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The force-frequency relationship in rat myocardium

The influence of muscle dimensions

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Abstract. Peak force and membrane potential were recorded from papillary muscles and trabeculae excised from the ventricles of adult rat hearts. Experiments were performed at 2.5 mM Ca^{2+} and 26° C. In thick preparations (diameter 0.2–1.2 mm) an increase of stimulation frequency caused a reduction of peak force and action potential duration as has been found in many studies previously. In thin preparations (diameter < 0.2 mm) both peak force and action potential duration were almost independent of stimulation frequency. When the flow of Tyrode solution through the muscle bath was reduced an increase of stimulation frequency caused a reduction of peak force and action potential duration in thin preparations. We conclude that the reduced peak force and action potential duration in papillary muscles at high stimulation frequencies is due to insufficient exchange of metabolites and oxygen between the medium and the core of the muscle. The results indicate that the critical diameter for the preparations is about 0.2 mm.

Key words: Myocardium – Contractility – Ischemia – Stimulation frequency

Introduction

Preparations of mammalian myocardium generally show a positive force staircase upon a stepwise increase of stimulation frequency. The result is a high contractility at high frequencies in myocardium of cat (Brutsaert 1967; Allen 1977; Boyett 1978; Maylie 1982), sheep (Braveny and Sumbera 1970), guinea pig (Sleator et al. 1964; Rumberger and Reichel 1972; Schulze 1981) and rabbit (McCans et al. 1974; Edman and Johannsson 1976). In contrast, a negative force staircase has been consistently reported for rat myocardium. The negative force-frequency relationship has been regarded as a genuine property of that species (Hoffmann and Kelly 1959; Forester and Mainwood 1974; Siegl and McNeill 1981).

We could, however, not reproduce the negative force-frequency relationship in thin trabeculae of the right ventricle of adult rat. The present study revealed that the frequency response of rat ventricular preparations depends strongly on the diameter of the preparation.

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Methods

Three types of ventricular preparations were isolated from rat heart: papillary muscles of the left ventricle with a diameter of about 1 mm, and of the right ventricle with a diameter of 0.15 to 0.5 mm, and right ventricular trabeculae with a diameter of 0.06 to 0.25 mm. The diameter was measured from the middle part of the preparation with a dissection microscope and occular micrometer. When the cross-section of the preparation was oval only the shorter diameter was measured. In several experiments 3 or 4 preparations from one heart were used. In total 51 preparations from 37 hearts were used. The preparation was suspended in a 1.5 ml perfusion bath between the lever of a force transducer and a length adjustment device. The solution had the following composition (in mM): 120 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2.0 NaH_2PO_4, 1.2 Na_2SO_4, 27 $NaHCO_3$, 10 glucose. The solution was recirculated via a 0.5 l reservoir which was in equilibration with 95% O_2 and 5% CO_2. The flow through the muscle bath was 10 ml/min, pH was 7.35–7.45 and temperature was 26 ± 0.3° C. Stimulus pulses of 3 ms duration and of a strength of 50% above threshold were derived from a programmable timing unit via a stimulus isolator and two platinum wire electrodes. The intracellular potential was recorded by means of glass microelectrodes with a long (3 mm) and thin (20 μm) flexible shaft. Stable impalements of a single cell frequently lasted several hours. The resistance of the microelectrodes filled with 3 M KCl was 70–150 MΩ in the physiological salt solution. In combination with a preamplifier with capacitance compensation the response time was about 0.3 ms. The potential signal was fed into an action potential analyser which produced at its outputs resting membrane potential (V_{rest}) sampled 20 ms prior to the stimulus pulse, and a voltage proportional to the duration of the action potential at 20% of its amplitude (APD_{20}). The experimental protocol consisted of an equilibration period of one h at a stimulation frequency of 0.2 Hz, followed by a frequency series as indicated in Fig. 1. After mounting the muscle was stretched to a length where passive force was 2–5% of active force.

Results

During the frequency series peak force and often membrane potential were continuously recorded. Figure 1 shows typical results from three preparations from one heart. Peak force of thin trabeculae was almost constant over the entire frequency range from 0.03 to 3.3 Hz. APD_{20} was constant from 0.03 to 0.33 Hz. The increase from 0.1 to 1.0 Hz caused a transient

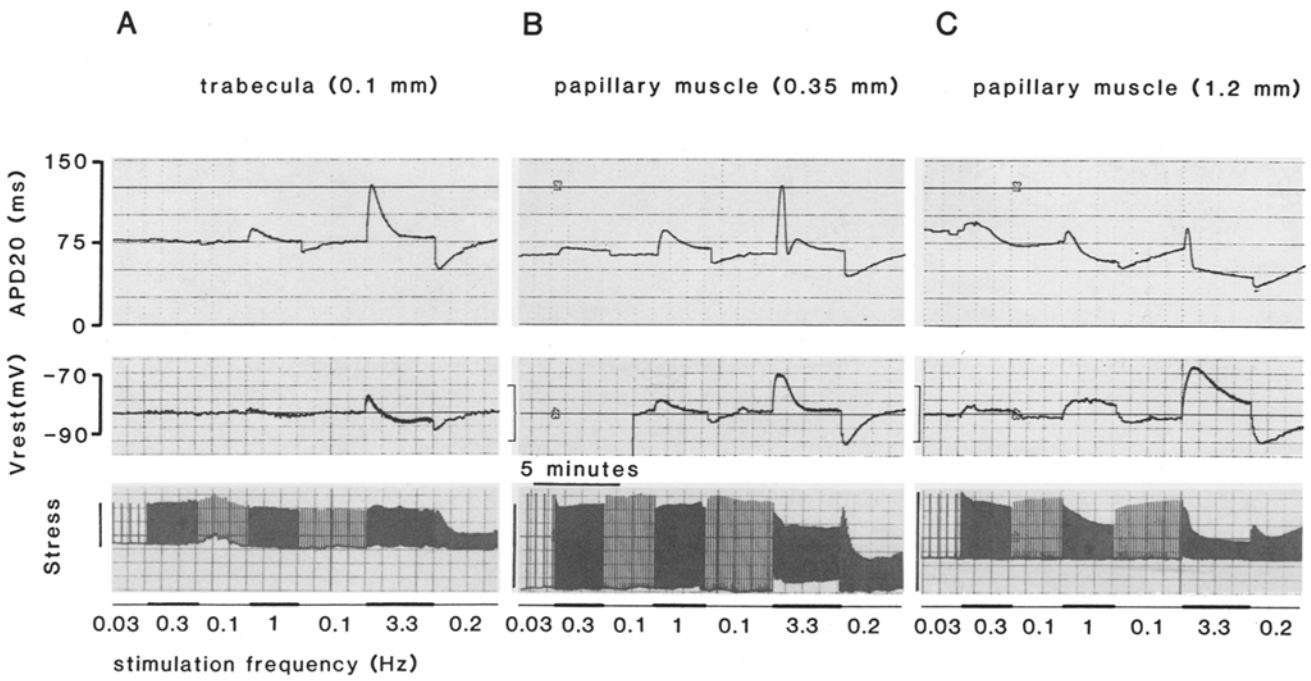

Fig. 1A–C. Frequency response of ventricular preparations of rat heart. Three preparations from one heart were used for this experiment: a right ventricular trabeculae (**A**), a right ventricular papillary muscle (**B**), and a left ventricular papillary muscle (**C**). Stimulation frequency was varied from 0.033 to 3.3 Hz, as indicated below the force traces. The traces show continuous recordings of APD_{20} (*upper panels*), V_{rest} (*middle panels*) and force (*lower panels*). The bar at the left of each force trace represents a stress of 50 mN/mm^2

prolongation of APD_{20} and a small depolarization of the membrane. The same type of transients, however more pronounced, were observed upon an increase of frequency from 0.1 to 3.3 Hz. The steady state APD_{20} was slightly prolonged and the membrane was hyperpolarized at 3.3 Hz (Fig. 1a). Upon the change from 3.3 Hz to 0.2 Hz the opposite transients in V_{rest} (hyperpolarization) and in APD_{20} (shortening) were observed, and peak force was depressed. V_{rest} and APD_{20} returned to the steady state values within 3–5 min, whereas the depression of peak force lasted about 15 min. In right ventricular papillary muscles the increase of frequency from 0.1 Hz to a higher frequency initially resulted in about the same peak force, but after 10–20 s a decline occurred. The transient prolongation of APD_{20} and depolarization of the membrane were present, but the time course was significantly different from that in thin trabeculae. The transient depolarization was much larger, and lasted longer, and in the steady state at 3.3 Hz the hyperpolarization was absent. The transient prolongation of APD_{20} at 3.3 Hz showed two peaks (Fig. 1b). Stimulation frequency had more drastic effects on left ventricular papillary muscles. After 10–25 s at 3.3 Hz peak force rapidly dropped to about 30%, the membrane depolarized by about 20 mV and did not recover within 4 min, and the transient prolongation was followed by a gradual shortening during the next 4 min. Similar mechanical (electrical) data were obtained from 33 (18) trabeculae, 16 (12) right papillary muscles and 2 (2) left ventricular muscles. The stress (mean ± standard deviation) developed by the muscles was: 56 ± 21 mN/mm^2 in trabeculae and 40 ± 9 mN/mm^2 in papillary muscles.

In Fig. 2 steady state peak force at 3.3 Hz is plotted, as a fraction of peak force at 0.1 Hz, against the diameter of the preparations. Obviously there is a critical diameter of 0.2 mm or less, beyond which peak force shows frequency

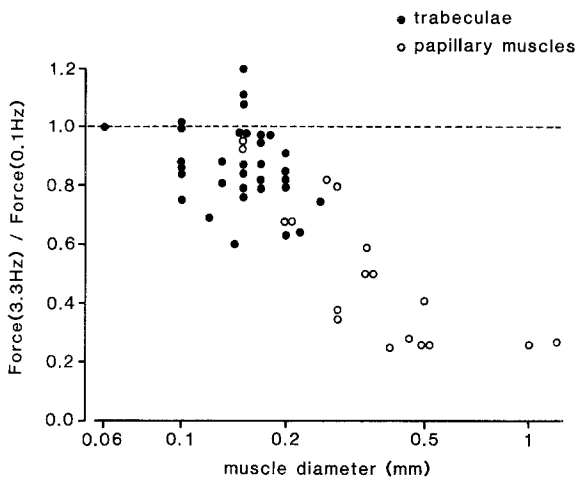

Fig. 2. Steady state peak force at 3.3 Hz as a function of the diameter of the preparations. Peak force is given as a fraction of peak force at 0.1 Hz. Muscle diameter is given on a logarithmic scale

induced depression. The results indicate that the frequency response does not depend on a fundamental difference between trabeculae and papillary muscles because thin papillary muscles behaved like trabeculae with the same diameter. The most likely explanation is that the thicker preparations suffered from inadequate diffusion of metabolites and oxygen to and from the core of the muscle. In order to test this hypothesis the influence of zero flow of Tyrode on three thin trabeculae was studied. Figure 3 shows a typical result. First the effect of a stepwise increase of stimulation frequency from 0.2 to 3.3 Hz was recorded when the flow of Tyrode was 10 ml/min (Fig. 3A). Then the flow

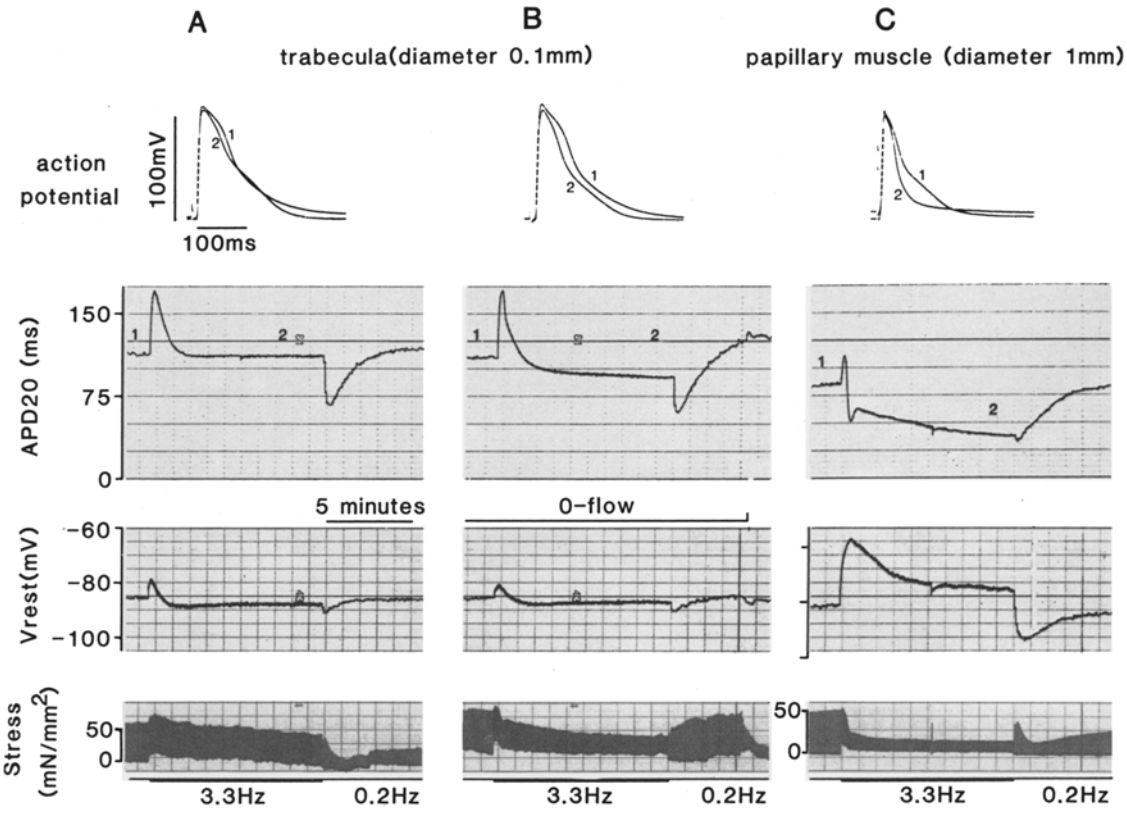

Fig. 3A–C. Influence of muscle diameter and flow on the frequency response of ventricular preparations of rat myocardium. A thin trabecula (diameter 0.15 mm) was paced at 0.2 and 3.3 Hz when the flow of the superfusate was 10 ml/min (**A**) and when the flow was zero (**B**). Steady state action potentials at 0.2 Hz and at 3.3 Hz are shown in the *upper panels*. The *other panels* show APD_{20}, V_{rest} and force. The numbers near the action potentials correspond to the numbers on the APD_{20} trace. In **C** the response of a papillary muscle (diameter 1.0 mm) from the same heart is shown

was reduced to zero, which had no significant effect at a stimulation frequency of 0.2 Hz. At 3.3 Hz, however, peak force decreased to 40% and the transient prolongation of APD_{20} was followed by a slow shortening which continued for 10 min or more (Fig. 3B). It was remarkable that, at zero flow, the change from 3.3 Hz to 0.2 Hz was not followed by a transient depression of peak force. If the flow was switched on after a few minutes, then a delayed depression was observed (compare Fig. 3A, B). With respect to the reduced peak force and the continuous shortening of APD_{20} at 3.3 Hz the thin trabeculae at zero flow were comparable to the thick papillary muscles (compare Fig. 3B, C).

Discussion

The reduction of peak force and APD_{20} in rat myocardium at high stimulation frequencies was shown to depend on muscle diameter (Fig. 2). Probably the long diffusion pathway caused depletion and accumulation of different metabolites in the core of the papillary muscles. Some effects of global ischemia, hypoxia or inhibitors of glycolysis have been described in literature. A common finding is reduced contractility and shortening of the action potential (McDonald et al. 1971; Vleugels et al. 1976; Alvarez 1981; Marshall et al. 1981; Senges et al. 1981; Weiss and Shine 1982). Intracellular acidosis also causes a reduction of contractility (Jacobus et al. 1982) and shortening of the action potential (Kurachi 1982). It is in accordance with these facts to ascribe the reduced force and APD_{20} at high frequencies to hypoxia and acidosis in papillary muscles and in thin trabeculae at zero flow. In Fig. 4 the mean value of peak force is given of 25 preparations with a diameter of 0.2 mm or less, and of 5 preparations with a diameter of 0.40 mm or more. In the same figure literature data of papillary muscles are plotted. From this figure it is clear that all papillary muscle data are similar. We conclude that the negative inotropic effect of stimulation frequency often reported for rat myocardium is typical for ischemic preparations. The critical diameter of 0.2 mm for rat myocardium (Fig. 2) is relatively small. For myocardium of other mammalia values of 0.5–1.2 mm have been reported (Paradise et al. 1981; and references therein). The critical diameter may depend on experimental conditions and species. In this respect it is important to note that contractility of isolated myocardium of rat is much higher than that of other species (Allen and Kurihara 1980) and thus the rate of energy metabolism may be much higher.

It was remarkable that a decrease of stimulation frequency from 3.3 to 0.2 Hz caused a depression of peak force (Figs. 1 and 3). The nature of this phenomenon is unclear.

It has been reported that the action potential of rat myocardium is shortened with increasing stimulation

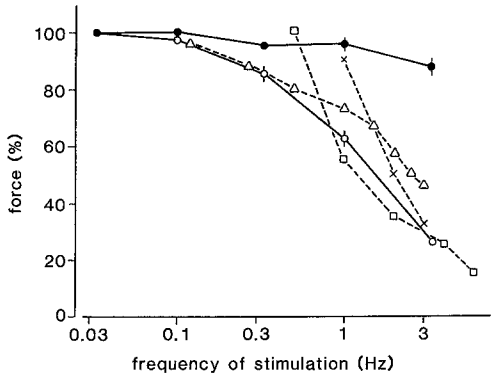

Fig. 4. Force-frequency relationships of rat myocardium. (●——●) mean and standard deviation of the mean of 25 preparations with a diameter less than 0.2 mm. (○——○) mean and standard deviation of the mean of five papillary muscles with a diameter of 0.4 mm or more; (□-----□) data from Hoffmann and Kelly (1959) from left ventricular papillary muscles at 2.7 mM Ca^{2+} and 37°C; (△-----△) data from Forester and Mainwood (1974) from left ventricular papillary muscles at 2.5 mM Ca^{2+} and 26°C; (×-----×) data from Siegl and McNeill (1981) from papillary muscles at 1.0 mM Ca^{2+} and 37°C. Peak force at the lowest stimulation frequency applied in each study was taken as 100% in this figure

frequency (Keung and Aronson 1981). Figures 1 and 3 evidence that such shortening is typical for thick (i.e. ischemic) preparations. Boyett and Jewell (1978) argued that action potentials of superficial fibers would not be affected by the hypoxic state of the core of the muscle. Obviously this hypothesis does not apply to rat myocardium. We recorded action potentials from superficial cells only, and although these cells were in close contact with well oxygenated Tyrode, their action potentials showed signs of insufficient transport of oxygen in thick preparations (Figs. 1, 3). Our results indicate that thin trabeculae are suitable preparations for the in vitro study of electrical and mechanical properties of heart muscle at high frequencies of stimulation.

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