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THE CONTRACTILE STATE OF RABBIT PAPILLARY MUSCLE IN RELATION TO STIMULATION FREQUENCY

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SUMMARY

1. The relationship between active force and stimulation frequency (0·25–5/sec) was studied at 36–37° C in isolated papillary muscles of the rabbit.

2. The muscle's force producing capability at a given frequency was determined as the isometric twitch response to a test stimulus that was applied at various times after a priming period. The optimum contractile response was obtained at an interval of 0·8 sec between the test pulse and the last stimulus of the priming period.

3. The optimum contractile response exceeded the steady-state twitch amplitude at all stimulation frequencies higher than 1/sec. While the steady-state twitch response declined at frequencies higher than 4/sec, the optimum contractile response was steadily increased as the stimulation frequency was raised.

4. The optimum contractile response was also determined after priming the muscle with a sinusoidal a.c. pulse (field strength, 10 V (r.m.s.)/cm; frequency, 20 c/s; duration, 2·5 sec). The optimum contractile response obtained after a.c. stimulation was 2·2 times greater than the maximal steady-state response. Its absolute value was $67{\cdot}3 \pm 6{\cdot}1$ mN/mm^2 (mean $\pm$ S.E. of mean, $n = 6$).

5. The twitch potentiation produced by priming the muscle at a given frequency decayed exponentially in two phases after optimum contractile response had been attained. The time constants of the two phases, determined after a.c. stimulation, were $2{\cdot}6 \pm 0{\cdot}8$ ($n = 4$) and $92{\cdot}0 \pm 13{\cdot}3$ sec ($n = 7$), respectively.

6. The optimum contractile response determined at various stimulation frequencies was linearly related to the fraction of time during which the cell membrane was depolarized (beyond -40 mV) by the action potentials.

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7. The results are interpreted in terms of a two-component model of the metabolism of activator calcium in the excitation–contraction coupling.

INTRODUCTION

Isolated cardiac muscle alters its contractile output in response to a change in stimulation rate. This force–frequency relationship varies substantially between atrial and ventricular myocardium and also from species to species. The frequency dependence, first described by Bowditch (1871), has continued to attract much interest (for references, see Koch-Weser & Blinks, 1963; Morad & Goldman, 1973; Langer, 1968), and there is reason to believe that the force–frequency relationship reflects a fundamental cellular mechanism in the control of the heart muscle's performance. Recent evidence suggests that the contractile effects seen after a change in stimulation rate are based on an altered metabolism of activator calcium in the excitation–contraction coupling. A change in stimulation frequency has thus been shown to affect both the transport of calcium across the membrane and the content of calcium in the myocardial cell (Langer, 1965; Sands & Winegrad, 1970).

It has been the aim of the present investigation to examine the kinetics of the contractile change induced by activation of the rabbit papillary muscle. The capacity of the muscle to produce twitch tension was determined at different stimulation frequencies by applying a test pulse at various times after a priming period. Attempts were made to correlate the twitch potentiation with the fraction of time during which the cell membrane is depolarized by the action potentials. Some of the results have already been reported briefly (Edman & Jóhannsson, 1973).

METHODS

Preparation. Papillary muscles were dissected from the right ventricle of heparinized rabbits (weight 0·8–1·5 kg). The techniques used for dissection and mounting of the muscles have been described (Edman & Nilsson, 1968). The length and thickness of the muscles were measured at 20 and 40 × magnification, respectively, using a Zeiss Stereo II microscope fitted with an ocular micrometer. The thickness (at approximately 1 mm from the base) was 0·4–0·8 mm and the length of the muscles was 3·0–5·5 mm in the various experiments.

In certain experiments, specified below, papillary muscles were dissected from rabbits after reserpinization. To these animals a single dose (4 mg/kg) of reserpine (Serpasil, Ciba) was given intraperitoneally 18–24 h before the experiment. As demonstrated by Spilker & Cervoni (1969) this amount of reserpine is sufficient to reduce the noradrenaline content of the heart to approximately 2·5 % of the normal value.

Muscle chamber and mounting of preparation. The muscle chamber was an open trough fitted on top of a Lucite block. The trough was 80 mm long and 5 mm wide

and contained 2 ml. solution. The muscle was mounted horizontally between two glass hooks, one of which was fitted on the lever of a tension transducer. The resting length of the muscle was adjusted by altering the position of the tension transducer by means of a micrometer screw. The rest length used in these experiments was approximately 95 % of the length at which maximal isometric tension was recorded.

Water was circulated from a thermostatically controlled tank through a jacket that surrounded the chamber. The trough was continuously perfused with bathing solution at a rate of 5 ml./min. This provided a 90 % exchange of solution in about 2 min as determined colorimetrically from the dilution of a dye ($KMnO_4$) added to the perfusion fluid. The bath solution had been prewarmed to 37° C and equilibrated with a mixture of 95 % O_2 and 5 % CO_2 immediately before entering the trough. The bath temperature was 36–37° C in the different experiments and did not vary by more than ± 0·2° C throughout any given experiment.

Tension transducer. Tension was recorded by means of a semiconductor strain-gauge transducer. The lever of the transducer was made of a thin-walled glass tube, the distal end of which was provided with a hook for attachment of the muscle. The frequency response of the transducer with the lever immersed in solution was 800 Hz. A linear response was obtained for forces up to at least 50 mN. The compliance of the transducer was 1·4 μm/mN.

The transducer signal, after amplification (Philbrick SQ-10a operational amplifier), was recorded on paper using a Grass polygraph ink writer. In certain experiments the tension signal was also displayed on a Tektronix 502A oscilloscope and photographed on 35 mm film.

The amplitude of the isometric myogram was measured (to the nearest 0·5 mm) on the original paper records, the size of the myogram usually being 35–50 mm.

Intracellular recording of membrane potential. Membrane action potentials were recorded by means of conventional micro-electrode technique. Glass capillary electrodes filled with 2·5 M-KCl were used. The electrodes had a resistance of 15–25 MΩ and a tip potential of less than 5 mV. An Ag-AgCl electrode embedded in a gel of agar and bath solution in a polyethylene tube served as the reference electrode in the grounded muscle bath. The electrodes were connected differentially to an amplifier with high input impedance and capacitance neutralization (Holmer & Lindström, 1972). The signals, after amplification, were displayed on a Tektronix 502A oscilloscope and photographed on 35 mm film.

Stimulation. The muscle was stimulated by passing current between two platinum plate electrodes that were placed 3·7 mm apart on either side of the muscle. Current was delivered from a power amplifier that was operated by a Grass S-44 stimulation unit (point 1, see below) or a sinewave generator (point 2, see below). Stimulation was performed in two different ways. (1) A rectangular d.c. pulse of 2 msec duration was applied at a frequency of 0·25–5 stimuli/sec. The stimulus strength exceeded the threshold value by approximately 20 %. This mode of stimulation was employed unless otherwise stated. (2) Sinewave a.c. stimulation was used in some experiments. The characteristics of the a.c. pulse are given under Results.

Procedure of stimulation. After mounting, the muscle was stimulated to contract isometrically at a frequency of 1/sec (d.c. pulses, see above) for about 1 h before the experiment was started. The procedure for stimulation of the muscle during the actual experiment is described under Results. For the successful performance of an experiment it was essential to have a precise timing of the stimulation sequence. This was achieved by means of a programmable control unit which alternatingly gated the output of two d.c.-pulse stimulators (set at 1/sec and at another selected frequency) and a sinewave generator used for the a.c. stimulation. This control unit made it possible to pre-set the time of onset and the duration of the different

events during a stimulation sequence to within ± 2 %. The values of the 'test pulse interval' (definition, see below) were read from a calibrated potentiometer scale on the control unit.

Solution. The bath solution had the following composition (mM): NaCl 100·0, KCl 4·0, $NaHCO_3$ 20·0, $MgSO_4$ 1·5, NaH_2PO_4 1·5, $CaCl_2$ 2·0, Na acetate 20·0 and glucose 10·0. The solutions were equilibrated with a gas mixture containing 95 % O_2 and 5 % CO_2. The pH of the solution was 7·4. The chemicals used were of analytical grade. The water used for washing of glassware and for preparation of solutions was double-distilled in borosilicate glass distillers.

Statistical evaluation. The statistical significance of the results has been calculated using a *t* test for paired observations.

RESULTS

1. *Post-stimulation potentiation and the optimum contractile response*

The purpose of these experiments was to determine the inotropic state of the papillary muscle at different contraction frequencies. The procedure of an experiment was as follows: the muscle was paced to contract at a frequency of 1/sec for 3–4 min (control period). This was followed by a *priming period*, during which a train of stimuli of a frequency varying between 0·25/sec and 5/sec was applied. The stimulation was continued until steady-state responses were obtained, i.e. 3–10 sec within the frequency range 2–5/sec and 180 sec at 0·25/sec priming frequency (see further below). At a selected instant after a completed priming period a *test pulse* was given. The *test pulse interval* is defined as the time from the last stimulus during the priming period to the occurrence of the test pulse. The test stimulus was actually the first pulse of a new control stimulation period. A complete stimulation sequence thus included: (1) control period, (2) priming period, (3) test stimulus and (4) control period.

Fig. 1 illustrates oscillograph traces from a typical experiment. In Fig. 2*A* the amplitude of the test twitch has been plotted as a function of the test pulse interval at four priming frequencies (2–5/sec) in the same muscle. It can be seen that as the test pulse interval was prolonged, the twitch amplitude was steadily increased reaching a maximum at an interval of approximately 0·8 sec at the different frequencies. After attainment of the maximum there was a slow decline of the peak twitch tension as will be described in more detail in section 3. In six muscles primed at stimulation frequencies ranging between 2 and 5/sec the twitch potentiation was investigated at 0·6, 0·7, 0·8, 0·9 and 1·0 sec test pulse intervals. The twitch amplitude recorded at 0·6 and 1·0 sec test pulse intervals was, respectively, 97 % (range 91–100) and 96 % (range 93–98) of the maximally potentiated twitch response at each frequency. In all cases maximum twitch potentiation was obtained at a test pulse interval

of 0·8 sec. The maximal isometric twitch response to the test stimulus, i.e. at optimal test pulse interval, will be referred to in the following as the '*optimum contractile response*'.

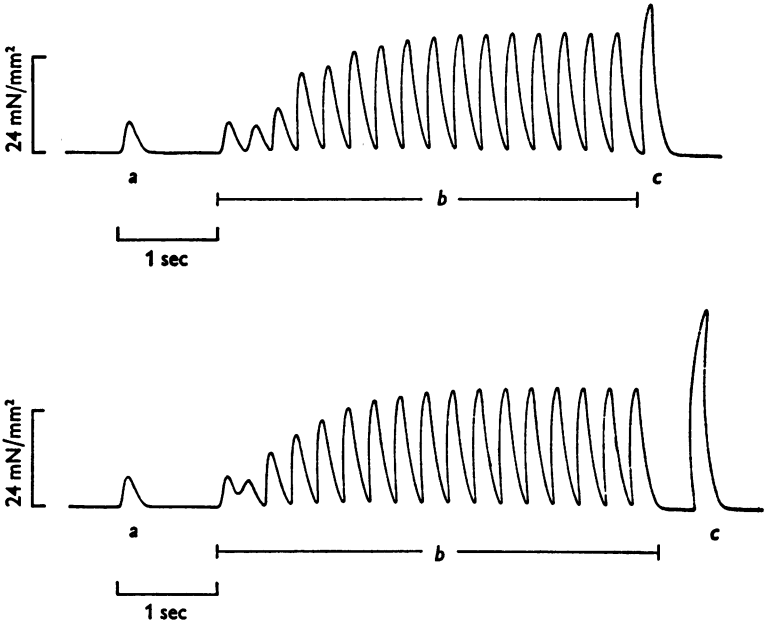

Fig. 1. Oscillograph traces illustrating isometric twitches: *a*, during control period (1/sec stimulation frequency), *b*, during priming period (4/sec stimulation frequency) and *c*, in response to a test stimulus. Test pulse interval: 0·3 sec (upper) and 0·6 sec (lower). Note difference in amplitude of test twitches.

The influence of the duration of the priming period (and accordingly the number of priming pulses) on optimum contractile response was studied at stimulation frequencies of 2, 3, 4 and 5/sec. Maximum value of the optimum contractile response at each of these frequencies was obtained as the isometric twitch amplitude attained steady-state level during the priming period. This occurred after 10–15 contractions at the stimulation frequencies studied, i.e. after a priming period of 2–3 sec at the highest (5/sec) stimulation frequency and 5–7 sec at the lowest (2/sec) frequency. Increasing the number of priming pulses to 25–30 contractions (an approximate doubling of the priming period) did not increase the optimum contractile response further. Longer priming periods were not studied to avoid fatiguing the muscle. The twitch amplitude could be seen to decay slowly as the number of stimuli exceeded about 30 at frequencies $\geqslant$ 3/sec.

The variation in the optimum contractile response with the priming

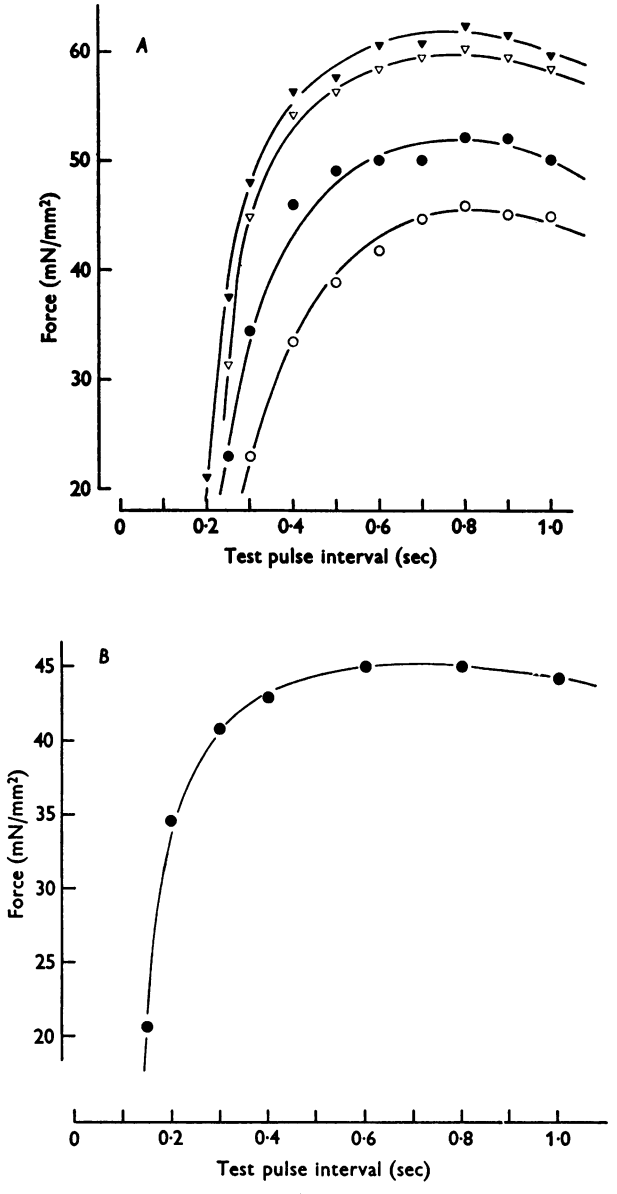

Fig. 2. Amplitude of the isometric test twitch in relation to the test pulse interval: *A*, after priming periods of 2 (○), 3 (●), 4 (▽) and 5 (▼) stimuli/sec and *B*, after a 2·5 sec priming period of 20/sec a.c. stimulation. Data in *A* and *B*, respectively, obtained from two different muscles. Note that the ordinates start from 20 mN/mm².

frequency (0·25–5/sec) was investigated in six papillary muscles. A test pulse interval of 0·8 sec was employed at all frequencies studied during the priming period. The results are presented in Fig. 3, which also includes, for comparison, the curve relating steady-state twitch tension and stimulation frequency in the same muscles. It can be seen that the steady-state

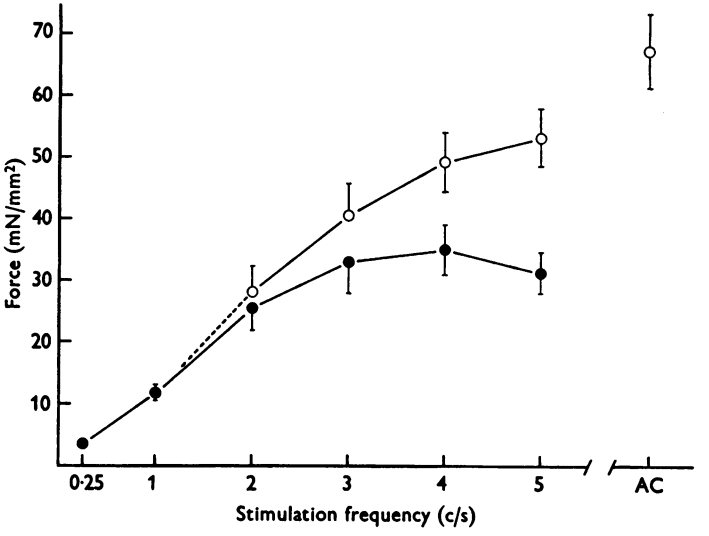

Fig. 3. Peak isometric twitch force of rabbit papillary muscles as a function of stimulation frequency. Filled circles: amplitude of steady-state twitch responses. Open circles: optimum contractile response at different frequencies of d.c. stimulation and at 20/sec a.c. stimulation (top right). Each symbol represents the mean of six experiments. Vertical bars indicate S.E. of mean. The same experiments used for determination of steady-state twitch responses and the optimum contractile response.

amplitude of the isometric twitch increased with increasing stimulation frequency and reached a maximum at 4/sec. A further increase to 5/sec reduced the peak isometric twitch tension to 90% of the maximal value. These findings accord with results previously obtained in studies of the ventricular myocardium of rabbit (Bautovich, Gibb & Johnson, 1962; Teiger & Farah, 1968), guinea-pig (Rumberger & Reichel, 1972), cat (Trautwein & Dudel, 1954; Koch-Weser, 1963), dog (Endoh & Hashimoto, 1970) and man (Gennser, Jóhannsson & Nilsson, 1972; Buckley, Penefsky & Litwak, 1972). Fig. 3 furthermore shows that at stimulation rates equal to or higher than 2/sec, the optimum contractile response exceeded the steady-state twitch response. It should be noted that whereas the steady-state response declined when going to a higher frequency than 4/sec, the

optimum contractile response continued to rise. The differences between the optimum contractile response and the corresponding steady-state value are statistically significant (for paired observations) at stimulation rates higher than 1/sec ($P < 0{\cdot}01$).

2. *Optimum contractile response following a.c. stimulation*

As demonstrated in Fig. 3, the optimum contractile response curve did not attain a clear plateau at the stimulation frequencies studied, i.e. up to 5 stimuli/sec. Higher priming frequencies were not feasible to investigate, as the muscle generally failed to propagate action potentials at stimulation rates exceeding 5/sec. It was therefore of interest to explore an alternative way of depolarizing the preparation at higher frequencies. Such a possibility is offered by using a.c. stimulation (Sten-Knudsen, 1960).

For determination of the optimum contractile response after a.c. stimulation the same general approach was used as described in section 1. The oscillograph records in Fig. 4*A* illustrate the contractile response to a 20 c/s a.c. pulse (2·5 sec duration) and the response to a subsequent test stimulus. Typically the a.c. stimulation induced an initial twitch-like contraction which reached approximately the same amplitude as obtained during an ordinary isometric twitch at a stimulation frequency of 1/sec. After the initial peak the tension attained a somewhat lower level which was maintained during the rest of the a.c. stimulation period. It can be seen that the contractile response to the test stimulus was substantially increased as the test pulse (measured from the end of the a.c. pulse) was prolonged from 0·15 to 0·6 sec.

Experiments were performed to determine the optimal frequency, duration and field strength of the a.c. pulse used for priming of the muscle. For this purpose the isometric twitch response to a test stimulus that was applied at a fixed time (2 sec) after the priming a.c. pulse was recorded. On the basis of results obtained in such experiments (Fig. 4*B*, *a–c*) the following characteristics of the sinusoidal a.c. pulse were considered to be optimal for the analysis of the optimum contractile response after a.c. stimulation: field strength 10 V (r.m.s.)/cm, frequency 20 c/s, duration 2·5 sec. Under the conditions used, repeated a.c. stimulation (at 3–4 min intervals) did not cause any noticeable deterioration of the muscle over at least 2 h experimentation.

Fig. 2*B* shows the response to the test stimulus applied at various times after the priming a.c. pulse. In similarity with the results shown in Fig. 2*A* the greatest twitch amplitude was attained at a test pulse interval of about 0·8 sec. This test pulse interval was therefore used as a standard also in the analysis of the optimum contractile response after a.c. stimulation.

Data concerning optimum contractile response after a.c. stimulation obtained in six experiments (on the same muscle as used in section 1) are illustrated in Fig. 3. It is evident that the optimum contractile response could be increased considerably above the value that was obtained after

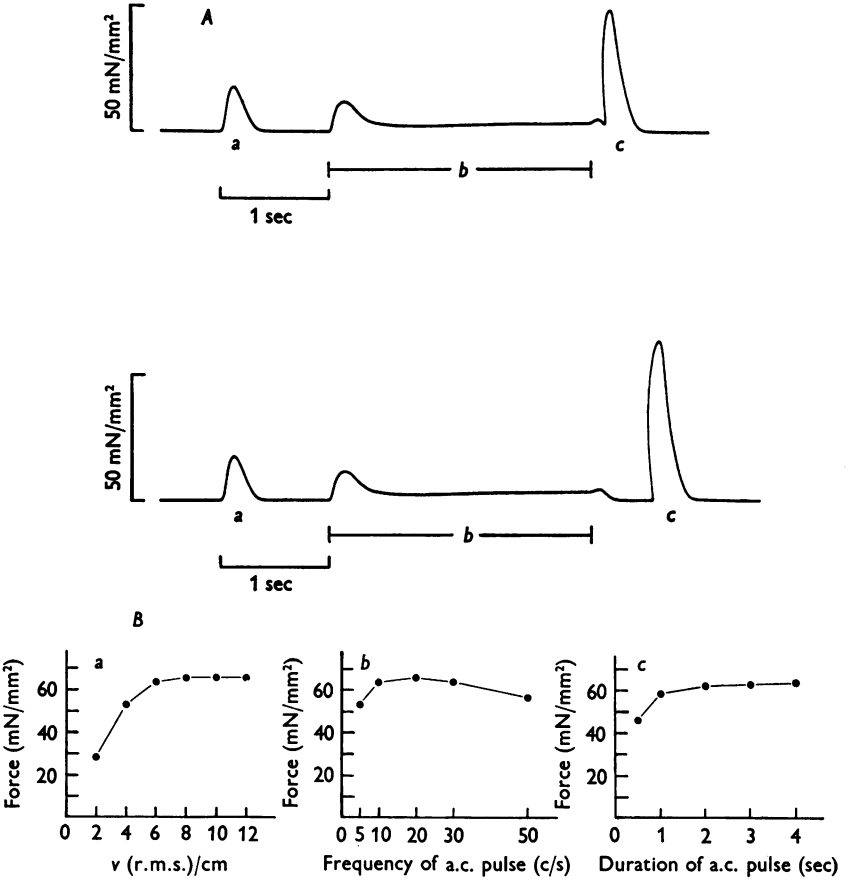

Fig. 4. *A*, oscillograph traces illustrating: *a*, isometric twitch during control period (1/sec stimulation frequency), *b*, the mechanical response during priming period (20/sec a.c. stimulation) and *c*, the twitch response to a test stimulus. Test pulse interval: 0·15 sec (upper) and 0·6 sec (lower). *B*, peak isometric twitch tension in response to a test stimulus applied 2 sec after a priming sinusoidal a.c. pulse. Influence of (*a*) field strength (20 c/s, 3 sec duration), (*b*) frequency (3 sec duration, 8 V/cm) and (*c*) duration (20 c/s, 8 V/cm) of the priming a.c. pulse.

ordinary 5/sec d.c. stimulation. The amplitude of this response obtained after a.c. stimulation can be seen to be 2·2 times greater than the maximal steady-state twitch response. Its absolute value was $67{\cdot}3 \pm 6{\cdot}1$ mN/mm² (mean ± S.E. of mean, $n = 6$).

3. *Disappearance of the twitch potentiation*

Fig. 5 shows the decay of twitch potentiation as the test pulse interval is increased beyond the optimal 0·8 sec. Illustrated are results obtained from the same muscle after priming with a.c. stimulation and 5/sec d.c. stimulation, respectively. In this experiment the optimum contractile response derived after a.c. stimulation was 22% greater than that after

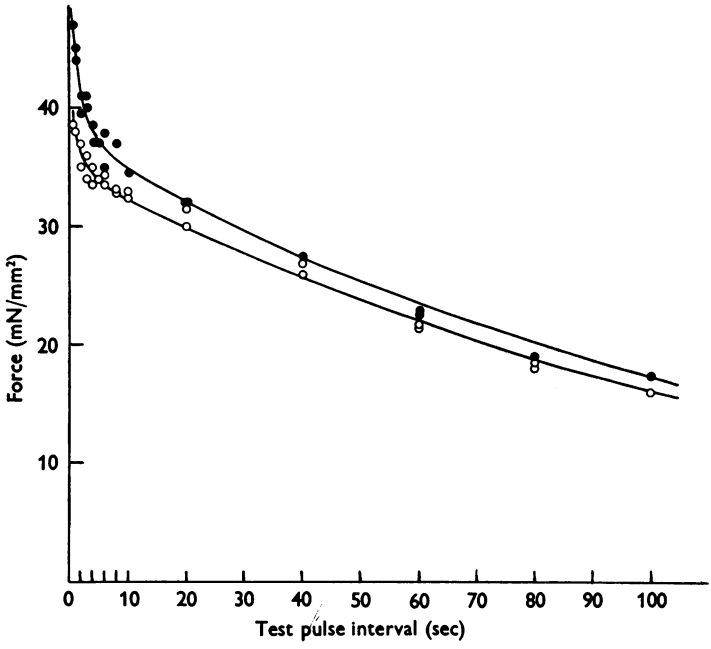

Fig. 5. The disappearance of twitch potentiation in reserpinized papillary muscle after priming with 20/sec a.c. stimulation (filled circles) and 5/sec d.c. stimulation (open circles). Uppermost symbol in each curve represents the optimum contractile response. Curves drawn from the empirical equations

$$F = 12e^{-0.555t} + 36{\cdot}8e^{-0.008t} + 0{\cdot}8 \text{ (upper)}$$

and

$$F = 12e^{-1.053t} + 34{\cdot}4e^{-0.008t} + 0{\cdot}8 \text{ (lower),}$$

in which F denotes force (mN/mm²) and t the test pulse interval (sec).

5/sec d.c. stimulation. As can be seen the time course of disappearance of the twitch potentiation was very similar in the two cases. The decay of twitch potentiation can be fitted with a two-phasic exponential function. There was an initial rapid phase and a subsequent slower phase, the latter accounting for the major portion of the tension decline.

In some experiments the decay curve exhibited a slight upwards convex shape at 5–20 sec after the priming period. This peculiarity

disappeared after treating of the muscle with 3 μM propranolol (Inderal, I.C.I.), suggesting that the aberrant shape of the curve represented an inotropic action of released noradrenaline. Supplementary experiments were therefore carried out on reserpinized muscles, and these regularly yielded a smooth two-phasic exponential decay curve. The time constants of the two decay phases, determined after a.c. stimulation, were: 2·6 ± 0·8 sec (mean ± S.E. of mean, $n = 4$; all muscles reserpinized) and 92·0 ± 13·3 sec ($n = 7$; four muscles reserpinized).

4. *Relationship between potentiation and membrane depolarization*

It was of interest to investigate whether there existed a simple relationship between twitch potentiation and the fraction of time during which the cell membrane was depolarized during the priming period. For this purpose the summated intervals (horizontal bars, Fig. 6*A*) during which the cell membrane was depolarized beyond −40 mV during a 1 sec period was determined at different stimulation frequencies (Table 1). The results obtained in six experiments are presented in Fig. 6*B*. It can be seen that over the range of stimulation frequencies investigated, the optimum contractile response increased as a rectilinear function of the total depolarization time per sec. Since the shape of the action potential was not fundamentally different at the various frequencies studied, results similar to those illustrated in Fig. 6*B* exist at other depolarization levels. The rationale behind the choice of −40 mV for determination of the total depolarization time per sec was the previous demonstration that an inward calcium current is activated at this level (Beeler & Reuter, 1970*a*; Ochi, 1970; New & Trautwein, 1972).

TABLE 1. Resting membrane potential, action potential duration at −40 mV level and overshoot at different stimulation frequencies. Mean ± S.E. of mean for six experiments

Stimulation frequency (c/s)	Membrane resting potential (mV)	Action potential duration (msec)	Overshoot (mV)
0·25	−77·0 ± 2·7	89·5 ± 7·0	18·8 ± 0·9
1	−80·4 ± 1·9	115·8 ± 8·6	23·3 ± 1·5
2	−75·2 ± 2·2	109·3 ± 10·9	21·9 ± 1·4
3	−71·6 ± 2·8	101·3 ± 12·0	18·9 ± 3·3
4	−72·3 ± 1·8	88·5 ± 11·9	18·2 ± 2·5
5	−67·2 ± 3·0	82·0 ± 7·9	19·5 ± 2·0

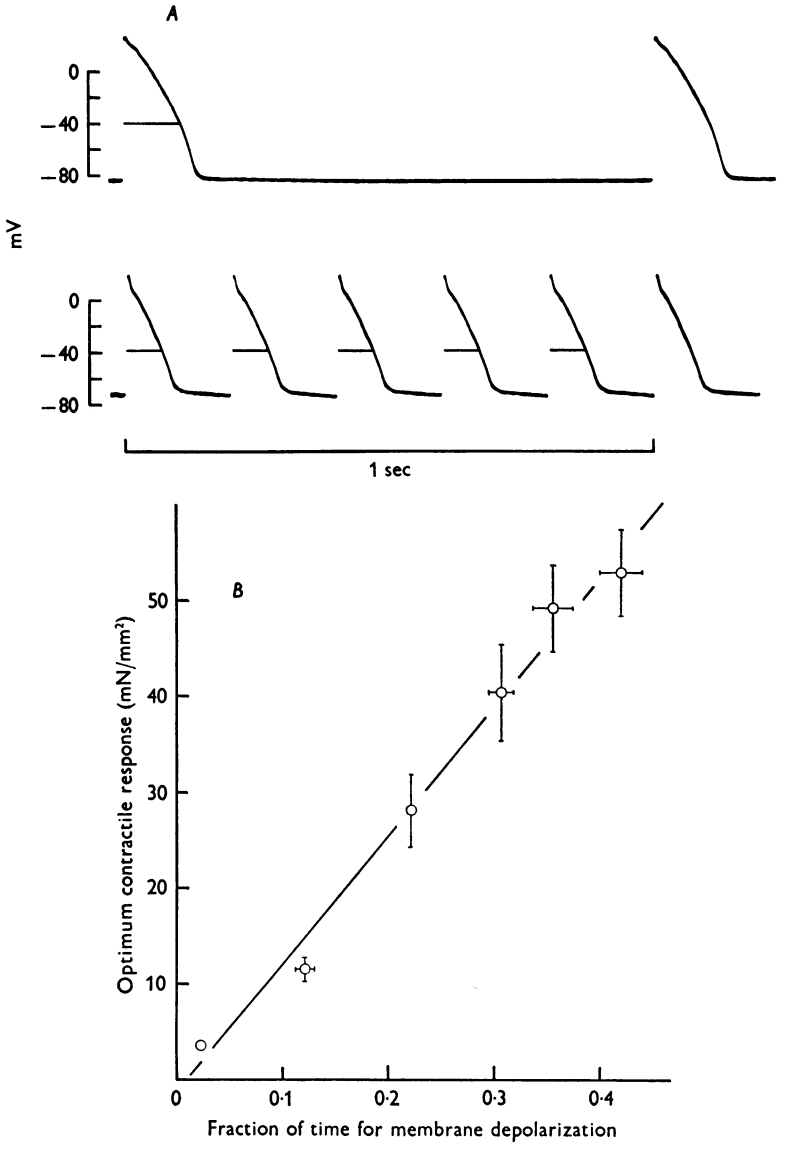

Fig. 6. *A*, oscilloscope traces illustrating action potentials at stimulation frequencies of 1/sec (upper) and 5/sec (lower). The time during which the cell membrane is depolarized beyond the −40 mV level during a 1 sec interval is indicated by horizontal bars. *B*, relationship between optimum contractile response and the fraction of time during which the cell membrane is depolarized beyond −40 mV at stimulation frequencies varying between 0·25 and 5/sec. Each symbol denotes the mean of six experiments. Vertical and horizontal bars indicate S.E. of mean. The straight line represents the least-squares fit to the data (r = 0·993).

DISCUSSION

Steady-state force and optimum contractile response

Several attempts have been made in the past to analyse the force–frequency relationship in cardiac muscle as being the net result of a positive and a negative effect of activation (Koch-Weser & Blinks, 1963; Rumberger & Reichel, 1972). According to these views the contractile force of myocardium increases with stimulation frequency as long as the positive inotropic effect of activation (PIEA, Koch-Weser & Blinks, 1963) increases more than the negative inotropic effect of activation (NIEA, Koch-Weser & Blinks, 1963). Similarly the decline in force that occurs at supraoptimal stimulation frequencies reflects a greater increase of NIEA than of PIEA due to the increase in stimulation rate.

In the present analysis of the force–frequency relationship the assumption is made that activation of cardiac muscle only results in a *positive* inotropic effect. However, a finite time is assumed to be required for the full development of this effect. The time course of development of the positive inotropic effect was determined by measuring the isometric twitch response to a test stimulus that was applied at different times after a preceding stimulation period. Optimum contractile response was obtained approximately 0·8 sec after the last stimulus. Peak value of this response at any given frequency was attained as the isometric twitch amplitude reached a steady-state level during the priming period. This occurred after nearly the same number of responses (10–15) at different frequencies above the control rate (1/sec), and no further increase of the optimum contractile response was obtained by prolonging the priming period beyond this point. The optimum contractile response increased steadily with the stimulation frequency and reached its highest value at 20 c/s a.c. stimulation.

Cellular mechanisms of twitch potentiation

Evidence has been presented (Wood, Heppner & Weidmann, 1969; Lee, Mainwood & Korecky, 1970; Gibbons & Fozzard, 1971; Bassingthwaighte & Reuter, 1972; Tritthart, Kaufmann, Volkmer, Bayer & Krause, 1973; Morad & Goldman, 1973) favouring the idea that the excitation–contraction process involves two distinct cellular compartments for calcium. Fig. 7 illustrates a tentative model for the metabolism of activator calcium based on the previous results. The calcium entering the cell during the action potential (Beeler & Reuter, 1970*a*; Ochi, 1970; New & Trautwein, 1972) is assumed to be largely taken up by an intracellular compartment I, with only a minor portion reaching the contractile proteins to initiate mechanical activation. The fact that the first contraction after a long

pause is very small (approximately one-tenth of the steady-state twitch tension recorded at optimal frequency, Fig. 3) is concordant with this view. Calcium is assumed to be transported from compartment I to another cellular compartment, II, from which calcium can later be released into the myofibrillar space during an action potential. The calcium that is released from compartment II activates the contractile system, the peak twitch tension being related to the amount of calcium released. The released calcium is taken up during relaxation by compartment I and from this temporary storage site it would be transported to compartment II, to be utilized again for activation of the contractile system. It is

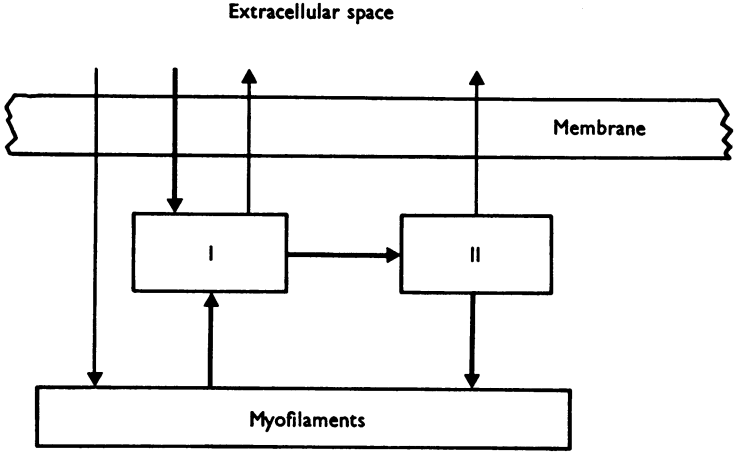

Fig. 7. Model of the calcium metabolism in the myocardial cell. Arrows indicate calcium movement. For further explanation, see text.

assumed that there is a continuous loss of calcium from compartments I and II out of the cell. The precise location of the two compartments is unclear. It has been proposed (Bassingthwaighte & Reuter, 1972; Morad & Goldman, 1973), analogous to the situation in skeletal muscle (Winegrad, 1968), that the longitudinal components of the sarcoplasmic reticulum in the myocardium are responsible for the immediate uptake of the free cellular calcium (compartment I, Fig. 7). On the same basis it has been suggested that the releasable store of calcium (compartment II, Fig. 7) is located in the lateral sacs and subsarcolemmal cisternae.

The finding that the optimum contractile response is steadily increased by raising the stimulation frequency is explainable in terms of the model of calcium metabolism presented above. The results have shown that over the wide range of stimulation frequencies investigated (0·25–5/sec) the optimum contractile response is linearly related to the fraction of time

during which the membrane is kept depolarized by the action potential (Fig. 6*B*). An increase in stimulation frequency will increase the duration of membrane depolarization per unit of time and this could lead to an increased inflow and accumulation of calcium in the cellular pool. According to the scheme presented in Fig. 7 this would result in an increased content of calcium in compartment II, provided that enough time were allowed for the uptake (see below). This would lead to a greater release of activator calcium into the myofilament system during the action potential and a potentiation of the mechanical response. That a rise in stimulation frequency is paralleled by a greater inflow of calcium and an increased content of calcium in the myocardial cell has been reported previously (Langer, 1965; Sands & Winegrad, 1970). It is important to emphasize that a change in stimulation rate does not markedly affect the membrane conductance of calcium (Beeler & Reuter, 1970*b*). The increased inflow of calcium recorded at a higher frequency is therefore most likely attributable to the increased fraction of time occupied by membrane depolarization.

The time interval for attainment of the optimum contractile response was 0·8 sec, i.e. more than 5 times the total action potential duration, and it was found to be very nearly the same at several different stimulation frequencies, including a.c. stimulation. If it is assumed that the release of calcium from compartment II takes approximately 0·1 sec (the action potential duration at -40 mV), then the remaining 0·7 sec would represent the time required for recirculation of calcium, i.e. for the transport to the contractile system, to compartment I and back to compartment II. This is a considerably longer time than that needed (approximately 0·2 sec) for complete relaxation of the muscle. It is therefore reasonable to conclude that calcium after being released into the myofibrillar space is first taken up by a cellular structure that is different from the releasable store. A possibility which cannot yet be excluded would be that the cellular store becomes refractory for release of calcium after the action potential and that the 0·8 sec required for the optimum contractile response reflects the time for complete reactivation of the release mechanism.

The disappearance of twitch potentiation

The increase in contractile strength induced by stimulation of the cardiac muscle disappears exponentially in two distinct phases. The time constants of the two phases were found to be 2·6 and 92 sec, respectively, yielding a half-time for the entire course of disappearance of 62 sec (data refer to potentiation by a.c. stimulation). A similar rate of disappearance (half-time of 50–100 sec) has been reported for twitch potentiation produced by longlasting depolarization pulses (Wood *et al.* 1969; calf and sheep ventricular fibres) and by isotonic shortening of cardiac muscle (Jewell &

Rovell, 1973; cat papillary muscles). It is reasonable to assume that the decay of twitch potentiation reflects the disappearance of calcium from the releasable store (compartment II, Fig. 7). The fact that the twitch potentiation can be repeated numerous times seems to imply that calcium, after leaving the releasable store in the resting muscle, is eventually transported out of the cell. This accords with the view that there exists a calcium pump in the cell membrane that transports calcium out of the cell in exchange of sodium (Glitsch, Reuter & Scholz, 1970). The two-phasic decline of the contractile strength suggests that two different transport mechanisms are involved in the elimination of calcium from the store. Alternatively, the releasable calcium store may be composed of two separate cellular structures with different affinities for calcium.

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