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EFFECTS OF REPETITIVE ACTIVITY ON DEVELOPED FORCE AND INTRACELLULAR SODIUM IN ISOLATED SHEEP AND DOG PURKINJE FIBRES

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SUMMARY

1. When cardiac muscle is stimulated after a rest there is a gradual increase in force development over several minutes. The origin of this 'force staircase' was investigated in experiments on sheep and dog Purkinje fibres. Particular attention was paid to the possible role of changes in the intracellular Na^+ activity (a^i_{Na}).

2. The first line of evidence for a role for a^i_{Na} came from a comparison of sheep and dog Purkinje fibres generating action potentials: after a change in the stimulus rate the slow changes of both a^i_{Na} and force were monophasic in sheep but biphasic in dog preparations.

3. In the remaining experiments changes in a^i_{Na} and force in sheep preparations were measured during 4 min trains of voltage-clamp pulses at a frequency of 2·5 Hz.

4. A number of these voltage-clamp experiments also indicated that changes in a^i_{Na} are involved. Depending on the preparation and the duration of the pulses a^i_{Na} rose or fell during a train – a rise in a^i_{Na} was always associated with a gradual rise in force, whereas a fall in a^i_{Na} was usually accompanied by a gradual fall in force. The addition of tetrodotoxin (TTX) or the use of a low holding potential reduced the progressive rises of both a^i_{Na} and force, whereas the inclusion of a 10 mV hyperpolarization between pulses potentiated the progressive rises of both.

5. The effect of TTX on the staircase was more marked the longer the pulses during the train; this possibly indicates that the effect of a^i_{Na} on the force staircase is complex and is more marked with longer pulses.

6. A rise in a^i_{Na} was shown not to be the only factor underlying the progressive increase in force, because in many preparations a gradual rise in force occurred in spite of no change or even a fall of a^i_{Na}.

7. It is concluded that an increase in a^i_{Na} is involved in the slow increase in force during the staircase accompanying a train of action potentials, and that other factors are also involved; various possibilities are discussed.

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INTRODUCTION

It has been known since the time of Bowditch (1871) that the force of contraction of the heart depends on the rate and rhythm of stimulation (for examples see Blinks & Koch-Weser, 1961; Koch-Weser & Blinks, 1963). It is now known that these changes of force are the result of rate-dependent changes of the intracellular Ca^{2+} concentration (or to be more precise the intracellular Ca^{2+} transient that underlies the twitch; Allen & Blinks, 1978; Allen & Kurihara, 1980; Orchard & Lakatta, 1985), but the cause of the rate-dependent changes of the intracellular Ca^{2+} concentration is not understood. Various explanations have been put forward. In 1968 Langer suggested that the changes of the intracellular Ca^{2+} concentration and force are the result of rate-dependent changes of the intracellular Na^+ activity (a^i_{Na}), evidence of which he and others had obtained from isotope flux studies (for review see Langer, 1968). It is now well established that changes of a^i_{Na} exert an important influence on the intracellular Ca^{2+} concentration and force via Na^+–Ca^{2+} exchange (for example see Eisner, Lederer & Vaughan-Jones, 1981*a*). Langer's hypothesis has become more widely accepted with the advent of Na^+-sensitive micro-electrodes and direct measurements of rate-dependent changes of a^i_{Na} (Cohen, Fozzard & Sheu, 1982; Lederer & Sheu, 1983. See also Daut, 1982; Kaila & Vaughan-Jones, 1986).

The purpose of the present study was to test the hypothesis that the rate-dependent changes of force are solely the consequence of rate-dependent changes of a^i_{Na}. Our principal approach has been to measure simultaneously the changes of force and a^i_{Na} during a train of voltage-clamp pulses in sheep cardiac Purkinje fibres. We have manipulated the changes of a^i_{Na} during the train and observed the effect on the force staircase. Our previous study of the rate-dependent changes of a^i_{Na} (Boyett, Hart & Levi, 1987) presented a number of ways of manipulating the changes of a^i_{Na} during a pulse train.

Aspects of this work have been presented at meetings of the Physiological Society (Boyett, Hart & Levi, 1985; Boyett & Hart, 1986).

METHODS

The methods employed in this study have been fully described elsewhere (Boyett, Hart & Levi, 1986; Boyett *et al.* 1987). To summarize, Purkinje fibres were dissected from sheep hearts obtained from a local abattoir or from dog hearts obtained at the end of experiments carried out by colleagues. Fibres with a single discernible core were shortened (to 2 mm or less for voltage-clamp experiments) and then mounted in the bath. The normal physiological solution used to bathe the preparations contained the following (mM): Na^+, 130; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 1; Cl^-, 129; acetate, 10; SO_4^{2-}, 1; glucose, 10; HEPES, 10. This solution was titrated to a pH of 7·4 by adding NaOH (4 mmol NaOH/l solution added) and was maintained at a temperature of 37 °C. One end of a preparation was pinned to the chamber floor and the other end was attached to a force transducer based on an Akers strain gauge element. Active force (the force developed during a twitch) was obtained by electronically subtracting the resting force from the total force (Boyett *et al.* 1986; cf. Eisner, Lederer & Vaughan-Jones, 1984). Because the force developed by Purkinje fibres is small, base-line drift of the force transducer signal can sometimes be a problem if small changes of force over a long period of time are being studied, and the use of active force circumvents this problem. In all experiments both total and active force were recorded, but only the records of active force are shown here (with one exception). Small changes in resting force were sometimes observed (e.g. Fig. 3*B*) but they were not studied. a^i_{Na} was measured with LIX-type (liquid ion exchanger)

Na^{+}-sensitive micro-electrodes. For reasons discussed elsewhere (Boyett *et al.* 1987), at the start and end of a voltage-clamp train there was a transient deflection of the a^{i}_{Na} signal, and during the train the a^{i}_{Na} signal could be shifted up or down. Both of these features are artifacts. In some Figures a dotted line (drawn by eye) is used to indicate a possibly more realistic level of a^{i}_{Na} during the train. In voltage-clamp experiments the membrane potential was controlled with the two-micro-electrode technique. In the Figures that show membrane current, during the trains of pulses, only the 'diastolic current' towards the end of the interpulse interval is shown. A total of thirty-four preparations were used in this study. A preparation was considered acceptable for study if, when initially placed in the chamber, it contracted in response to stimulation, and when it had been connected to the force transducer and had been successfully impaled with the full complement of micro-electrodes it had a resting potential of about −60 mV or more and it showed no signs of deterioration (e.g. transient inward currents and after-contractions).

In some experiments the bathing solution contained $2{\cdot}5 \times 10^{-5}$ M-TTX (obtained from Sigma). The TTX from Sigma contains five parts by weight citrate per one part TTX, and Deitmer & Ellis (1980) and Bhattacharyaa & Vassalle (1981) have indicated that a sizeable amount of Ca^{2+} is chelated by the citrate. For example, Deitmer & Ellis (1980) estimated that the bathing Ca^{2+} concentration is reduced by 0·3 mM (from 2 mM) in the presence of $6{\cdot}3 \times 10^{-5}$ M-TTX. Because such a problem would affect the interpretation of the present experiments this possibility was further tested. First, the fall in the Ca^{2+} concentration was calculated as described by Denton, Richards & Chin (1978) (these calculations made use of stability constants obtained from Martell & Smith, 1974), and secondly, the fall was measured directly with a Phillips Ca^{2+}-selective electrode: at a pH of 7·4 the fall was estimated to be 0·012 mM and was measured to be 0·055 mM (from 2 mM). In either case the fall in the Ca^{2+} concentration is small and it will be ignored.

RESULTS

The force staircase

Fig. 1*A* illustrates a typical force staircase when a sheep Purkinje fibre was stimulated to produce action potentials at a frequency of 2 Hz after a 20 min rest. The accompanying changes of the maximum diastolic potential, action potential duration and a^{i}_{Na} are also shown. The first beat after the rest was large but this was followed by a dramatic decrease in force in the next beat.

The reason for the abrupt reduction of force in the second beat will be considered briefly. A 'mechanical restitution curve' (Kruta & Braveny, 1961) was obtained under the conditions of the present experiments and is shown in Fig. 1*B*. In this experiment on a sheep Purkinje fibre control 200 ms voltage-clamp pulses were applied at a low frequency (one per 30 s) and test pulses were interpolated at shorter intervals. The effect of a test pulse was allowed to subside by allowing several control cycles before the next test pulse was applied. In Fig. 1*B* the force of contraction produced by the test pulse has been plotted as a function of the test interval. The curve represents the recovery or 'restitution' of mechanical activity after a previous contraction and it can be seen that in sheep Purkinje fibres this recovery is a slow process taking up to 30 s to approach completion. Similar results were obtained in one other sheep preparation. It is clear from Fig. 1*B* that a contraction after an interval of only 400 ms (point marked by arrow) will be greatly reduced because of incomplete mechanical restitution and this explains the decrease in force of the second beat during a train of action potentials or voltage-clamp pulses (see below) at 2 or 2·5 Hz (2·5 Hz corresponds to an interpulse interval of 400 ms). This experiment also demonstrates that at the high stimulus frequencies used in this study the preparations developed a small fraction of the force that they were capable of generating at low frequencies when mechanical restitution is complete.

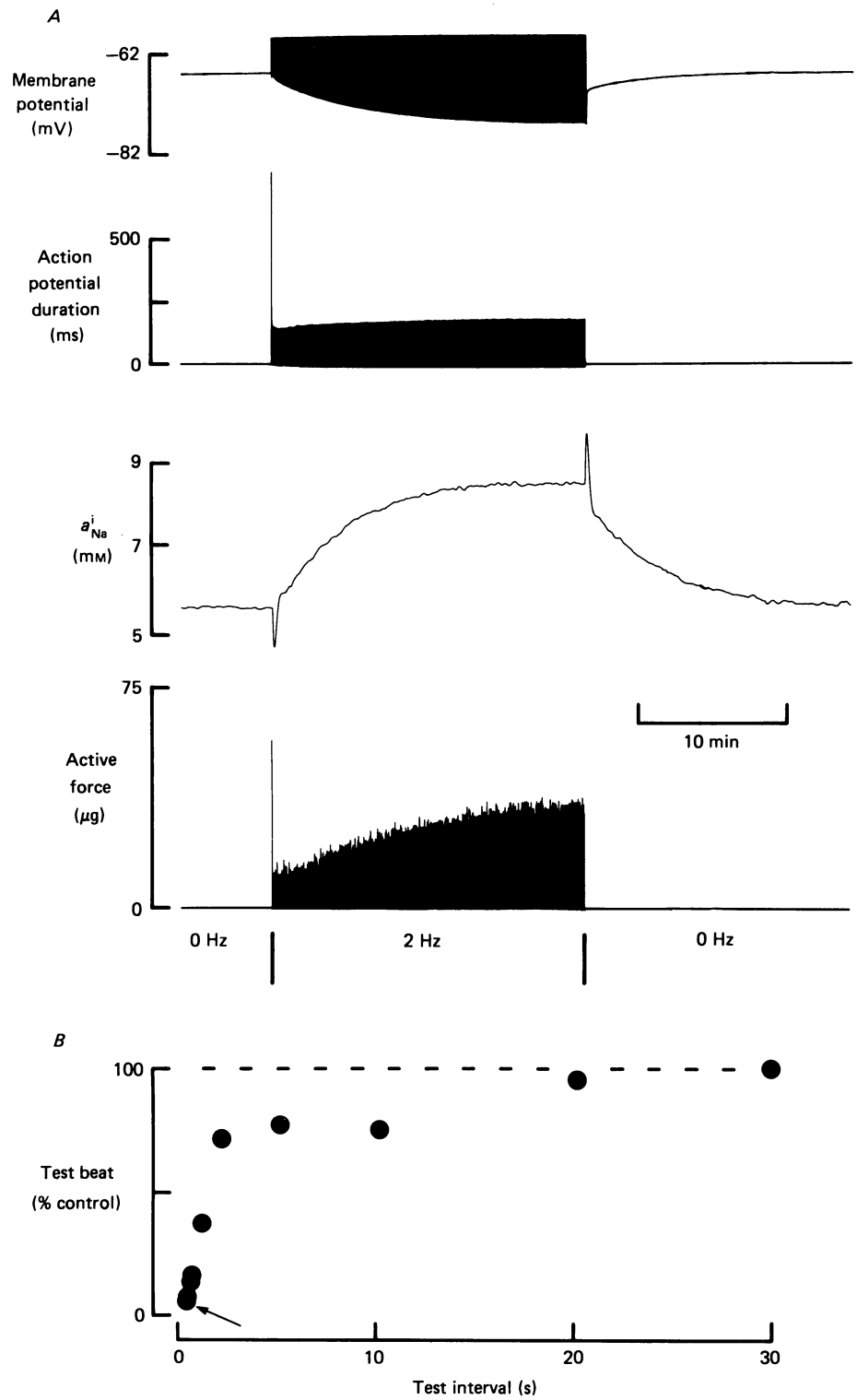

Fig. 1. For legend see opposite.

In Fig. 1*A* the initial fall in force was followed by a progressive increase in the force of contraction over about 20 min before force reached a new stable value. This increase in force was accompanied, over a similar time course, by an increase in a^i_{Na}. The remainder of the experiments to be described were designed to investigate whether the increase in force during the staircase is the result of this increase in a^i_{Na}.

Note that the increase in force was also accompanied, over a similar time course, by an increase in the maximum diastolic potential and a small prolongation of the action potential (Fedida & Boyett, 1985); the maximum diastolic potential increased by about 10 mV during the period of stimulation.

Experiments demonstrating a link between a^i_{Na} and the force staircase

A comparison of rate-dependent changes of force in sheep and dog Purkinje fibres. In sheep Purkinje fibres the slow changes of the maximum diastolic potential and a^i_{Na} after an alteration of the stimulus rate are monophasic, but we have observed that in dog Purkinje fibres this is not the case (Fig. 2*A*): the changes of a^i_{Na} in particular are biphasic. This novel behaviour has not been reported before. In the example shown in Fig. 2*A* the biphasic change is such that at steady state at the higher rate a^i_{Na} is actually lower than at the lower rate of stimulation. (It is intriguing that the maximum diastolic potential remains elevated at the higher stimulus rate, because it has been suggested that the increase in the maximum diastolic potential is the result of a stimulation of the Na^+–K^+ pump caused by an increase in a^i_{Na}!) The view that a^i_{Na} is involved in the rate-dependent changes of force is supported by a comparison of the changes of a^i_{Na} and force in sheep and dog Purkinje fibres (Fig. 2). Fig. 2*B* and *C* allows a comparison to be made of the changes in a^i_{Na}, force and the maximum diastolic potential after a change in rate from 1 Hz to a higher rate and then back to 1 Hz. On switching rate there were abrupt changes in force but attention should be focused on the slow changes of force. In the sheep Purkinje preparation (Fig. 2*B*) there were slow changes of a^i_{Na} and force lasting about 10 min after the changes in rate, whereas in the dog preparation (Fig. 2*C*) the pattern was different: after the increase in rate there was a relatively rapid rise in a^i_{Na} over about 3 min after which a^i_{Na} was steady (in contrast to the result in Fig. 2*A*); slow changes of force are not discernible. In the dog preparation (Fig. 2*C*) the changes after the decrease in rate are more interesting: there were relatively rapid falls of a^i_{Na} and force over about 3 min to values that were below the previous control values at 1 Hz. After a^i_{Na} and force reached minimal values both variables then slowly returned to their control values over about a further 10 min. The slow changes in both a^i_{Na} and force were therefore biphasic. Although not in this example, the slow changes in force can also

Fig. 1. *A*, the force staircase in a sheep Purkinje fibre stimulated to produce action potentials at a rate of 2 Hz. Changes in the maximum diastolic potential, action potential duration, a^i_{Na}, as well as active force are shown. The upper trace shows only the lower part of the action potentials in order to highlight the changes of the maximum diastolic potential. *B*, a mechanical restitution curve for a sheep Purkinje fibre. Test voltage-clamp pulses were applied at different test intervals after control pulses (frequency, 0·03 Hz). The point marked by the arrow was obtained after an interval of 400 ms. The active force of the test beat has been expressed as a percentage of the active force of the preceding control beat. Holding potential, −76 mV; pulse potential, +8 mV; pulse duration, 200 ms.

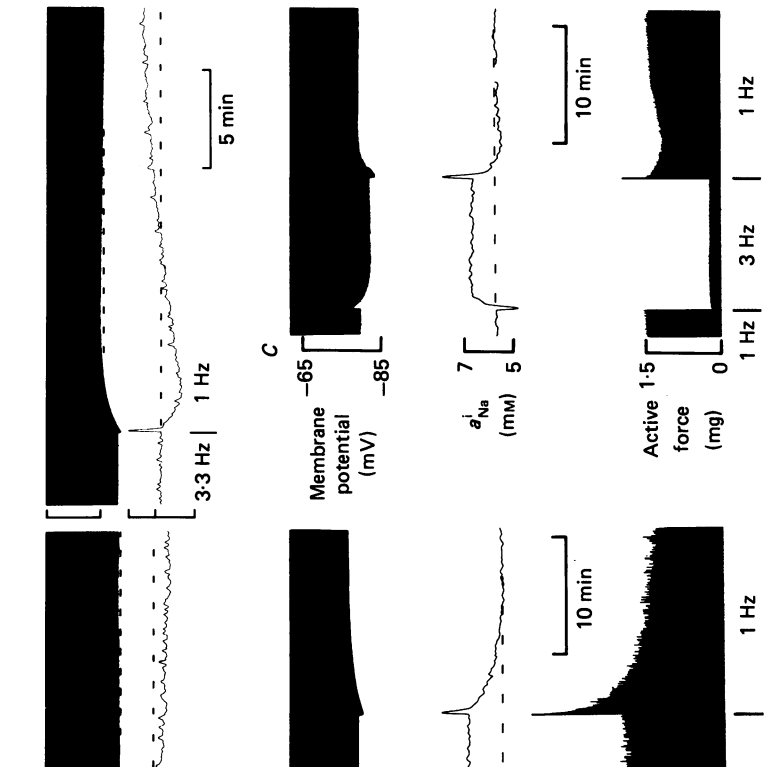

Fig. 2. Comparison of rate-dependent changes in a^i_{Na} and active force in sheep and dog Purkinje fibres generating action potentials. The stimulus rate is noted below the traces. In *A* (from a dog preparation) changes of the maximum diastolic potential and a^i_{Na} are shown; in *B* (from a sheep preparation) and *C* (from a dog preparation) the changes of the maximum diastolic potential, a^i_{Na} and active force are shown, whereas in *D* (from a dog preparation) only the changes of active force are illustrated. In *A* the dashed lines represent the level of a^i_{Na} at the initial frequency, whereas in *B* and *C* the dashed lines represent the level of a^i_{Na} at the lower frequency. Note that the two runs in *A* were separated by a substantial time. The results in *A*, *C* and *D* were obtained from three different dog preparations.

be biphasic after an increase in rate in dog Purkinje fibres. Fig. 2*D* shows rate-dependent changes in the force of contraction of another dog Purkinje fibre. The slow biphasic changes in force after the decrease in rate are very similar to those shown in Fig. 2*C*, but note that in this preparation the slow changes in force after the increase in rate were also biphasic. a^i_{Na} was not recorded in this preparation. Monophasic slow changes in a^i_{Na} and force have been observed in a total of six sheep preparations (in a further ten preparations, in which only a^i_{Na} was recorded, monophasic changes in a^i_{Na} were also seen), whereas biphasic slow changes in a^i_{Na} and force have been seen in a total of seven dog preparations. The fact that force behaves in a similar manner to a^i_{Na} despite the species difference in the rate-dependent changes of a^i_{Na} suggests that a^i_{Na} is involved in the staircase.

Positive and negative force staircases. All remaining experiments described in this paper were carried out on voltage-clamped sheep preparations. Fig. 3*A* illustrates the voltage-clamp protocol used. Initially the membrane potential was clamped a few millivolts negative to the original resting potential of the cell. When a^i_{Na} was steady a 4 min train of voltage-clamp pulses at a frequency of 2·5 Hz was applied. In the experiment of Fig. 3*A* the voltage-clamp pulses were 200 ms in duration, but the pulse duration was varied in different runs. It is important to note that because the pulse frequency was always 2·5 Hz, a change in the pulse duration affected the period between pulses. The changes of membrane current, a^i_{Na} and force were measured. After one train, a second train was not applied until a^i_{Na} had recovered and reached a steady state.

In all experiments and under all conditions force was large in the first beat of the train, but over the next few beats there was a marked reduction in force. Note that in this and many of the other Figures the force record in the first beat is off-scale. In Fig. 3*A* the initial decrease in force in the first few beats was followed by a progressive increase in force over the remainder of the 4 min period and this was accompanied by a progressive increase in a^i_{Na} during the train. This staircase is very similar to that accompanying a train of action potentials (Fig. 1*A*).

We have previously shown that the change of a^i_{Na} during a train of voltage-clamp pulses is variable and a^i_{Na} can rise or fall according to the experimental conditions and the preparation (Boyett *et al.* 1987). One of our earliest observations was that if a^i_{Na} rose during the train there was invariably a slow rise in force (which we describe as a 'positive staircase') whereas if a^i_{Na} fell there was frequently a progressive decline of force throughout much of the train (a 'negative staircase'). An example of a negative force staircase is shown in Fig. 3*B*. The results in Fig. 3*A* and *B* were obtained from different preparations but in both cases the pulses were 200 ms in duration. Similar positive and negative staircases accompanied by rises and falls of a^i_{Na}, respectively, have been seen in a total of thirteen preparations. This is further evidence that a^i_{Na} may be involved in the slow changes of force during the staircase. (Note, however, that there was one exception: in one preparation a negative staircase was not accompanied by a detectable fall of a^i_{Na}).

Notice that after the voltage-clamp trains membrane current and a^i_{Na} changed in a similar manner, but in opposite directions in Fig. 3*A* and *B*.

The effects of TTX and a low holding potential. The abolition of the Na^+ current by the application of tetrodotoxin (TTX) or the use of a low holding potential greatly

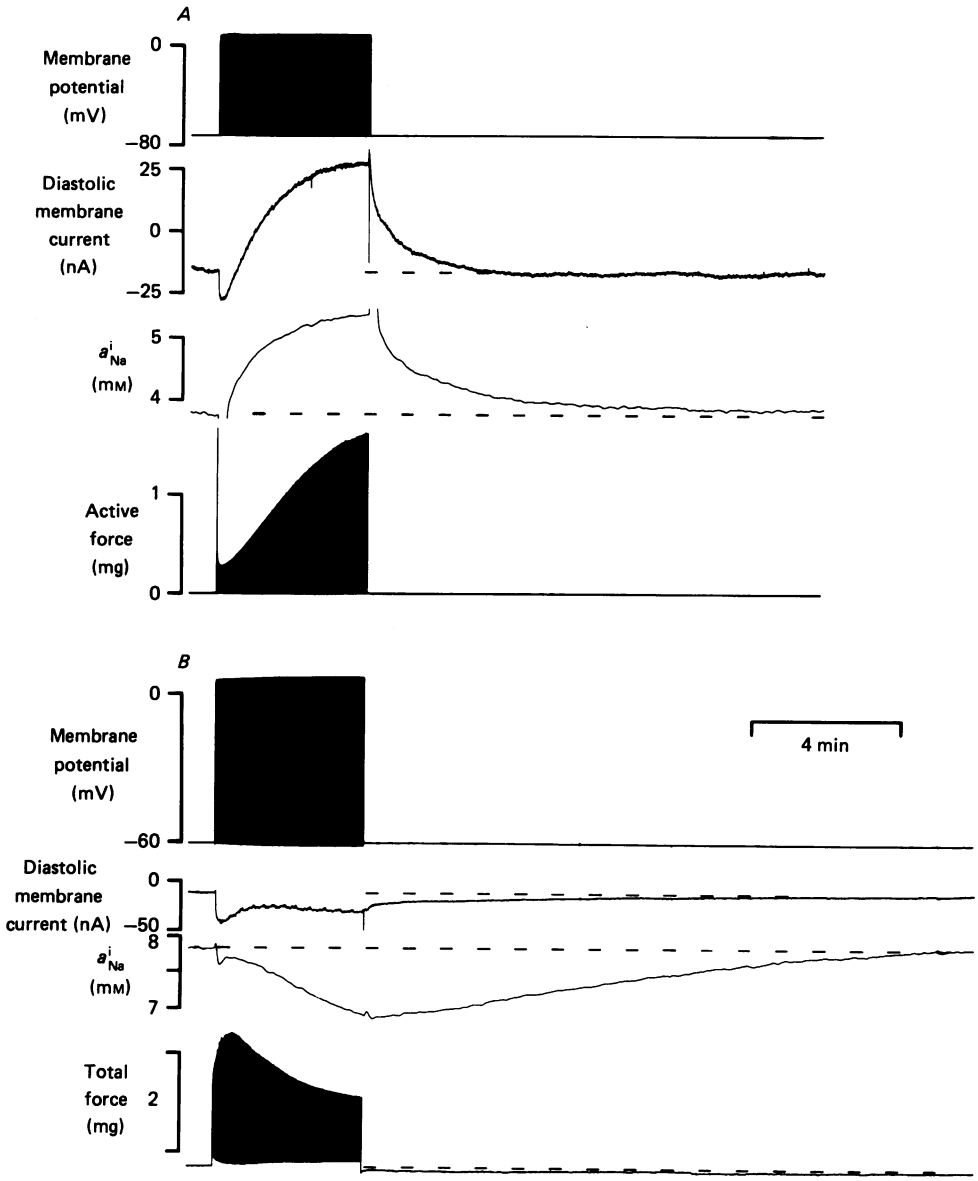

Fig. 3. Positive (*A*) and negative (*B*) force staircases. Voltage-clamp experiments on sheep Purkinje fibres. In both *A* and *B* changes of membrane potential, diastolic membrane current, a^i_{Na} and force are shown. The horizontal dashed lines indicate the resting level of a variable. *A*: holding potential, −73 mV: pulse potential, +7 mV; pulse duration, 200 ms; 2 mM-Cs^+ present. *B*: holding potential, −60 mV; pulse potential, +6 mV; pulse duration, 200 ms. In this and all subsequent Figures the trains of voltage-clamp pulses lasted 4 min and the pulse frequency was 2·5 Hz. The results in *A* and *B* were obtained from two different preparations.

reduces or abolishes a rise of a^i_{Na} during a train of voltage-clamp pulses (Boyett *et al.* 1987). If a^i_{Na} is involved in the staircase then either of these interventions should greatly reduce the slow rise of force during the staircase. In four preparations in the presence of TTX ($2{\cdot}5 \times 10^{-5}$ M) and in five preparations at a holding potential of about -50 mV this result was indeed observed. A result with TTX is illustrated in Fig. 4, which shows the changes of membrane current, a^i_{Na} and active force during and after trains of 100 ms pulses. In the presence of TTX the rise of a^i_{Na} was abolished, the outward tail of current after the train was lost, and the progressive rise of force during the staircase was greatly reduced. Note that with both TTX and a low holding potential there was a reduction in the resting a^i_{Na} (Fig. 4; see also Boyett *et al.* 1987) and this itself would be expected to produce a decrease in force. However, this might be expected to result in a rough scaling down of the staircase rather than a large reduction in the slow change of force. This argument is supported by the following observation. Another agent that results in a reduction of the resting level of a^i_{Na} is Cs^+; in the same preparation from which Fig. 4 was obtained, 2 mM-Cs^+ resulted in a fall of resting a^i_{Na} from 6·8 to 6·2 mM. During a train of 100 ms pulses the absolute level of force at the end of the staircase was indeed less in Cs^+ (120 μg, Cs^+ absent; 85 μg, Cs^+ present), whereas the percentage rise of force during the staircase was actually *greater* with Cs^+ (166%, Cs^+ absent; 202%, Cs^+ present); the latter was possibly the result of the greater rise of a^i_{Na} during the train in the presence of Cs^+ (0·5 mM, Cs^+ absent; 0·8 mM, Cs^+ present).

The actions of TTX and a low holding potential are considered in further detail below.

The effects of a 10 mV hyperpolarization between pulses. We have previously shown that a 10 mV hyperpolarization between pulses can potentiate a rise of a^i_{Na} during a voltage-clamp train (Boyett *et al.* 1987). In this section the effects of this voltage-clamp protocol on the force staircase are examined. This intervention is particularly interesting because as noted above during a train of action potentials there can be a comparable hyperpolarization of the membrane between action potentials (Fig. 1*A*); this protocol is therefore closer to the normal physiological situation than our standard protocol.

The effect of the 10 mV hyperpolarization between short pulses (50 ms long) is shown in Fig. 5. The control run is illustrated in Fig. 5*A*; during this run there was a small progressive rise of force and this was accompanied by a small rise of a^i_{Na}. When the membrane was hyperpolarized by 10 mV between the pulses during the train (Fig. 5*B*) the rise of a^i_{Na} was approximately doubled and the progressive rise of force was also more marked. Once again after the trains the changes of current reflected the changes of a^i_{Na}.

The effect of the 10 mV hyperpolarization between long pulses (300 ms long) is shown in Fig. 6. For simplicity only the changes of a^i_{Na} and active force are illustrated; the protocol is illustrated by the membrane potential traces in the insets. In this experiment the trace of a^i_{Na} was spoilt by transients at the beginning and end of the train and throughout the train the a^i_{Na} signal was shifted up; as before, the dotted line indicates a possibly more realistic level of a^i_{Na} at this time. The reader may wish to ignore the changes of a^i_{Na} during the train and concentrate on the level of a^i_{Na} immediately after the train – this approximate level is indicated by the arrows.

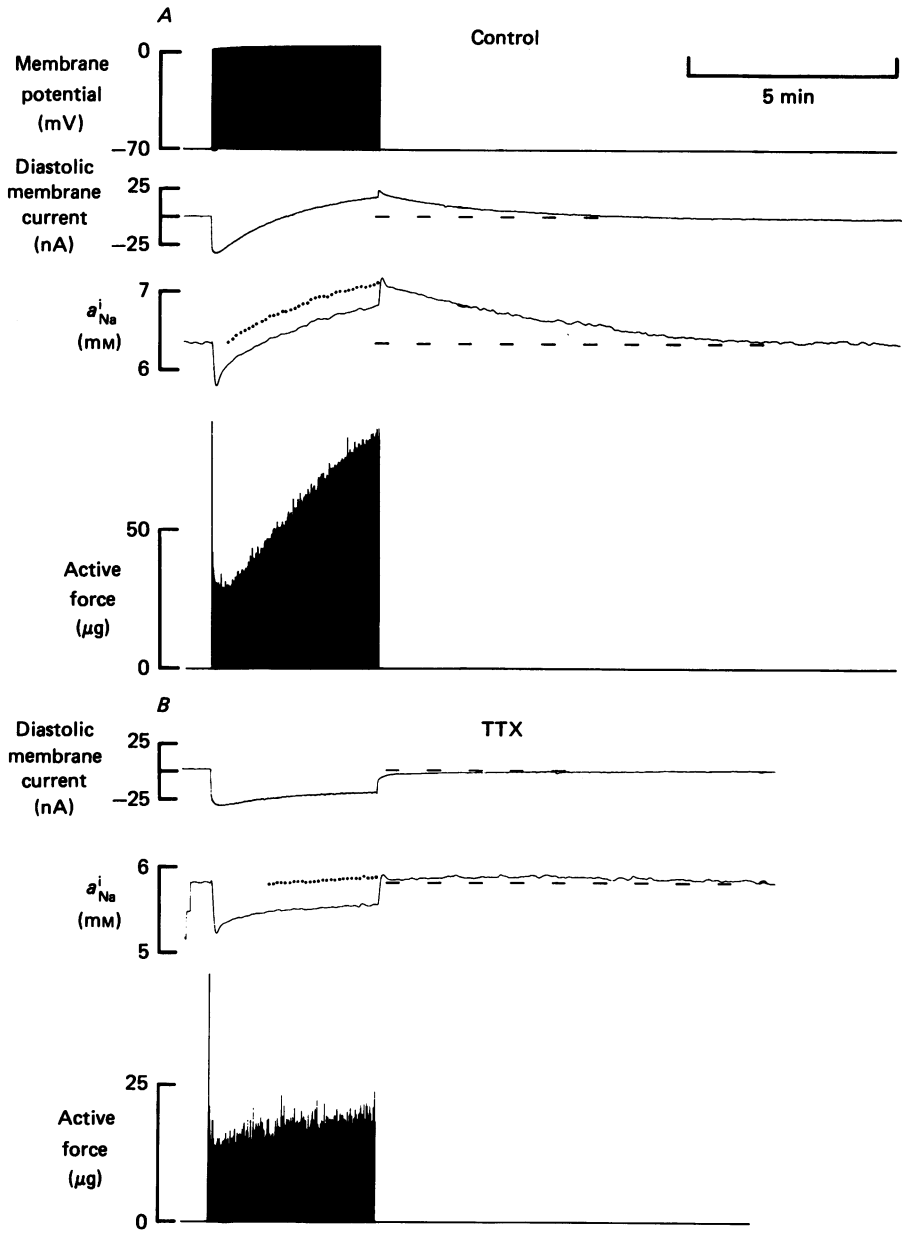

Fig. 4. The effect of TTX on the force staircase. The result in *A* was obtained under 'control' conditions whereas that in *B* was obtained in the presence of $2{\cdot}5 \times 10^{-5}$ M-TTX. The results in both *A* and *B* were obtained in the presence of 2 mM-Cs^+. Voltage-clamp experiment on a sheep Purkinje fibre. In *A* and *B* changes of membrane potential, diastolic membrane current, a^i_{Na} and active force are shown. The horizontal dashed lines indicate the resting level of the variable. *A* and *B*: holding potential, −70 mV; pulse potential, +4 mV; pulse duration, 100 ms. In this and some of the subsequent Figures there was an offset in the a^i_{Na} signal during the train, and in these cases the dotted lines (fitted by eye) possibly indicate a more realistic level of a^i_{Na}.

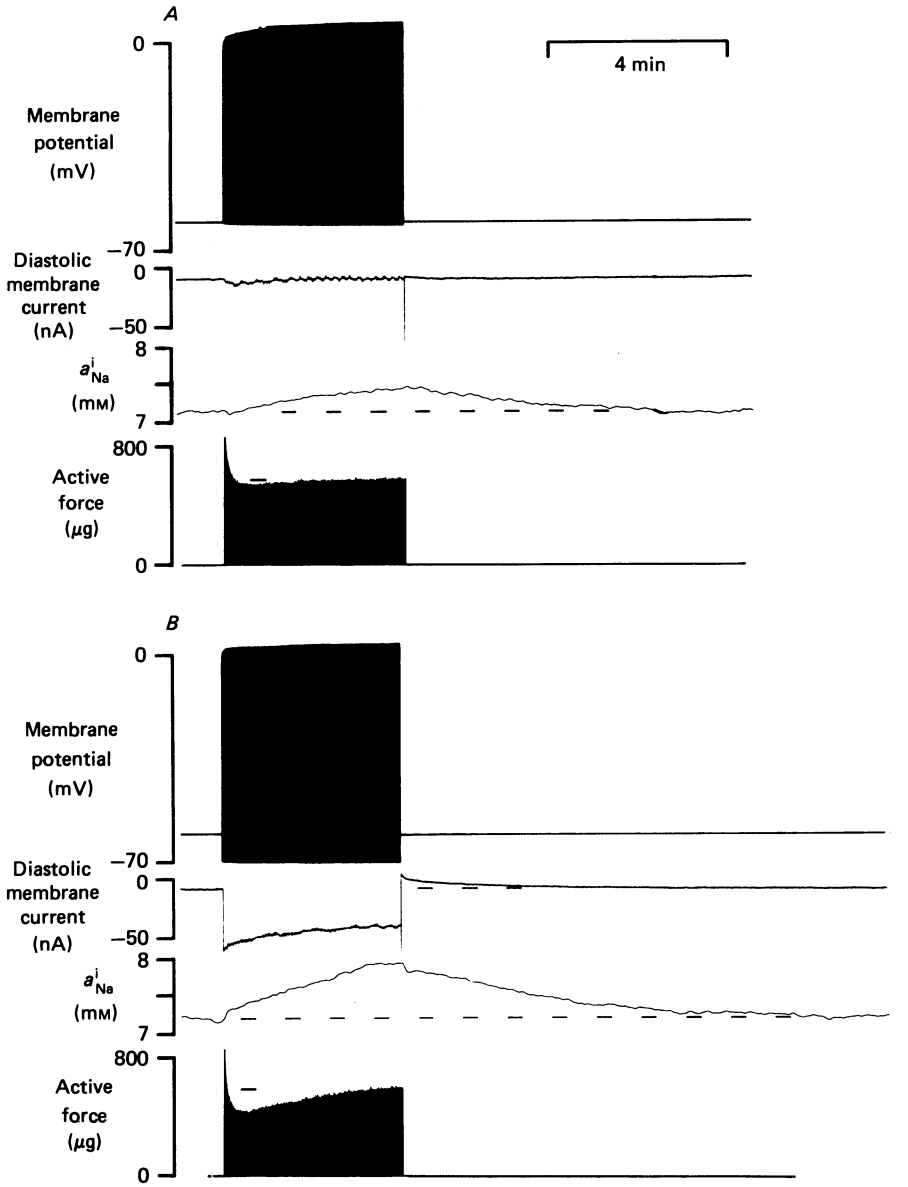

Fig. 5. The effect on the force staircase with a train of 50 ms pulses of a 10 mV hyperpolarization between the pulses. The result in *A* was obtained with the normal protocol whereas that in *B* was obtained when the membrane was hyperpolarized between pulses. Voltage-clamp experiment on a sheep Purkinje fibre. In both *A* and *B* changes of membrane potential, diastolic membrane current, a^{i}_{Na} and active force are shown. The horizontal dashed lines indicate the resting level of the variable. *A*: holding potential (both at rest and during the train), −60 mV; pulse potential, +4 mV. *B*: holding potential at rest, −60 mV; holding potential during the train, −70 mV; pulse potential, +4 mV. The short bars on the traces of active force represent the force at the end of the two trains.

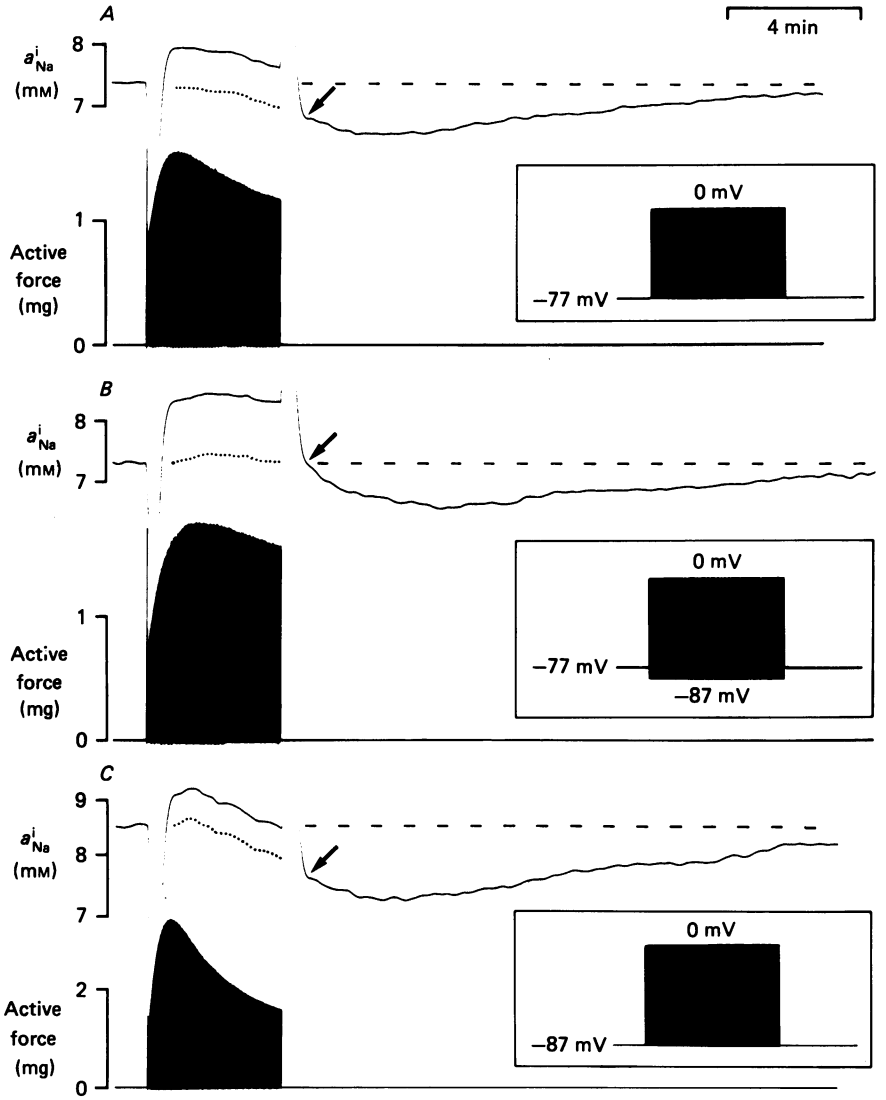

Fig. 6. The effect on the force staircase with a train of 300 ms pulses of a 10 mV hyperpolarization between the pulses. *A*, normal protocol: resting potential equal to −77 mV both prior to and during the train. *B*, membrane hyperpolarized by 10 mV between pulses: resting potential equal to −77 mV prior to and −87 mV during the train. *C*, membrane hyperpolarized by 10 mV prior to the train as well as between the pulses of the train: resting potential equal to −87 mV both prior to and during the train. Voltage-clamp experiment on a sheep Purkinje fibre. In *A*, *B* and *C* a^i_{Na} and active force are shown; membrane potential is shown in the inset. The horizontal dashed lines indicate resting a^i_{Na}. The arrows indicate the approximate level of a^i_{Na} at the end of the trains. Pulse potential, 0 mV.

During the control run (*A*) there was a fall of a^i_{Na} during the train and this was accompanied by a progressive decline in force throughout much of this period. During the run in *B* the membrane was hyperpolarized by 10 mV between pulses. As expected the fall of a^i_{Na} during the train was greatly reduced and note that the progressive decline of force was also less marked. During this run there appeared to be no net change of a^i_{Na} and therefore it may seem surprising that there was still a slight decline in force. However, during the train the change of a^i_{Na} appears biphasic – during the first 2 min there appeared to be a small rise of a^i_{Na} whereas during the second 2 min a^i_{Na} fell (after the train the changes of a^i_{Na} are unmistakably biphasic). Similar results to those shown in Figs. 5 and 6 have been obtained from a total of six preparations. It could be argued that the alteration of the force staircase with the addition of the 10 mV hyperpolarization between pulses is not the result of the changes of a^i_{Na} but is simply the result of the more negative holding potential during the train – it is known that the holding potential *per se* has an influence on force production (e.g. Gibbons & Fozzard, 1975*a*; Trautwein, McDonald & Tripathi, 1975). To counter this argument the experiment in *C* was conducted. After the run in *B* the holding potential was increased from −77 to −87 mV, and the holding potential was maintained at this value both during and after the run shown in *C*. Therefore in this run the holding potential during the train was the same as that during the train in *B* – the only difference is that in *B* the holding potential was −77 mV prior to the train whereas in *C* it was −87 mV prior to the train. Notice that the resting level of a^i_{Na} (the level prior to the train) was greater in *C* than in *A* or *B*. This is expected and it was a consequence of the voltage dependence of resting a^i_{Na} (after the increase of the holding potential at least 15 min was allowed to elapse to enable a^i_{Na} to reach a new stable value; Eisner, Lederer & Vaughan-Jones, 1981*b*). Notice also that the over-all level of force in *C* was greater than that in the two previous runs – this was presumably the consequence of the higher over-all level of a^i_{Na}. Contrary to the result in *B*, the progressive fall of force during the train in *C* was more marked compared to the control in *A*. Clearly the effect on the force staircase in *B* was unlikely to be the result of the increase in the holding potential *per se* during the train; it was more likely to be the result of the smaller fall of a^i_{Na} during the train as compared to the control (*A*). The more marked decline in force in *C* can also be explained in terms of a^i_{Na}: in the run in *C* the fall of a^i_{Na} during the train was more marked as compared to the control (*A*). The more marked decline in a^i_{Na} is to be expected because a fall of a^i_{Na} during a train depends on the extent of the depolarization during the pulses, and this was greater in *C* than in *B* (87 mV as compared to 77 mV). Similar results to those illustrated in Fig. 6*C* were obtained in two other preparations.

The effect of the level of depolarization. The left-hand panel of Fig. 7 shows a train of 300 ms voltage-clamp pulses; the holding potential was −85 mV and the pulse potential was 0 mV. During this train there was a fall of a^i_{Na} and during the later part of the train there was a fall of force. As explained above the extent of a fall of a^i_{Na} depends on the extent of the depolarization during the pulses (see also Boyett *et al.* 1987). Another way to reduce the extent of the depolarization is to reduce the pulse potential and this is illustrated in the right-hand panel of Fig. 7. It can be seen that the decrease in the pulse potential to −20 mV abolished the fall of a^i_{Na} during the train. The over-all level of force during the train was reduced – it is well known

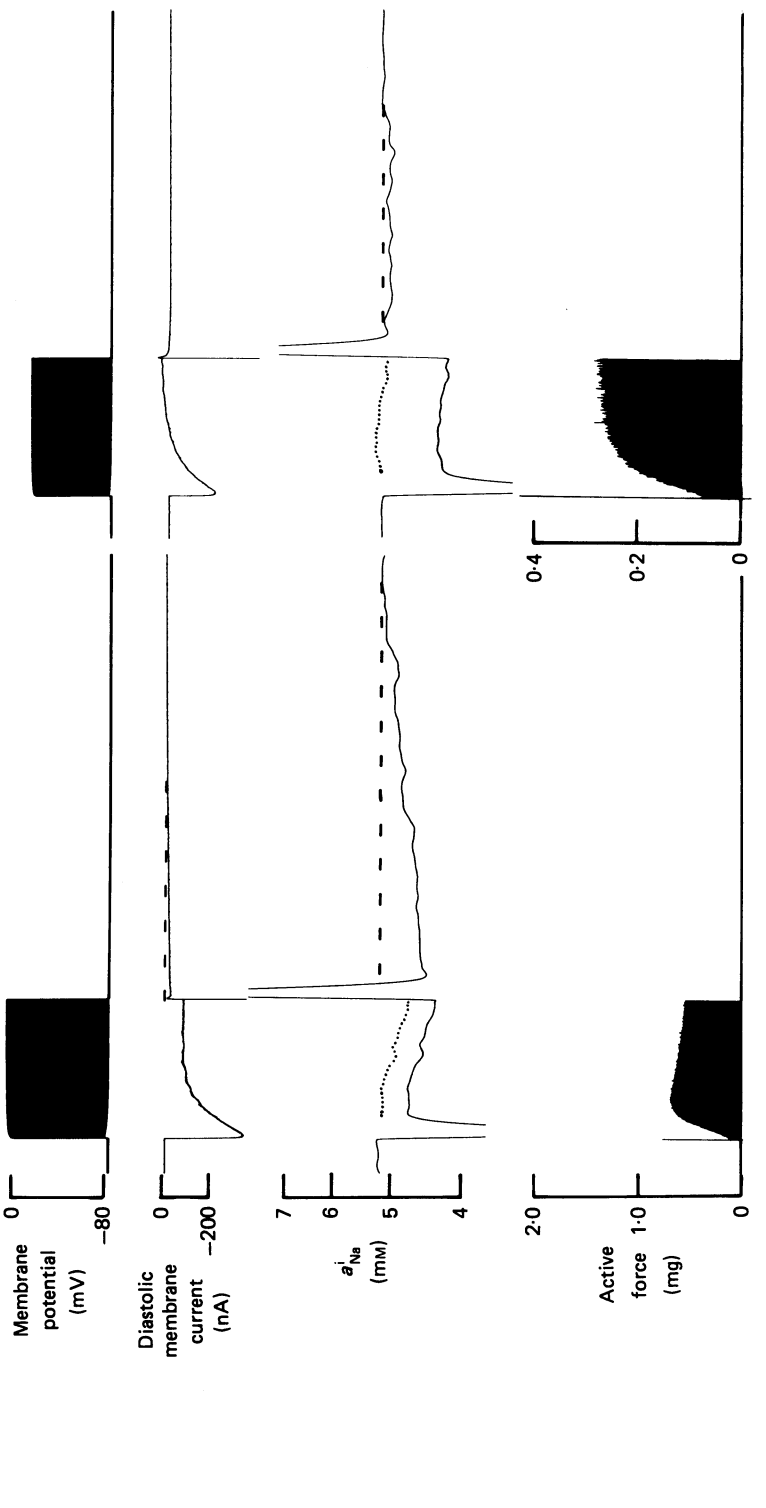

Fig. 7. The effect of the pulse potential on the force staircase. During the pulses the membrane potential was clamped to 0 mV in *A* and −20 mV in *B*. Voltage-clamp experiment on a sheep Purkinje fibre. In both *A* and *B* changes of membrane potential, diastolic membrane current, a^i_{Na} and active force are shown. The horizontal dashed lines indicate the resting level of the variable. Holding potential, −85 mV; pulse duration, 300 ms.

that the pulse potential *per se* has an important influence on force production (e.g. Gibbons & Fozzard, 1975*a*). However, more importantly notice that there was no longer a progressive decline of force during the staircase. Results similar to this were seen in a total of four preparations. This is the final line of evidence to be presented to show that a^{i}_{Na} may be an important factor in the force staircase.

As before the current after the trains in Fig. 7 reflects the changes in a^{i}_{Na}.

Evidence that the effect of a^{i}_{Na} on the force staircase is greater at longer pulse durations

The experiments described so far present a deceptively simple picture of the staircase. Experiments to be presented in this section indicate that the relation between a^{i}_{Na} and the staircase is more complicated.

The effect of pulse duration on the force staircase. Fig. 8 shows the changes of a^{i}_{Na} and active force during trains of pulses of different duration (in all other respects the trains were identical, but once again note that because the frequency was 2·5 Hz in each case, a change in the pulse duration also affected the time interval between pulses). During the train of 20 ms pulses there was a substantial rise of a^{i}_{Na} and this was accompanied by a small progressive rise of force. With the train of 50 ms pulses the rise of a^{i}_{Na} was slightly greater and the gradual rise of force was also more marked. With the train of 100 ms pulses the picture is more surprising: the rise of a^{i}_{Na} was less than with the trains of shorter pulses (to be expected: Boyett *et al.* 1987) but the gradual rise of force during the staircase was further enhanced. There appeared to be no net change of a^{i}_{Na} during the train of 200 ms pulses and yet the progressive rise of force was even more marked. During the train of 300 ms pulses there was a net fall of a^{i}_{Na} and during the second part of the staircase there was a slight fall of force. These results are summarized graphically in Fig. 9*A*, *B*, and *C*. The changes of a^{i}_{Na} are plotted against pulse duration in Fig. 9*A*. Two measurements were made to quantify the force staircase: first, the active force at the end of the train (Fig. 9*B*) and, secondly, the slow change of force was quantified by expressing the force at the end of the train as a percentage of the minimum force at the start of the train (Fig. 9*C*). Both measurements can be criticized individually but the combination of both measurements presents a fairly complete picture of the staircase.

Before the implications of these results for the possible role of a^{i}_{Na} in the staircase can be discussed, the effect of pulse duration *per se* on force production (e.g. Fozzard & Hellam, 1968) has to be considered; this is illustrated in Fig. 9*D*. This panel shows mean results from three preparations. During these experiments 200 ms voltage-clamp pulses were applied every 20 or 30 s. At intervals a test pulse with a duration less than or greater than 200 ms was applied. Active force was allowed to return to its control value before another test pulse was applied. In Fig. 9*D* the force during the test pulse (expressed as a percentage of the preceding control) has been plotted as a function of the duration of the test pulse. It can be seen that at pulse durations less than about 100 ms force production is curtailed.

Fig. 9*B* and *C* shows that over a range of pulse durations from 20 to 200 ms the staircase was greater the longer the pulses during a train – this is true whether one considers the force at the end of the train or the progressive increase in force during the train. The increase in force with longer pulses must have been partly the result of pulse duration *per se* (as illustrated by Fig. 9*D*). However, this is unlikely to be

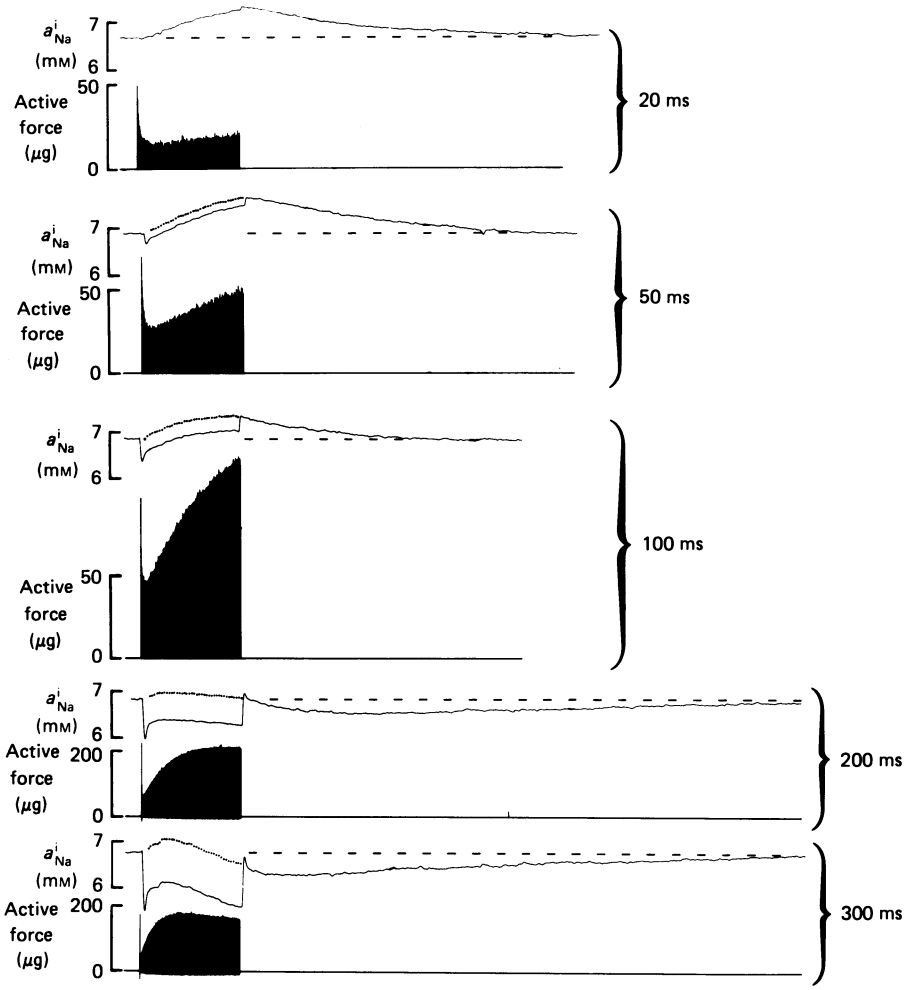

Fig. 8. The effect of pulse duration on the force staircase. The duration of the pulses during each train is shown adjacent to each pair of traces. Voltage-clamp experiment on a sheep Purkinje fibre. Each pair of traces shows a^i_{Na} and active force. The horizontal dashed lines indicate resting a^i_{Na}. Notice that force is shown on a lower gain in the two lower records. Holding potential, −70 mV; pulse potential, 0 mV. There is no time bar on this Figure as the trains lasted 4 min.

the complete story because on this basis one would not expect the force to be greater with a train of 200 ms pulses as compared to 100 ms pulses, and also the reduction in force with a train of 20 ms pulses (as compared to 100 ms pulses) is also much greater than would be expected (cf. Fig. 9*B* and *D*). Finally, it is difficult to explain the effect of pulse duration on the progressive increase in force during the staircase (Fig. 9*C*) in terms of the action of pulse duration illustrated in Fig. 9*D*; the effect illustrated in Fig. 9*D* might be expected to result in a uniform scaling down of the staircase during a train of short pulses rather than a reduction in the gradual rise of force. In conclusion, over the range of pulse durations from 20 to 200 ms, there was an increase in the staircase that could not be attributed to the effect of pulse

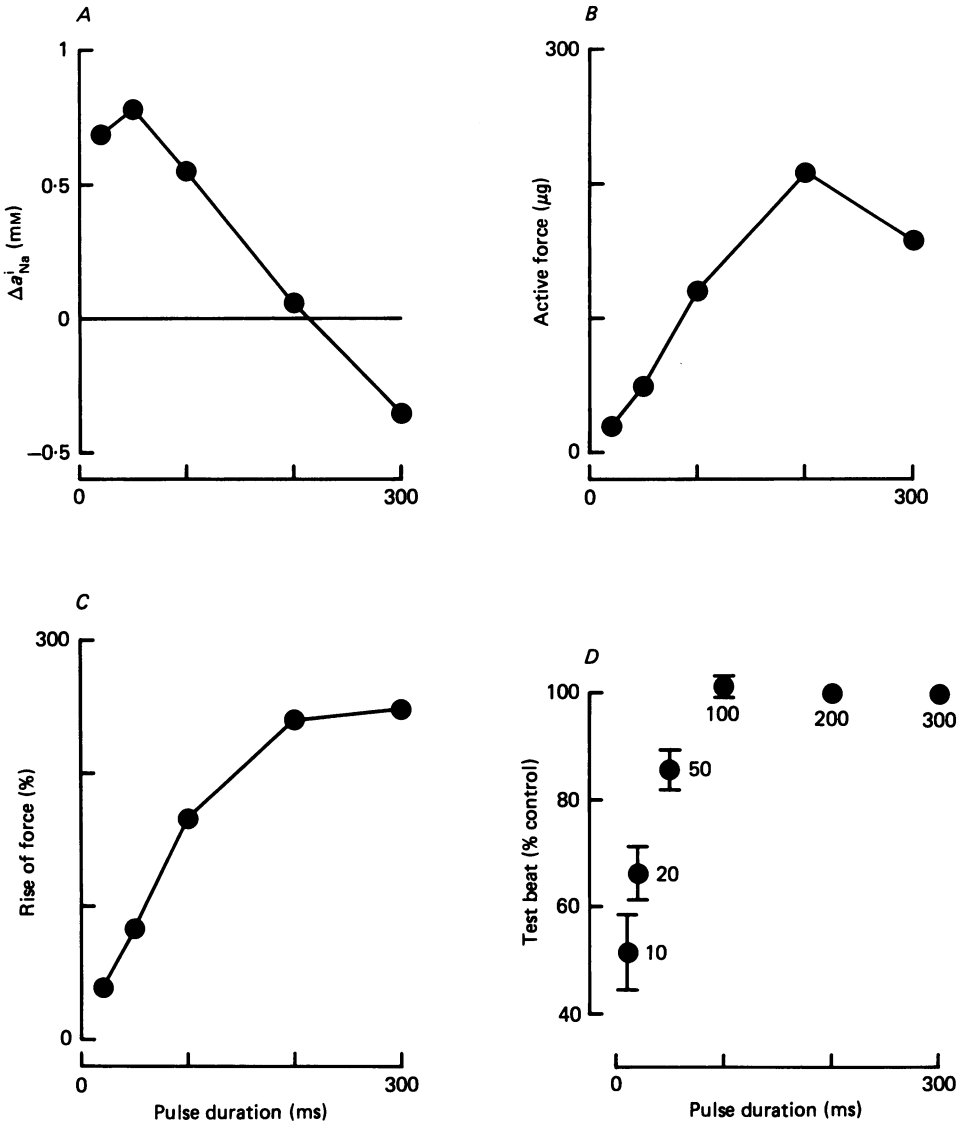

Fig. 9. *A*, *B* and *C*, the effect of pulse duration on the force staircase. The change of a^{i}_{Na} during the train (*A*), the active force at the end of the train (*B*) and the percentage rise of force during the train (*C*; the force at the end of the train has been expressed as a percentage of the minimum force early during the train) are plotted as a function of the pulse duration. The data are taken from the experiment illustrated in Fig. 8. *D*, the effect of pulse duration on the twitch. The active force during single test pulses of different duration (given beside each point in milliseconds) has been expressed as a percentage of the active force in the preceding control pulse (frequency, 0·03 or 0·05 Hz; pulse duration, 200 ms) and plotted as a function of the test pulse duration. The means and S.E. of means for three preparation have been plotted. The holding and pulse potentials for the three preparations (in millivolts) were: −76, +8; −69, +5; −58, +12.

duration *per se*, and this increase occurred despite the fact that the rise of a_{Na}^{i} during the trains was less with longer pulses (with the exception of 20 *versus* 50 ms pulses). This is difficult to reconcile with the view that a rise of a_{Na}^{i} is responsible for the progressive increase in force during the staircase. Similar results to that shown in Fig. 8 were obtained in a total of nine preparations. The use of TTX or a low holding potential, however, sheds some light on this issue.

The effects of TTX and a low holding potential on the force staircase with different pulse durations. The effect of TTX on the dependence of the force staircase on pulse duration is shown in Fig. 10; the staircases before and after TTX are shown in Fig. 10*A* and the data, plotted graphically, are shown in Fig. 10*B*. The control set of data is very similar to that shown in Fig. 8. The effect of TTX on the changes of a_{Na}^{i} was the expected one (Boyett *et al.* 1987): the curve of the change of a_{Na}^{i} *versus* pulse duration was displaced downwards. The effect of TTX on the force staircases with different pulse durations was more surprising: the force staircase was greatly reduced (and this is true whether the force at the end of the staircase is considered or whether the slow changes of force are considered) regardless of the pulse duration. This result was observed in a total of three preparations and it is a surprising result for the following reason. With the data in Fig. 8 or the control data in Fig. 10 it could be argued that the small gradual rise of force with a train of short pulses (for example 50 ms long) is the result of the accompanying substantial rise of a_{Na}^{i}. If this is the case then it follows that the prominent rise of force with longer pulses (for example 200 ms long), which is always accompanied by a smaller rise of a_{Na}^{i}, is the result of another factor. However, this may not be the case because TTX greatly reduced the gradual rise of force with both short and long pulses. It is possible that the effect of a_{Na}^{i} on the force staircase is dependent on pulse duration. This then explains why in Fig. 8, for example, there was a larger progressive rise of force and yet a smaller rise of a_{Na}^{i} with a train of 100 ms pulses than with a train of 20 ms pulses. (Note that an alternative explanation of these findings is that TTX is having another unknown action.)

The findings with TTX were unexpected and it was considered important to verify them by another means: Fig. 11 shows the effect of a low holding potential on the dependence of the force staircase on pulse duration. Once again under control conditions, when the holding potential was -71 mV, similar data to those already shown were obtained. The holding potential was then reduced to -45 mV, time was allowed for a_{Na}^{i} to reach a new steady value, and then the experiment was repeated. As expected the curve of the change of a_{Na}^{i} *versus* pulse duration was displaced downwards with the low holding potential (Boyett *et al.* 1987). As with TTX the force staircase was greatly reduced regardless of the pulse duration at the low holding potential. A similar result was observed in one other preparation. This result with the low holding potential appears to confirm the view that the action of a_{Na}^{i} on the staircase is dependent on the duration of the pulses during the train, and a possible reason for this will be considered in the Discussion.

Evidence that a_{Na}^{i} is not the only factor involved in the slow changes of force during the staircase

In this section of the Results evidence is presented that other factors are also involved in the slow changes of force during the staircase. Evidence of another factor

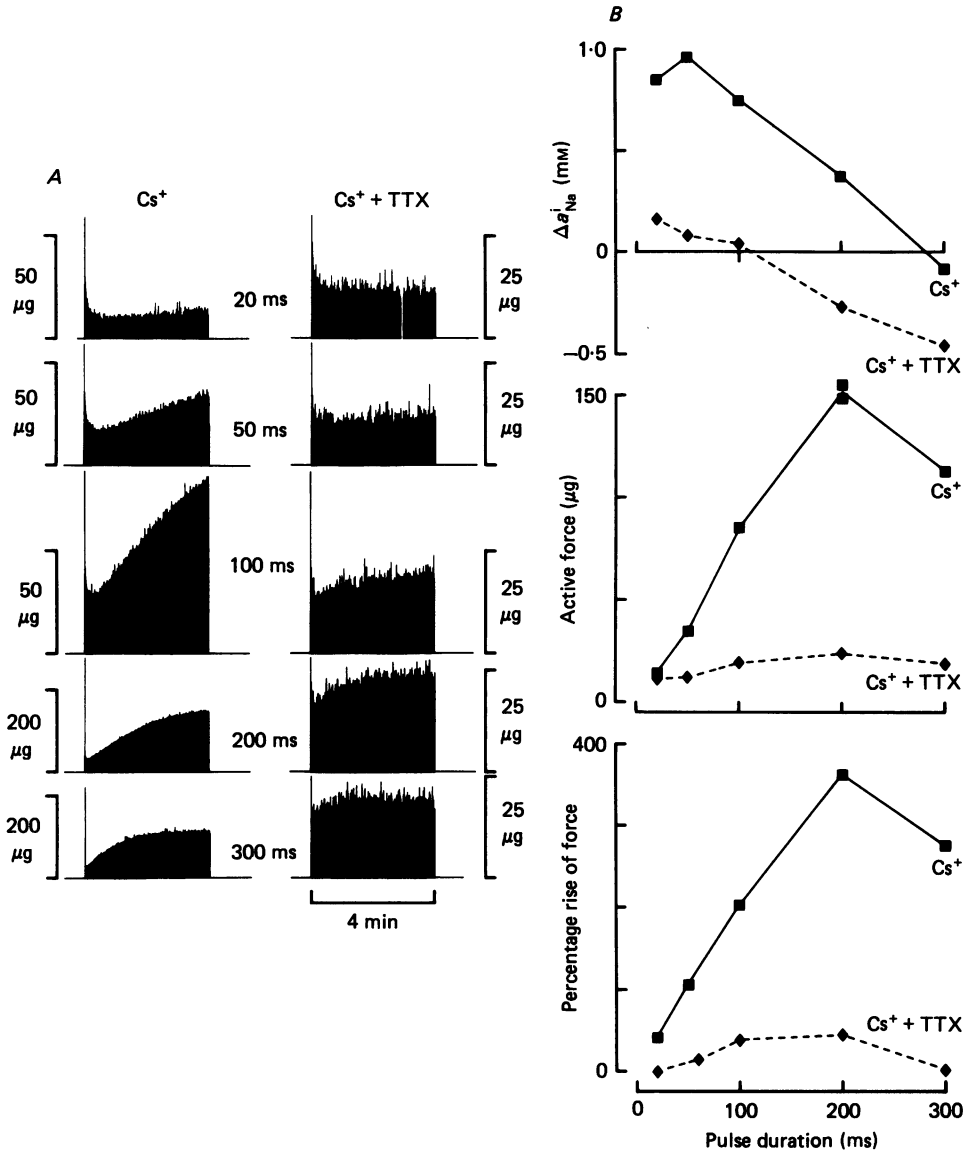

Fig. 10. The effect of TTX on the force staircase. *A*, the force staircase during trains of pulses of different duration (shown next to each trace) in the presence of 2 mM-Cs^+ alone (left) and 2 mM-Cs^+ and $2{\cdot}5 \times 10^{-5}$ M-TTX (right). Note that force is shown on a number of different gains. Voltage-clamp experiment on a sheep Purkinje fibre. Holding potential, −70 mV; pulse potential, +4 mV. *B*, the change of a^i_{Na} during the train, the active force at the end of the train, and the percentage rise of force during the train plotted as a function of the duration of the pulses. Data obtained in the presence of Cs^+ (■) and Cs^+ and TTX (◆) are shown.

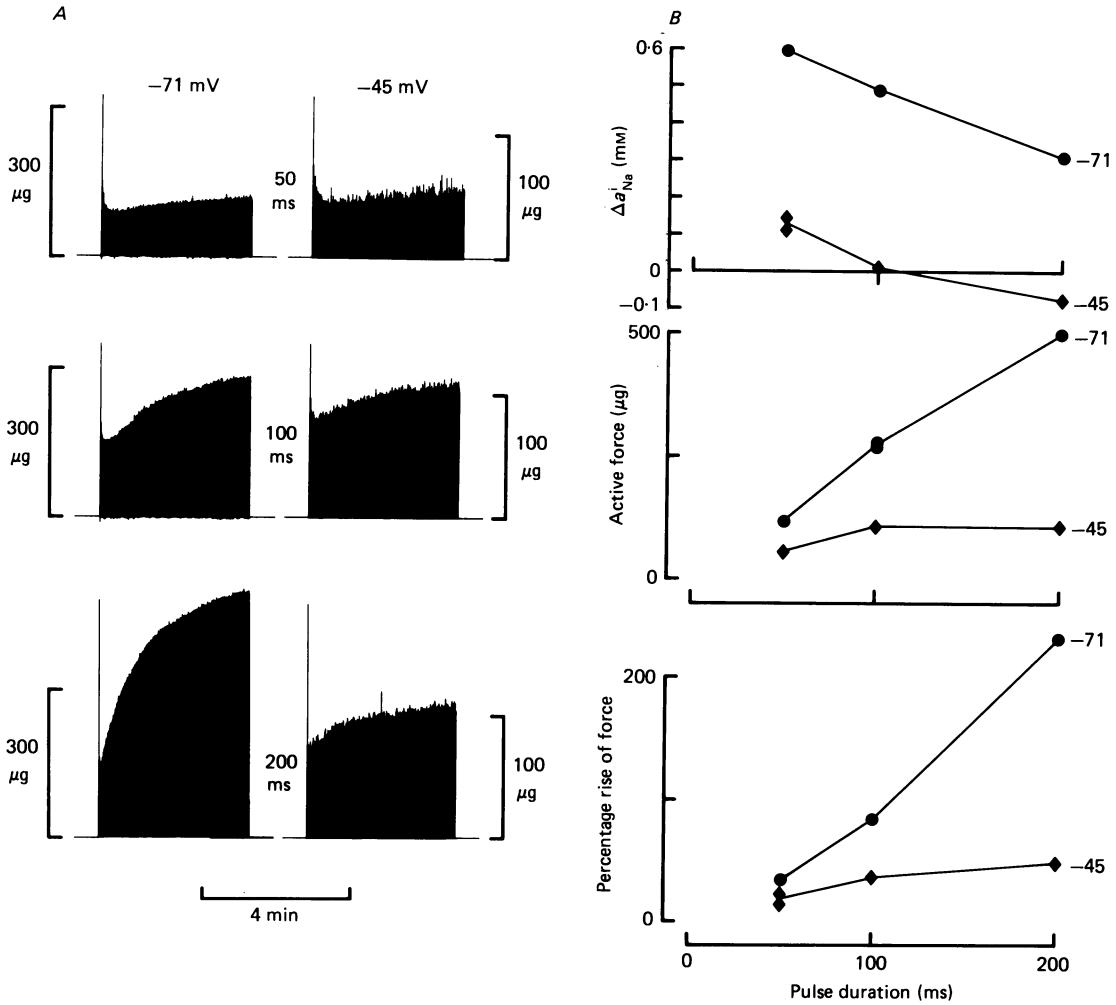

Fig. 11. The effect of the holding potential on the force staircase. *A*, the force staircase during trains of pulses of different duration (shown next to each trace) at holding potentials of −71 and −45 mV. Voltage-clamp experiment on a sheep Purkinje fibre. Pulse potential, +5 mV. *B*, the change of a^i_{Na} during the train, the active force at the end of the train, and the percentage rise of force during the train plotted as a function of the duration of the pulses. Data obtained at the holding potentials of −71 mV (●) and −45 mV (◆) are plotted.

is to be seen in some of the earlier Figures: in Fig. 8 during the train of 200 ms pulses there was a substantial gradual rise of force but apparently no net change of a^i_{Na}; in Fig. 10 with the train of 200 ms pulses in the presence of TTX there was a small slow rise of force and yet a fall of a^i_{Na}; and in Fig. 11 with the train of 200 ms pulses at the low holding potential there was also a slow rise of force but fall of a^i_{Na}. Clearly in these instances the gradual rise of force during the staircase cannot be attributed to a rise of a^i_{Na}. One of the most dramatic examples of a dissociation of the slow changes of force and a^i_{Na} during the staircase is illustrated in Fig. 12. During this train

of 300 ms pulses, which was applied in the presence of Cs^+, there was a gradual rise of force throughout much of the staircase and this was despite an apparent fall of a^i_{Na}; again, the changes of active force in this example cannot be attributed to a^i_{Na}. During a train of 300 ms pulses, four out of fifteen preparations exhibited a dissociation between a^i_{Na} and force similar to that illustrated in Fig. 12. A gradual rise of force accompanied by a slow fall of a^i_{Na} (not necessarily with 300 ms pulses) was observed in a total of seven preparations during voltage-clamp trains. These results were obtained under a variety of conditions: under control conditions, at a low holding potential, in the presence of Cs^+, and in the presence of Cs^+ and TTX.

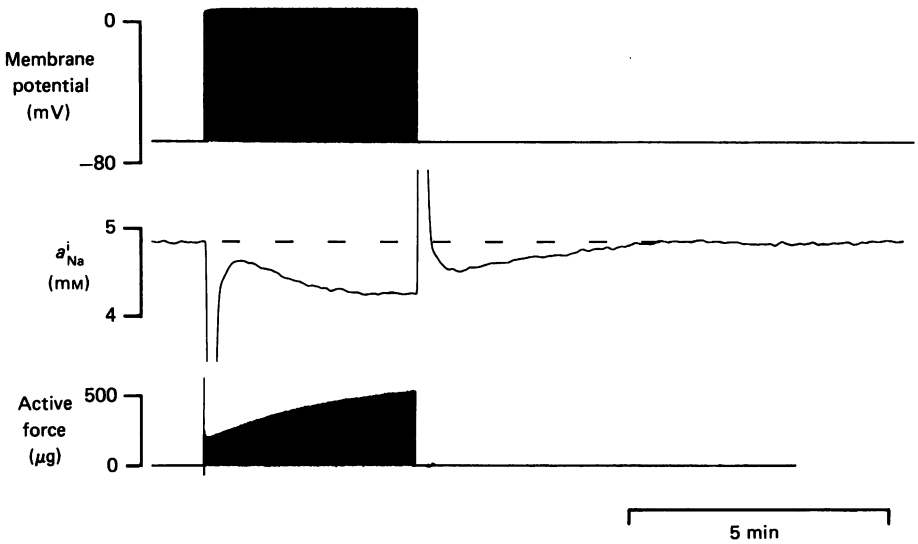

Fig. 12. An example of a positive staircase associated with a fall of a^i_{Na}. Voltage-clamp experiment on a sheep Purkinje fibre. The horizontal dashed line indicates resting a^i_{Na}. Holding potential, −67 mV; pulse potential, +7 mV; pulse duration, 300 ms. 2 mM-Cs^+ present.

DISCUSSION

The force staircase in cardiac Purkinje fibres

The experiments described here provide information about the force staircase. It could be argued that Purkinje fibres are not appropriate preparations in which to study force, because Purkinje tissue is specialized for conduction and is poorly contractile. The reason for working with Purkinje fibres is that these experiments, which involve measuring force and a^i_{Na} as well as voltage clamping for many hours, would not be possible with vigorously contracting ventricular muscle. Another advantage in working with sheep Purkinje fibres is that previous studies on the relationship between a^i_{Na} and force have also been carried out on this tissue (for example the various studies of Eisner, Lederer and Vaughan-Jones) and knowledge of this relationship is vital for the interpretation of the present results.

It is reassuring to note that the force staircase in sheep Purkinje fibres (Figs. 1*A*

and 2*B*) is similar to the staircase in other tissues (for examples see: Blinks & Koch-Weser, 1961; Boyett, 1978). In most tissues, including sheep Purkinje fibres, there is an abrupt fall of force in the first beat after an increase in rate, or in the second beat on stimulation after a rest, although the extent of the fall varies in different preparations: it is small or absent in, for example, cat ventricular muscle (Boyett, 1978), whereas it is very prominent in atrial muscle (Blinks & Koch-Weser, 1961) as it is in sheep and dog Purkinje fibres (Figs. 1*A* and 2). Blinks & Koch-Weser (1961) refer to this as the 'negative inotropic effect of activation' (n.i.e.a.). In all tissues, including sheep Purkinje fibres, there is a progressive increase in force after an increase in rate – Blinks & Koch-Weser (1961) refer to this as the 'positive inotropic effect of activation' (p.i.e.a.).

The effects of n.i.e.a. and p.i.e.a. can be seen in dog Purkinje fibres (Fig. 2*C* and *D*) but in addition there is a third effect in this tissue. This third effect can also be described as a negative inotropic effect of activation but in contrast to the n.i.e.a. described by Blinks & Koch-Weser (1961) this effect develops very slowly with time. Schouten & ter Keurs (1986) have observed a similar phenomenon in rat ventricular muscle.

The possible factors underlying the staircase will now be surveyed.

Pulse duration

Fig. 9*D* illustrates the effect of pulse duration *per se* on the twitch. It shows that the twitch is curtailed if the pulse is less than about 100 ms in duration. This result is similar to those obtained by others (Fozzard & Hellam, 1968; Beeler & Reuter, 1970). Clearly this effect is important in experiments such as that shown in Fig. 8 and is one reason why active force during the staircase is depressed at pulse durations less than 100 ms. However, there is no reason to suspect that this effect is involved in the time-dependent changes of force during a staircase.

Incomplete mechanical restitution

Fig. 1*B* illustrates the process of 'mechanical restitution' after a contraction. This mechanical restitution curve is similar to those obtained by Gibbons & Fozzard (1975*a*, *b*) in sheep Purkinje fibres. Incomplete mechanical restitution is responsible for the rapid n.i.e.a., i.e. the abrupt decrease in active force of the second beat when stimulation is commenced, whereas it is unlikely to be involved in the slow changes in force although a slow change in the mechanical restitution curve cannot be ruled out. What process underlies mechanical restitution? It has been suggested to be the result of Ca^{2+} recycling from an uptake site to a release site (e.g. Allen, Jewell & Wood, 1976; Orchard & Lakatta, 1985), or the reactivation of the Ca^{2+} current (Trautwein *et al.* 1975); it could also reflect the recovery from inactivation of the Ca^{2+} release mechanism of the sarcoplasmic reticulum (Fabiato, 1985*a*). Finally, Hilgemann & Noble (1986) have proposed a scheme that is based on the postulate that the transient rise of intracellular Ca^{2+} during contraction is an important stimulus for Na^+–Ca^{2+} exchange. It follows from this that there is a large efflux of Ca^{2+} via the exchanger during the large contraction after a rest, and Hilgemann and Noble propose that this depletes an internal store of Ca^{2+}. Part of the mechanical restitution curve, according to this scheme, could therefore represent the gradual replenishment of the internal

store. Because the exact nature of the underlying mechanism is not known, in this paper this process will continue to be referred to as mechanical restitution.

The effect of a^i_{Na} *on* Na^+–Ca^{2+} *exchange*

It is concluded from this study that a^i_{Na} *is involved in the staircase*; this conclusion is based on a comparison of the behaviour of sheep and dog preparations (Fig. 2), a comparison of positive and negative staircases (Fig. 3), the effects of the holding and pulse potentials on the staircase (Figs. 5, 6, 7 and 11), and the action of TTX (Fig. 4). Although a role for a^i_{Na} in the staircase has been proposed before (Langer, 1968; Cohen *et al.* 1982; Daut, 1982; Lederer & Sheu, 1983) this is the first time that the hypothesis has been rigorously tested. The action of a^i_{Na} on the staircase may be mediated via Na^+–Ca^{2+} exchange. The change of a^i_{Na} during the staircase is modest and it is rarely greater than 1 mM, but it should be remembered that Eisner *et al.* (1984) have demonstrated that in sheep Purkinje fibres twitch force can be a steep function of a^i_{Na} – they reported that twitch force is proportional to a^i_{Na} raised to the power of 3·2. Changes in a^i_{Na} are of course only likely to be involved in the slow changes of force attributed to the p.i.e.a.; the changes of force as a result of the n.i.e.a. occur within a few beats – a very rapid time course compared to any change of a^i_{Na}. Another important conclusion from this study is that *other factors, apart from* a^i_{Na}, *are also involved in the slow rise of force during the staircase.* This is based on data such as those illustrated in Fig. 12. No evidence has been obtained in this study about the identity of these other factors but a number of possibilities are considered below.

The effect of membrane depolarization on Na^+–Ca^{2+} *exchange*

Evidence is mounting that Na^+–Ca^{2+} exchange is electrogenic and that it is affected by membrane potential (for review see Eisner & Lederer, 1985). It is constructive to consider the consequences of membrane depolarization *per se* on Na^+–Ca^{2+} exchange. On the basis of simple thermodynamic considerations depolarization is expected to decrease Ca^{2+} extrusion via the exchanger or even cause a net influx of Ca^{2+} via the exchanger if the reversal potential is exceeded (see Eisner & Lederer, 1985, for further discussion). Evidence for these effects of membrane depolarization has been obtained (Mullins, Tiffert, Vassort & Whittembury, 1983; Eisner, Lederer & Vaughan-Jones, 1983*b*). The effect of membrane potential on Na^+–Ca^{2+} exchange may be an important factor in the force staircase, because the membrane is being repetitively depolarized. Furthermore the effect of membrane depolarization will be greater with longer pulses – this is dramatically illustrated if one calculates the time-averaged membrane potential during trains of pulses of different duration. It is not necessary to assume that during each pulse the Na^+–Ca^{2+} exchange fluxes are reversed – it could simply be that the ability of the exchanger to extrude Ca^{2+} is depressed with membrane depolarization. The net result would be the same in each case: an accumulation of Ca^{2+} in the cell. If the extra Ca^{2+} was sequestered by the sarcoplasmic reticulum it could lead to a gradual increase in twitch force during the staircase. Therefore it could explain the gradual rise in force in the absence of a rise of a^i_{Na} that has been observed in this study (this is only one possible explanation of this observation – see below).

Another conclusion from this study is that *the effect of a change of* a^i_{Na} *on the*

staircase is dependent on pulse duration. A possible explanation is considered here. Mullins *et al.* (1983) and Eisner *et al.* (1983*b*) have obtained evidence that the effect of a^i_{Na} on Ca^{2+} entry via Na^+-Ca^{2+} exchange is greatly enhanced by membrane depolarization (see also Eisner & Lederer, 1985). For example, Eisner *et al.* (1983*b*) concluded that both an increase of a^i_{Na} and a depolarization are required to increase tonic tension in sheep Purkinje fibres. This result may be the explanation of why in this study a given rise of a^i_{Na} apparently resulted in a much larger rise of force if the pulses during the train were long (and consequently the time-averaged membrane potential was more positive). Again it may not be necessary to assume that net Ca^{2+} entry occurs via the exchanger during the depolarizing pulses – if in the face of a large Na^+ influx–Ca^{2+} efflux exchange component, the combination of a rise of a^i_{Na} and membrane depolarization was to produce an increase in the smaller Na^+ efflux–Ca^{2+} influx exchange component, the result would be a decrease in the net Ca^{2+} extrusion via the exchanger and the consequence of this could again be accumulation of Ca^{2+} in the cell and a gradual increase in force.

Ca^{2+} loading via the Ca^{2+} current

As a result of the Ca^{2+} current that flows during the action potential, it is to be expected that Ca^{2+} influx is greater during repetitive activity than at rest. Such an increase in Ca^{2+} influx is expected to lead to a gradual accumulation of Ca^{2+} within the cell (in particular within the sarcoplasmic reticulum) and therefore a gradual increase in force during the staircase. This has been put forward as an explanation of the staircase by a number of authors (e.g. Allen *et al.* 1976; Edman & Johannsson, 1976). In support of this possibility Fabiato (1985*b*) has recently shown that repetitively exposing skinned dog Purkinje cells to aliquots of Ca^{2+} (designed to simulate the Ca^{2+} current) does lead to a force staircase, which Fabiato interpreted as Ca^{2+} loading, via the 'Ca^{2+} current', of the sarcoplasmic reticulum. Therefore Ca^{2+} loading via the Ca^{2+} current is another mechanism that could explain the gradual rise in force in the absence of a rise of a^i_{Na} which has been observed in this study. However, it should be noted that Fabiato studied a staircase lasting less than 10 'beats', whereas in the present experiments the staircase was followed for 600 beats.

Potentiation of Ca^{2+} inflow

Bers (1983, 1985) has recently suggested that in certain species activation of the contractile apparatus may be largely dependent on the Ca^{2+} inflow across the surface membrane during the action potential rather than a release of Ca^{2+} from the sarcoplasmic reticulum. Furthermore he presents evidence that the slow rise of force during the staircase is the result of a progressive increase of Ca^{2+} inflow with each beat. A rate-dependent increase in the Ca^{2+} current has indeed been observed by a number of authors (Noble & Shimoni, 1981; Payett, Schanne & Ruiz-Ceretti, 1981; Boyett & Fedida, 1984). Bers (1985) argues that this hypothesis is not inconsistent with a role for a^i_{Na}, because part of the Ca^{2+} inflow may be via Na^+ efflux–Ca^{2+} influx exchange and a rise of a^i_{Na} may potentiate this. It is interesting to note that the use of ryanodine (a blocker of Ca^{2+} release from the sarcoplasmic reticulum) indicates that force development in both sheep (Valdeolmillos & Eisner, 1985) and dog (Bers, 1985) Purkinje fibres may be largely dependent on release of Ca^{2+} from the sarcoplasmic reticulum rather than Ca^{2+} inflow across the sarcolemma in that beat.

Changes of the intracellular pH (pH_i)

Recently Kaila & Vaughan-Jones (1986) have measured a small rate-dependent decrease in the pH_i of sheep Purkinje fibres generating action potentials. A. J. Levi (unpublished observations) has shown that a similar fall of pH_i occurs during trains of voltage-clamp pulses (similar to those used in the present study) and that the fall is greater during trains of longer pulses. A fall in pH_i is known to depress contraction in cardiac tissues (e.g. Fabiato & Fabiato, 1978; Eisner, Lederer & Vaughan-Jones, 1983*a*). The effect of this will be to curtail the rise of force or even result in a slow decline in force during the staircase. Negative staircases have been observed in this study and although they can be explained by the accompanying falls of a^{i}_{Na} the fall of pH_i may contribute to the decline in force.

Working hypothesis

As a working hypothesis the possible events taking place during a train of action potentials at 2 Hz after a rest (such as that illustrated in Fig. 1*A*) will be postulated. The scheme incorporates the major findings from this study. (It will be assumed that contraction is largely the result of Ca^{2+} release from the sarcoplasmic reticulum.) The first contraction is large principally because the process of mechanical restitution is complete. The action potential is longer than 100 ms and therefore the duration of the action potential will not limit the size of the twitch. The next contraction is greatly reduced in amplitude because of incomplete mechanical restitution. The next phase is more complex. Ca^{2+} influx per unit time into the cell is now greater. The result of this is that Ca^{2+} accumulates in the cell and is sequestered by the sarcoplasmic reticulum. More Ca^{2+} is available for release and so the contractions begin to increase in amplitude. Because of the increase in Ca^{2+} influx, Ca^{2+} extrusion via the exchanger must increase if the cell is to remain in ionic balance; the stimulus for the increase in Ca^{2+} extrusion via the exchanger is an increase in the intracellular Ca^{2+} concentration. The transient rise of intracellular Ca^{2+} with each beat will itself stimulate Ca^{2+} extrusion via the exchanger. In addition as the train of action potentials continues the intracellular Ca^{2+} concentration (both the diastolic and systolic concentrations) increases further and this further promotes Ca^{2+} extrusion. However, the time-averaged membrane potential is now more positive than at rest and this by itself tends to depress net Ca^{2+} extrusion via the exchanger. The result is that the intracellular Ca^{2+} concentration (and hence force) must rise higher than it would have done otherwise to overcome this influence. As the train of action potentials continues a^{i}_{Na} begins to rise; this is mainly the result of the large influx of Na^+ via the Na^+ current, but partly the result of the increase in Na^+ influx–Ca^{2+} efflux exchange, and is in spite of a decrease in Na^+ influx via both the pace-maker current (i_f) and the Na^+-leak current. As a^{i}_{Na} rises net Ca^{2+} extrusion via the exchanger tends to be depressed (furthermore, this effect is enhanced because of the decrease in the time-averaged membrane potential on going from rest to stimulation). Therefore intracellular Ca^{2+} (and hence force) must rise higher than it would have done otherwise to overcome this influence. During the train there is a slight intracellular acidification and this tempers the rise of force.

This hypothesis will explain one other finding. During certain staircases such as those in Figs. 3*B*, 6 and 8 there is a fall of a^{i}_{Na} during the staircase and although force

falls in the later part of the staircase, early in the staircase there can be a very prominent rise of force. According to the hypothesis above the effects of the increase in Ca^{2+} influx and the decrease in the time-averaged membrane potential will commence immediately at the start of a voltage-clamp train and could therefore account for the early rise of force, whereas any effect of a^i_{Na} will be delayed as a^i_{Na} changes only slowly.

We wish to express our sincere gratitude to D. Ellis for advice on the construction and use of Na^+-sensitive micro-electrodes. In addition, we also wish to thank G. Harrison for expert technical assistance, J. Kelly of the Leeds abattoir who provided fresh sheep hearts, F. Karim who provided dog hearts at the end of his own experiments, and B. R. Jewell and C. H. Orchard for useful discussion and comments on the manuscript. This work was supported by a grant from the British Heart Foundation (grant number: 84/64). A. J. L. is a British Heart Foundation Research Fellow (grant number: 83/13) and A. R. is a British Heart Foundation scholar (grant number: F70).

REFERENCES

ALLEN, D. G. & BLINKS, J. R. (1978). Calcium transients in aequorin-injected frog cardiac muscle. *Nature* **273**, 509–513.

ALLEN, D. G., JEWELL, B. R. & WOOD, E. H. (1976). Studies of the contractility of mammalian myocardium at low rates of stimulation. *Journal of Physiology* **254**, 1–17.

ALLEN, D. G. & KURIHARA, S. (1980). Calcium transients in mammalian ventricular muscle. *European Heart Journal* **1**, suppl. A, 5–15.

BEELER, G. W. & REUTER, H. (1970). The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. *Journal of Physiology* **207**, 211-229.

BERS, D. M. (1983). Early transient depletion of extracellular Ca during individual cardiac muscle contractions. *American Journal of Physiology* **244**, H462-468.

BERS, D. M. (1985). Ca influx and sarcoplasmic reticulum Ca release in cardiac muscle activation during postrest recovery. *American Journal of Physiology* **248**, H366–381.

BHATTACHARYAA, M. L. & VASSALLE, M. (1981). Role of calcium and sodium in strophanthidin inotropy in cardiac Purkinje fibres. *American Journal of Physiology* **240**, H561–570.

BLINKS, J. R. & KOCH-WESER, J. (1961). Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *Journal of Pharmacology and Experimental Therapeutics* **134**, 373–389.

BOYETT, M. R. (1978). An analysis of the effect of the rate of stimulation and adrenaline on the duration of the cardiac action potential. *Pflügers Archiv* **377**, 155–166.

BOYETT, M. R. & HART, G. (1986). Dissociation between force and intracellular sodium activity (a^i_{Na}) during the force staircase in isolated sheep Purkinje fibres. *Journal of Physiology* **374**, 37*P*.

BOYETT, M. R., HART, G. & LEVI, A. J. (1985). The role of the intracellular sodium activity (a^i_{Na}) in the force staircase in isolated sheep Purkinje fibres. *Journal of Physiology* **366**, 85*P*.

BOYETT, M. R., HART, G. & LEVI, A. J. (1986). Dissociation between force and intracellular sodium activity with strophanthidin in isolated sheep Purkinje fibres. *Journal of Physiology* **381**, 311–331.

BOYETT, M. R., HART, G. & LEVI, A. J. (1987). Factors affecting intracellular sodium during repetitive activity in isolated sheep Purkinje fibres. *Journal of Physiology* **384**, 405–429.

BOYETT, M. R. & FEDIDA, D. (1984). A decrease of the second inward current, rather than an increase in transient outward current, causes action potential shortening at low stimulus rates in sheep Purkinje fibres. *Journal of Physiology* **346**, 76*P*.

BOWDITCH, H. P. (1871). Über die Eigenthümlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. In *Berichte der sächsischen Akademie der Wissenschaften*, pp. 652–689.

COHEN, C. J., FOZZARD, H. A. & SHEU, S.-S (1982). Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circulation Research* **50**, 651–662.

DAUT, J. (1982). The role of intracellular sodium ions in the regulation of cardiac contractility. *Journal of Molecular and Cellular Cardiology* **14**, 189–192.

DEITMER, J. W. & ELLIS, D. (1980). The intracellular sodium activity of sheep heart Purkinje fibres: effects of local anaesthetics and tetrodotoxin. *Journal of Physiology* **300**, 269–282.

DENTON, R. M., RICHARDS, D. A. & CHIN, J. G. (1978). Calcium ions and the regulation of NAD^+-linked isocitrate dehydrogenase from the mitochondria of rat heart and other tissues. *Biochemical Journal* **176**, 899–906.

EDMAN, K. A. P. & JOHANNSSON, M. (1976). The contractile state of rabbit papillary muscle in relation to stimulation frequency. *Journal of Physiology* **254**, 565–581.

EISNER, D. A. & LEDERER, W. J. (1985). Na–Ca exchange: stoichiometry and electrogenicity. *American Journal of Physiology* **248**, C189–202.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981*a*). The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep Purkinje fibres. *Journal of Physiology* **317**, 163–187.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981*b*). The effects of rubidium ions and membrane potential on the intracellular sodium activity of sheep Purkinje fibres. *Journal of Physiology* **317**, 189–205.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1983*a*). The relationship between intracellular pH and contraction in sheep cardiac Purkinje fibres. *Journal of Physiology* **334**, 106–107*P*.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1983*b*). The control of tonic tension by membrane potential and intracellular sodium activity in the sheep cardiac Purkinje fibre. *Journal of Physiology* **335**, 723–743.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1984). The quantitative relationship between twitch tension and intracellular sodium activity in sheep cardiac Purkinje fibres. *Journal of Physiology* **355**, 251–266.

FABIATO, A. (1985*a*). Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *Journal of General Physiology* **85**, 247–289.

FABIATO, A. (1985*b*). Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *Journal of General Physiology* **85**, 291–320.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

FEDIDA, D. & BOYETT, M. R. (1985). Activity-dependent changes in the electrical behaviour of sheep cardiac Purkinje fibres. *Proceedings of the Royal Society* B **225**, 457–479.

FOZZARD, H. A. & HELLAM, D. C. (1968). Relationship between membrane voltage and tension in voltage-clamped cardiac Purkinje fibres. *Nature* **218**, 588–589.

GIBBONS, W. R. & FOZZARD, H. A. (1975*a*). Relationship between voltage and tension in sheep cardiac Purkinje fibres. *Journal of General Physiology* **65**, 345–365.

GIBBONS, W. R. & FOZZARD, H. A. (1975*b*). Slow inward current and contraction of sheep cardiac Purkinje fibres. *Journal of General Physiology* **65**, 367–384.

HILGEMANN, D. W. & NOBLE, D. (1986). Excitation–contraction coupling and extracellular calcium transients in rabbit atrium: reconstruction of basic cellular mechanisms. *Philosophical Transactions of the Royal Society B* (in the Press).

KAILA, K. & VAUGHAN-JONES, R. D. (1986). The effect of stimulation frequency upon intracellular pH, intracellular sodium and tension in sheep cardiac Purkinje fibres. *Journal of Physiology* **371**, 171*P*.

KOCH-WESER, J. & BLINKS, J. R. (1963). The influence of the interval between beats on myocardial contractility. *Pharmacological Reviews* **15**, 601–652.

KRUTA, V. & BRAVENÝ, P. (1961). Restitution de la contractilité du myocarde entre les contractions et les phénomènes de potentiation. *Archives internationales de physiologie et de biochimie* **69**, 645–667.

LANGER, G. A. (1968). Ion fluxes in cardiac excitation and contraction and their relation to myocardial contractility. *Physiological Reviews* **48**, 708–757.

LEDERER, W. J. & SHEU, S.-S. (1983). Heart rate-dependent changes in intracellular sodium activity and twitch tension in sheep cardiac Purkinje fibres. *Journal of Physiology* **345**, 44*P*.

MARTELL, A. E. & SMITH, R. M. (1974). *Critical Stability Constants*, vol. 1. London: Plenum Press.

MULLINS, L. J., TIFFERT, T., VASSORT, G. & WHITTEMBURY, J. (1983). Effects of internal sodium and hydrogen ions and of external calcium ions and membrane potential on calcium entry in squid axons. *Journal of Physiology* **338**, 295–319.

NOBLE, S. & SHIMONI, Y. (1981). The calcium and frequency dependence of the slow inward current 'staircase' in frog atrium. *Journal of Physiology* **310**, 57–75.

ORCHARD, C. H. & LAKATTA, E. G. (1985). Intracellular calcium transients and developed tension in rat heart muscle. A mechanism for the negative interval–strength relationship. *Journal of General Physiology* **86**, 637–651.

PAYETT, M. D., SCHANNE, O. F. & RUIZ-CERETTI, E. (1981). Frequency dependence of the ionic currents determining the action potential repolarization in rat ventricular muscle. *Journal of Molecular and Cellular Cardiology* **13**, 207–215.

SCHOUTEN, V. J. A. & TER KEURS, H. E. D. J. (1986). The force–frequency relationship in rat myocardium: the influence of muscle dimensions. *Pflügers Archiv* (in the Press).

TRAUTWEIN, W., MCDONALD, T. F. & TRIPATHI, O. (1975). Calcium conductance and tension in mammalian ventricular muscle. *Pflügers Archiv* **354**, 55–74.

VALDEOLMILLOS, M. & EISNER, D. A. (1985). The effects of ryanodine on calcium-overloaded sheep cardiac Purkinje fibres. *Circulation Research* **56**, 452–456.