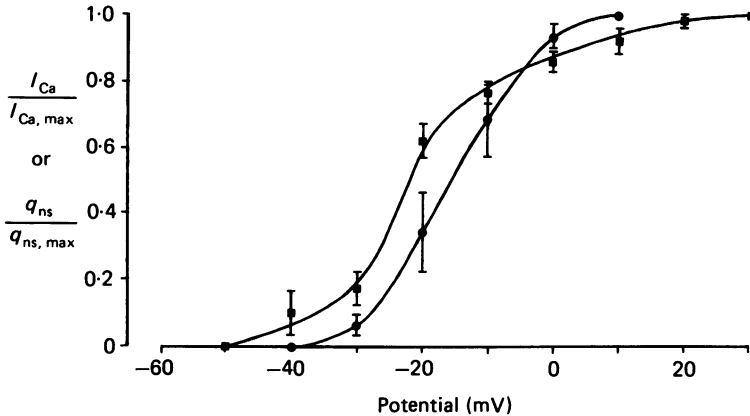


Fig. 7. Potential dependence of  $q_{ns}$  and calcium current ( $I_{Ca}$ ). The amount of nifedipine-sensitive charge movement at each potential ( $q_{ns}$ ) was ascertained for each of five e.d.l. fibres, from the difference in the amount of charge movement before and after addition of  $10 \mu\text{M}$ -nifedipine. The squares indicate the mean amount of  $q_{ns}$  at each potential expressed as a fraction of the maximum ( $q_{ns,max}$ ); the error bars indicate  $\pm$ s.e. of the mean. In the same five e.d.l. fibres, the peak calcium current at each potential between  $-50$  and  $+10$  mV, elicited by 2 s depolarizations, was expressed as fraction of the maximum peak current; the circles show the mean values. The relative calcium current will be greater than the relative conductance of the calcium channel at each potential, and thus the movement of  $q_{ns}$  occurs over a more negative potential range than the change in calcium conductance.

channel. This further supports the hypothesis that  $q_{ns}$  is part of the gating current of the slow calcium channel.

When racemic bay K8644 (i.e. both isomers) was applied ( $10 \mu\text{M}$ , two fibres), the calcium tail current was increased and prolonged and there was a partial suppression of the charge movement ( $\sim 25\%$ ); replacement of the bay K8644 with nifedipine led to the further suppression of a similar amount of charge movement and to complete block of the calcium current. This is consistent with the (+) isomer of bay K8644 being a calcium channel antagonist in skeletal muscle, as has been proposed for smooth and cardiac muscle (Franckowiak *et al.* 1985), though it is clear that agonist action of the (-) isomer determines the net effect of the racemic mixture.

#### *Binding of dihydropyridine*

The amount of specific binding of the labelled dihydropyridine, [ $^3\text{H}$ ](+)PN200/110, in bundles of intact, depolarized fibres from both soleus and e.d.l. muscles, was measured by the method of Schwartz *et al.* (1985). This technique allowed the saturatable (i.e. specific) component of DHP uptake in each bundle to be distinguished from the non-saturatable (i.e. non-specific) component. Figure 9 shows the decrease in the concentration of labelled DHP in the bathing solution (filled circles) as the drug is taken up by a fibre bundle (open circles) into both the saturatable and non-saturatable pools. When a very large amount of unlabelled DHP ( $50 \mu\text{M}$ , in ethanol) was added to the bathing solution (arrow) as the total uptake was close to equilibrium (i.e. after about 9 h), virtually all of the labelled

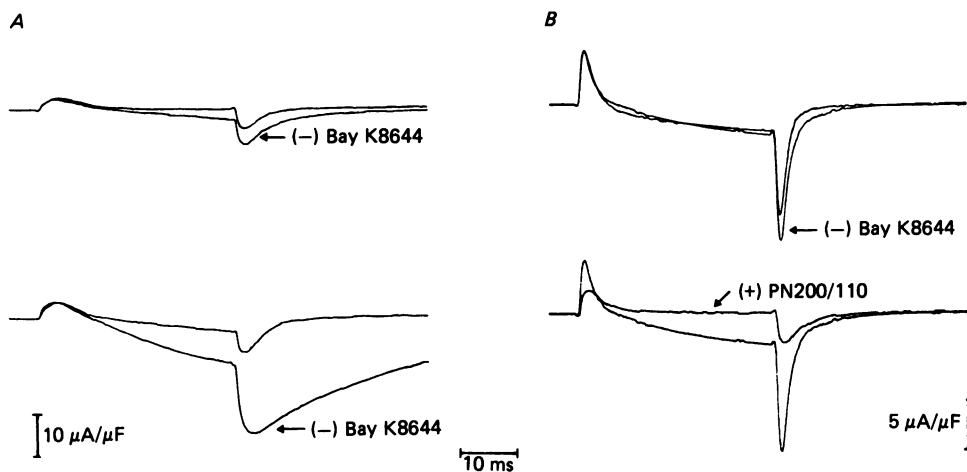


Fig. 8. The calcium agonist, (-)bay K8644, does not suppress charge movement. *A*, superposition of charge movement and calcium current before and after the addition of  $10 \mu\text{M}$ -(-)bay K8644, for depolarizations to  $-20 \text{ mV}$  (upper panel) and  $-10 \text{ mV}$  (lower panel). The calcium current at potentials near threshold was increased in the presence of the drug, and the calcium tail current was greatly increased at all potentials (e.d.l. fibre,  $8 \text{ mM-CaSO}_4$ ). *B*, similar superposition for another e.d.l. fibre in a low-calcium ( $0.6 \text{ mM-CaSO}_4$ ) solution, for a depolarization to  $0 \text{ mV}$  (upper panel). The tail current was increased, but the charge movement was unaffected by the drug. When the (-)bay K8644 was replaced with  $10 \mu\text{M}$ -(+)PN200/110 (lower panel), the calcium current and part of the charge movement were suppressed.

DHP bound to specific sites should have been displaced, and this displaced ligand redistributed between the bathing solution and non-specific pool in the muscle bundle, thereby altering the final equilibrium values (continuous lines). Experiments with other fibre bundles showed that when only ethanol was added, the amount of label in the fibre bundle and in the bathing solution continued towards the equilibrium values expected if nothing at all were added (dashed lines). Three or four hours after adding the unlabelled DHP, all the label in the muscle should be in the non-specific pool. The amount of labelled DHP in this non-specific pool was found to be linearly related to its concentration in the bathing solution, as reported by Schwartz *et al.* (1985). Thus, the amount of specific DHP binding can be calculated using the equations given in Schwartz *et al.* (1985), which simply state that (a) the total content of label in each bundle is the sum of the specific and non-specific components, and (b) the amount of labelled DHP in the non-specific pool, before and after the addition of the unlabelled DHP, is in the same ratio as the respective concentrations of label in the bathing solution (Fig. 9). As there may be some error in extrapolating the curves of bathing concentration, for each bundle an upper limit for the specific uptake was calculated using the concentration indicated by the extrapolation and a lower limit was calculated using the concentration at the time the unlabelled DHP was added. Figure 10 is a graph of such upper and lower limits of the specific binding in soleus and e.d.l. fibre bundles, plotted against the corresponding concentration of labelled DHP in the bathing solution; at high

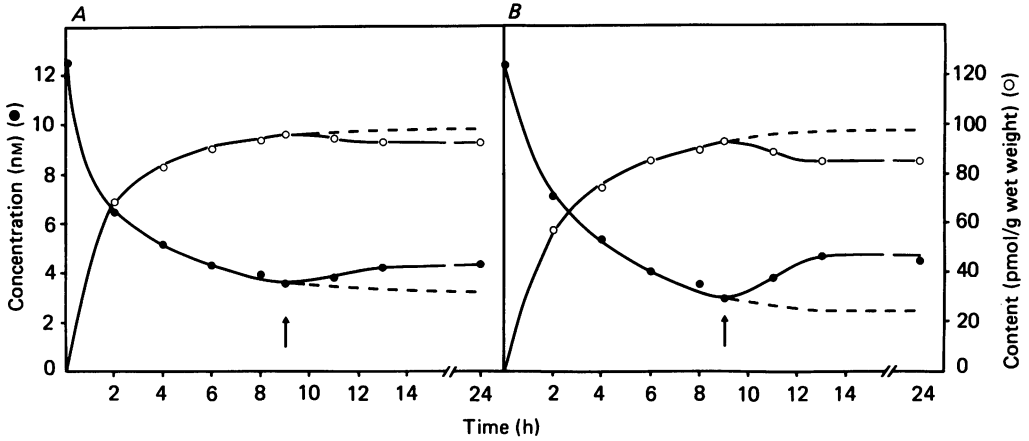


Fig. 9. Uptake of  $[^3\text{H}](+)\text{PN200/110}$  by a bundle of fibres from a soleus muscle (A) or an e.d.l. muscle (B). Bundles were incubated in a depolarizing (high- $\text{K}^+$ ) solution, and unlabelled (+)PN200/110 was added to give a concentration of  $50\ \mu\text{M}$ , at the time indicated by the arrows. ●: concentration of  $[^3\text{H}](+)\text{PN200/110}$  in bathing solution (scale on left). ○: calculated content of  $[^3\text{H}](+)\text{PN200/110}$  in the fibre bundle (scale on right). Dashed lines are extrapolations based on control fibres to which only ethanol was added at arrow.

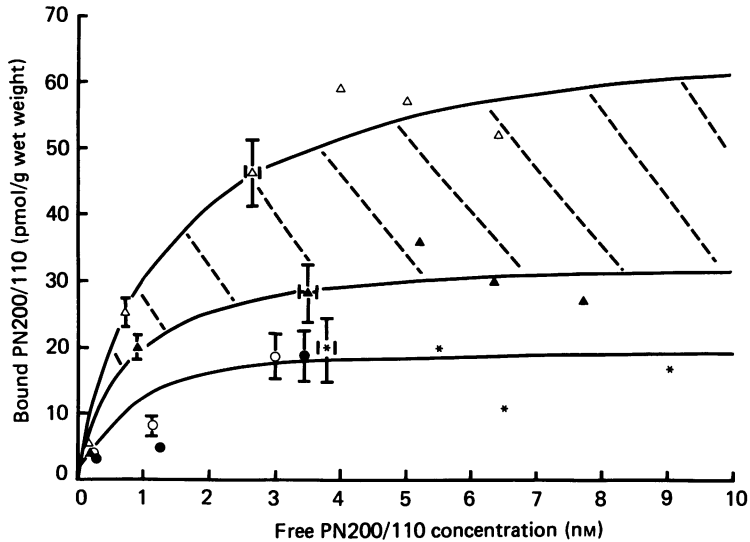


Fig. 10. Specific binding of  $[^3\text{H}](+)\text{PN200/110}$  by bundles of soleus fibres (○, ●) and e.d.l. fibres (Δ, ▲) versus the final concentration of free ligand in the bathing solution. Non-specific uptake of the ligand affected the estimate of the specific uptake; for each estimate, upper and lower limits (open and filled symbols respectively) were calculated as described in the text. The hatching indicates the region between these limits, for e.d.l. muscle. The lowest curve shows the upper limit for soleus muscle. Where there are error bars ( $\pm$  s.e. of mean) the symbols represent the mean of three to eight determinations. The symbols for the three highest free concentrations are individual measurements; for soleus muscle the non-specific binding at these high concentrations allowed only an upper limit for specific binding to be calculated (asterisks).



concentrations with soleus bundles, the continuation of slow non-specific uptake obscured the changes in specific uptake, and it was possible only to give an upper limit for specific binding. Using a non-linear least-squares regression program, the data were fitted by a hyperbolic function describing the simplest possible binding scheme, namely association with a site (or sites) having a single binding constant and no co-operative behaviour. From this analysis, it appears that the amount of specific binding of (+)PN200/110 reaches a maximum of between about 34 and 69 pmol/g wet weight of muscle in e.d.l. fibre bundles and less than about 22 pmol/g in soleus bundles. Analysis of paired observations (measurements made on the same day using the same ligand concentration in the two muscle types) revealed that the binding in the two muscle types was significantly different at the  $P < 0.005$  level (Student's  $t$  test), with the average ratio of the amount bound in e.d.l. to the amount bound in soleus being 2.2 (minimum estimates) to 3.0 (maximum estimates).

#### DISCUSSION

##### *Slow calcium currents and charge movement*

An important finding in this study is that the slow calcium current in slow-twitch fibres (soleus) is considerably smaller than in fast-twitch fibres (e.d.l.), regardless of whether the current is measured at its peak (where it may be affected by depletion effects), or on its rising phase, 35 ms after the start of the depolarizing stimulus. Apart from this difference in magnitude, the calcium currents seem very similar in the two muscle types. The most straightforward explanation of this would be that the calcium channels in the two muscle types are identical, but that the density of active channels in the T-system is about three times higher in e.d.l. muscle than in soleus muscle. This could account for the apparent relationship between the amount of DHP-sensitive charge movement and the size of the current. Nifedipine and (+)PN200/110, another calcium channel antagonist, suppressed the same component of charge movement ( $q_{ns}$ ), and the amount of this charge movement appeared to be linearly related to the size of the calcium current in each fibre, this relationship being the same in soleus, sternomastoid and e.d.l. muscles (Fig. 5A). This would be expected if the size of the calcium current were indicative of the number of activatable calcium channels, and the amount of  $q_{ns}$  per calcium channel were the same in every fibre, regardless of muscle type.

Thus, the observations that (a) the amount of  $q_{ns}$  is linearly related to the size of the slow calcium current, (b)  $q_{ns}$  and the calcium current are suppressed over the same concentration range by DHP antagonists of the calcium channel (Fig. 4A and B), (c) the two signals are suppressed with the same time course (Fig. 4C), and (d) (-)bay K8644, a DHP calcium channel agonist, does not suppress any charge movement, strongly suggest that  $q_{ns}$  is closely related to the slow calcium channel, possibly being part of the gating current of those channels. The relative potential dependence of  $q_{ns}$  and the current is also consistent with this (Fig. 7). The fact that the calcium current does not reach a peak until a long time after the start of the depolarization implies that there *must* be a slow step in the activation of the channels, and so the relatively fast time course of  $q_{ns}$  indicates that, if the movement of  $q_{ns}$  is involved in gating the channels, it must precede this slow step. Any charge

movement associated with this slow step would be very hard to detect because of its slow time course.

Many of the characteristics of the proposed 'calcium gating charge' ( $q_{ns}$ ) described here are very similar to those observed in *Aplysia* neurones (Adams & Gage, 1976, 1979*b*). In *Aplysia* neurones the calcium current does not peak until a long time after the movement of the calcium gating charge (though both currents are at least 5 times faster than those reported here). When normalized by cell capacitance the *Aplysia* gating charge is about  $3 \text{ nC}/\mu\text{F}$  and the peak calcium current is about  $6 \mu\text{A}/\mu\text{F}$  in  $5 \text{ mM-CaCl}_2$  (Adams & Gage, 1979*a, b*), which are very similar to the amount of  $q_{ns}$  ( $2.4 \text{ nC}/\mu\text{F}$ ) and the peak current ( $6.1 \mu\text{A}/\mu\text{F}$ ) observed in soleus muscle in this study with a solution of similar calcium ion activity.

Furthermore, the proposed connection between a component of the charge movement ( $q_{ns}$ ) and the gating of the T-system calcium channels in vertebrate muscle, is very similar to the conclusion of a recent study in invertebrate (scorpion) skeletal muscle (Scheuer & Gilly, 1986).

#### *Dihydropyridine binding sites and calcium channels*

Estimation of the number of DHP binding sites in skeletal muscle was made difficult by the presence of a large amount of non-specific binding. Nevertheless, when the relative amounts of specific DHP binding in e.d.l. and soleus muscle were compared under (a variety of) identical conditions, there were always about 2–3 times more specific binding sites in e.d.l. muscle than in soleus muscle. Clearly, this is similar to the ratio of the calcium current, and the presumed number of calcium channels, in the two muscle types. Such similarity would be expected if most of the DHP binding sites were on slow calcium channels (with the affinity of these sites being much higher when the muscle is depolarized (Cognard *et al.* 1986)).

In contrast, Schwartz *et al.* (1985) propose that there are 35–50 times more DHP binding sites than 'functional' calcium channels. However, their proposal is based on calculations of the number of calcium channels, found by comparing the maximal calcium current in intact frog fibres with the size of single-channel currents from other studies, and the assumptions underlying these calculations may not be valid. In particular they assume that *every* channel was *continuously* open under the conditions when the maximal current was recorded (near  $-20 \text{ mV}$ ). There are no single-channel data in skeletal muscle to support this assumption. Two studies of T-tubular calcium channels reincorporated into lipid bilayers show that the average fraction of time that a channel is open ( $P$ ), at potentials near  $0 \text{ mV}$  and in the absence of agonists such as bay K8644, is only about 0.08 (Coronado & Affolter, 1986) or 0.05–0.25 (Flockerzi, Oeken, Hofmann, Pelzer, Cavalie & Trautwein, 1986), though such values may not be comparable with those in intact fibres. It is quite possible that, as in heart muscle (Hess *et al.* 1984), a channel may not open at all on a particular stimulus, even though it may open for long periods on the preceding or following stimulus. This would be an example of a 'functional' or *activatable* calcium channel not contributing at all to the total current on a *particular* stimulus. Thus, if all channels exhibit similar behaviour independently, on each stimulus there may be many more non-conducting channels than there are conducting channels, even though all are activatable channels. Consequently, fluctuation analysis and tail

current analysis, which reflect only the behaviour of channels which open, do not necessarily indicate the number of activatable channels.

Thus, it is not presently possible to calculate reliably the number of activatable calcium channels in skeletal muscle. Nevertheless, the following calculation shows that there *may* be as few as one or two DHP binding sites per channel in mammalian muscle, assuming (i)  $P \approx 0.08$  under the conditions giving the maximal current in this study, (ii) the corresponding single-channel current is about 0.07 pA in the absence of depletion effects (taking into account the calcium activity in the  $\text{CaSO}_4$  solution used here; Fenwick, Marty & Neher, 1982; Hess & Tsien, 1984) and (iii) depletion effects cause the peak current to be underestimated by a factor of 2 (Almers *et al.* 1981). In e.d.l. muscle the maximum amount of specific DHP binding was approximately 34–69 pmol/g fibre weight, which is about  $2\text{--}4 \times 10^{13}$  sites/ml volume. The area of T-system membrane per unit volume is about  $1.9 \times 10^3$  cm<sup>2</sup>/ml in e.d.l. muscle (Dulhunty, Gage & Lamb, 1986), and the capacitance per unit area of T-system is assumed to be  $1.4 \mu\text{F}/\text{cm}^2$  (Mathias, Eisenberg & Valdiosera, 1977). Thus, taking depletion effects into account, the recorded peak current ( $14.8 \mu\text{A}/\mu\text{F}$  of total capacitance) corresponds to a 'true' current of about 79 mA/ml fibre volume, indicating about  $1.4 \times 10^{13}$  activatable channels/ml fibre volume, or about 1.5–2.5 DHP binding sites per activatable channel. A similar calculation for soleus fibres gives 1.5–3 DHP binding sites per activatable channel. Furthermore, if the amount of  $q_{\text{ns}}$  in each muscle type (8 nC/ $\mu\text{F}$  in e.d.l., and 2.4 nC/ $\mu\text{F}$  in soleus) is compared to the number of activatable calcium channels estimated above, the amount of  $q_{\text{ns}}$  per calcium channel is approximately seven electronic charges in soleus fibres and nine in e.d.l. fibres. This is similar to the minimum gating charge per calcium channel (about five or six electronic charges), estimated from the potential dependence of the current (Fig. 6).

However, if the assumptions involved in calculating the number of calcium channels are wrong, in particular if most channels are open at the peak of the current, then (a) only a very small proportion of the DHP binding sites are on activatable calcium channels (Schwartz *et al.* 1985) and (b) the amount of  $q_{\text{ns}}$  per channel would be so high as to suggest that most of  $q_{\text{ns}}$  must be associated with the other molecules which have DHP binding sites and not just with the activatable channels.

#### *Relationship between $q_{\text{ns}}$ , calcium channels and dihydropyridine binding sites*

Thus, the two extreme cases are as follows, depending on the actual number of activatable calcium channels: (a) in both e.d.l. and soleus fibres, most or all DHP binding sites are on activatable calcium channels (of which only a small proportion are activated on any particular stimulus), and each site is associated with a DHP-sensitive charge of about seven to nine electronic charges, which is probably involved in the channel gating; (b) alternatively, the molecules with the DHP binding sites are a heterogeneous group with only a very small proportion being activatable calcium channels, and with  $q_{\text{ns}}$  being associated with the majority of these molecules not just with the activatable channels. Furthermore, in order to explain the close relationship between  $q_{\text{ns}}$  and calcium current (both Figs 4 and 5), presumably most molecules with binding sites which are not activatable channels must be very closely related to such channels, possessing a 'gating' charge, even though its movement never leads to

channel opening. It must also be proposed that the number of activatable channels is roughly a fixed proportion of the total number of molecules with DHP binding sites, in order to explain why there should be such similarity of the ratios, in e.d.l. compared to soleus muscle, of (i) the number of DHP binding sites, (ii)  $q_{ns}$  and (iii) the size of the calcium current. Such a relationship between the activatable calcium channels and the other molecules with DHP binding sites could be analagous to the relationship between the fetal and adult forms of the muscle acetylcholine receptor-channel, in which the macromolecules differ only in one of their subunits (Mishina, Takai, Imoto, Noda, Takahashi, Numa, Methfessel & Sakmann, 1986).

#### *Charge movement components and contraction*

Nifedipine suppresses  $q_{ns}$  with a half-maximal effect at about  $0.5 \mu\text{M}$ , when approximately 20–25% of the 'total' charge is suppressed (Fig. 3 and Lamb, 1986*b*), though the relative amounts of the various components depends on the fibre type and the precise experimental paradigm (Lamb, 1987). The results of this study suggest that  $q_{ns}$  is part of the gating charge of the T-system calcium channels (or closely related molecules), and so the question then arises as to what role, if any, this component of charge movement plays in controlling calcium release from the s.r. and contraction. It was originally noted that even when nifedipine (up to  $50 \mu\text{M}$ ) had suppressed  $q_{ns}$  entirely, contractions could still be elicited in many fibres, with the threshold unchanged (Lamb, 1986*b*). (It was equivocal whether nifedipine had any effect on contraction in the other fibres, because with the cut-fibre preparation used, many fibres ceased to give contractions even in the absence of the drug.) Consistent with this, a recent study using a calcium-sensitive dye in the myoplasm of frog muscle, has shown that, at a holding potential of  $-100 \text{ mV}$ ,  $0.5 \mu\text{M}$ -nifedipine suppressed 29% of the charge movement without significant effect on calcium release from the s.r. (Rios & Brum, 1987). (The small non-significant reduction observed in *calculated* calcium release (6%), may be the result of nifedipine blocking calcium inflow from the T-system or of 'run-down' of release.) The amount of charge suppressed is very similar to that in mammalian muscle at such concentrations of nifedipine (see above). Rios & Brum (1987) did not examine the effects of higher concentrations of the drug. Nevertheless, McCleskey (1985) found that nifedipine, even at  $150 \mu\text{M}$ , did not block contractions in frog muscle, but in fact lowered the contraction threshold. Gallant & Goettl (1985) showed that  $25 \mu\text{M}$ -nifedipine had no effect on tetanic tension in mammalian muscle, and actually increased twitch tension. Finally, Ildefonse, Jaquemond, Rougier, Renaud, Fosset & Lazdunski (1985) showed that the tension produced during long (2 s) depolarizations appears to consist of two components, the slower of which probably results from calcium inflow through the T-system calcium channels, and which can be blocked by nifedipine. From the results displayed by Ildefonse *et al.* (1985), it is not possible to determine whether nifedipine had any effect on the faster component, which is presumably dependent on the calcium release from the s.r. Considering all these studies together, it seems reasonable to conclude that nifedipine, even at very high concentrations, does not reduce calcium release from the s.r. in normally polarized fibres, under these conditions. Nevertheless, under apparently the same conditions, nifedipine blocks calcium currents in the T-system and suppresses the  $q_{ns}$  component of the charge

movement. Thus, if  $q_{ns}$  is involved in controlling calcium release from the s.r. either (a) there is a highly non-linear relationship between the amount of charge moved and calcium release, as a large proportion of the charge can be suppressed without significant reduction of calcium release, or (b) nifedipine causes at least some of the charge to move permanently into an 'activated' or 'partially activated' state. Alternatively  $q_{ns}$  may not be involved in calcium release from the s.r.

Interestingly, when a muscle fibre is partially depolarized (holding potential  $-70$  mV) for a prolonged period, even low concentrations of nifedipine appear to reduce calcium release from the s.r. and suppress substantially more charge movement than is suppressed at a holding potential of  $-100$  mV (Rios & Brum, 1987). These authors suggest that most of the charge movement signal is involved with calcium release from the s.r. (The small component that they suggest may not be involved is probably part of  $q_a$ , a component which is unaffected by both prolonged depolarization and nifedipine (Lamb, 1987).) However, Rios & Brum (1987) did not examine the effect of nifedipine on the T-system calcium channel, and as they report that some charge, with the characteristics of  $q_{ns}$ , is suppressed by nifedipine at a holding potential of  $-100$  mV without significant reduction in calcium release from the s.r., their results could also be interpreted as indicating that only *part* of the charge movement,  $q_{ni}$  (as defined in Lamb, 1987), is involved in calcium release.

In conclusion, dihydropyridines (a) bind to T-system, (b) block T-system calcium channels, (c) reduce calcium release from the s.r. and (d) suppress particular components of the charge movement, *all* in a potential-dependent manner. It is possible that there are two different, closely related molecules in the T-system, one a calcium channel, with its associated DHP binding site and charge movement, and the other, a 'voltage sensor' which controls calcium release from the s.r., and which must also have an associated DHP binding site and charge movement. Alternatively, it is interesting to speculate that the calcium channel molecules in the T-system, each with a DHP binding site, may actually be the voltage sensors controlling calcium release from the s.r., and that depolarization induces them to undergo a number of transformations, one generating  $q_{ni}$  and releasing calcium from the s.r., and another generating  $q_{ns}$ , which permits a third relatively infrequent transformation, in which the channel actually opens.

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