Effect of substrates on the mechanical performance of rhesus monkey papillary muscle

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Summary. This study examines the effect of different substrates on mechanical performance of excised papillary muscles from rhesus monkeys which had been divided into a control group and an experimental group fed a high fat diet for 5 months prior to sacrifice. The results show that performance is affected by available substrate for both groups. The performance of the experimental group was depressed relative to control with the short chain fatty acid, butyrate (C4), producing a monotonically decreasing force-frequency response. Relative to other mammals, isolated rhesus papillary muscles exhibited a protracted treppe which was sensitive to β-adrenergic blockade with propranolol.

In addition to exhibiting clear preferences in substrate utilization[2], the mechanical performance of the mammalian myocardium is in part determined by the available exogenous substrate[3]. With adequate oxygen, the heart will preferentially use fatty acids over glucose[4]. However, under ischemic or hypoxic conditions, fatty acids may contribute to the decline in myocardial function[5,6]. Thus high levels of endogenous fatty acids may be deleterious to the challenged heart. In addition to these effects, the available substrates influence both the mechanical and metabolic performance of the well oxygenated myocardium. The peak developed tension[7], resting heats and heats of activation[8] are affected by the available substrate. Studies have also shown that both the resting redox state of the respiratory chain[3] and the extent of oxidation accompanying increased activity[7] are influenced by the exogenous substrate.

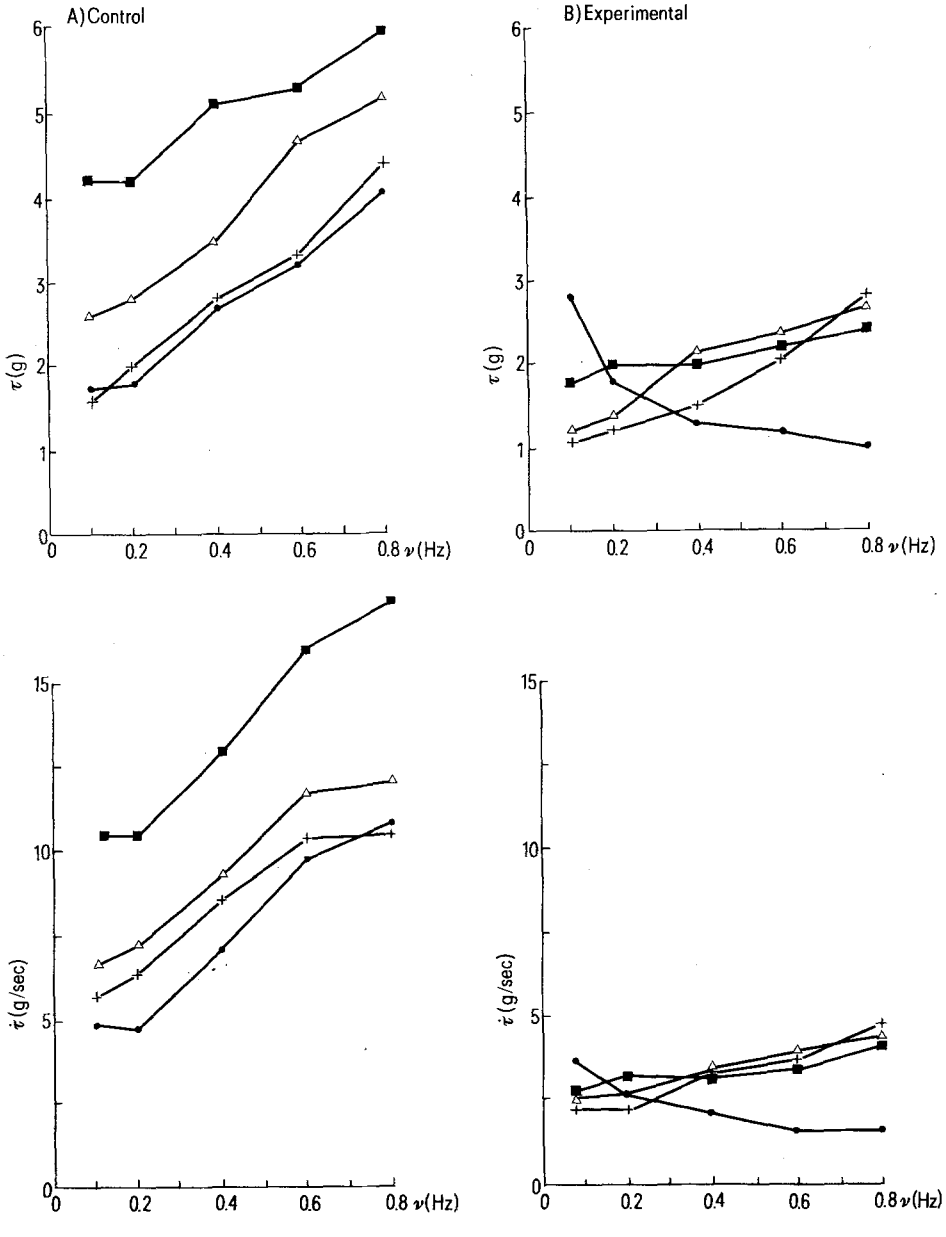

Fig. 1. Force frequency (τ vs ν) response of a rhesus monkey papillary muscle from the control group and from the experimental (high fat diet) group. ■ = pyruvate, △ = lactate, + = glucose and ● = butyrate. Concentration for all substrates was 10 mM.

In fact, recent studies with isolated mitochondria have shown that the PO_2 (50%) for cytochrome reduction is influenced by the available substrate[9].

The results from these studies show that in addition to providing the energy necessary for ADP rephosphorylation, substrates play an important role in regulating myocardial performance. However, there is a question as to the applicability of these results to the performance of the primate myocardium. The studies described here examine this question by determining the mechanical performance as a function of substrate in rhesus monkey papillary muscle. In addition, these studies also examine the change in the relation between available substrate and mechanical performance induced by chronic administration of a high fat diet. Since cyclic AMP plays such an important role in metabolic and mechanical performance[10], the effect of β-adrenergic blockade on function was also examined in a subset of the muscles used.

Methods. The papillary muscles used in this study were obtained from adult rhesus monkeys. In addition to providing tissue for this study, the animals also provided data on the difference in transplanted vascular patency between the control group and the experimental ones. As such, all animals had successfully undergone iliac arterial bypass surgery at least 5 months prior to sacrifice. The monkeys used in this study were randomly divided into 2 groups: a control group (n=6) fed standard monkey chow and an experimental group (n=4) fed a high fat, athrogenic diet containing 0.5% cholesterol, 25% lard, 13% cassein and other nutrients[11].

The monkeys were anesthetized with ketamine and then halothane, the peritoneal cavity opened and the abdominal iliac artery graft removed. During this period, the animal continued breathing oxygen and halothane on its own. Following removal of the graft, a thoracotomy was performed, the aorta cross clamped, the heart removed and one of the papillary muscles excised from the right ventricle (Δ t<2 min). The muscle was tied at both ends with 6×0 silk suture, immersed in oxygenated (P_{O_2}=550 Torr), HCO_3 Ringer and transported to the laboratory. The muscle was then vertically mounted in a bath (T=37 °C), with the septal end secured and the tendonous end attached to a isometric force transducer. The maximum time from thoracotomy to mounting of the muscle was 14 min. The bath solution was HCO_3^- Ringer with 10 mU/cm^3 insulin and 1 of 4 substrates (10 mM): glucose (G), pyruvate (P), lactate (L) or butyrate (B). Insulin has been shown to affect papillary muscle mechanical performance to different degrees depending upon the exogenous substrate present[12]. The level of insulin used is on the plateau region of the dose response curve for all of the substrates. The substrate concentration was selected for a similar reason. Under a 1 g load, the mean diameter of the papillary muscles in the control group was 1.8 mm (0.8-3.6 mm) and the experimental group 1.5 mm (0.8-2.1 mm). The control mean weight was 8.5 mg (2.1-12 mg) and the experimental was 6.8 mg (4.3 and 9.4 mg).

Although several of the muscles used in this study had diameters greater than 1.0 mm, there is no evidence of the development of a hypoxic core. If a hypoxic core did develop, then the τ vs ν curve would be expected to peak and begin to decrease as the frequency of stimulation is increased[13]. Such a turnover of the τ vs ν curve, indicative of hypoxia, was not observed in any of the muscles tested. The failure of a hypoxic core to develop in these experiments is consistent with the results from more thorough studies of the relation between papillary muscle diameter and the development of a hypoxic core[13,14].

The experimental protocol was an equilibration period (30 min) followed by a test period. This was repeated for each of the 4 substrates with the order of substrate presentation randomly varied for each muscle. During the equilibration period, the muscle was continuously stimulated at a frequency of 0.1 Hz. The test period was comprised of a sequence of stimulation trains (20 twitches/train at ν=0.1-0.8 Hz) separated by a quiescent period (1 min). Following the test period, the bath was changed to one containing a different substrate and the next equilibration period begun. For each muscle, the order of presentation of the stimulation frequencies was identical for all substrates[15].

Abbreviations used are: $\dot{\tau}$ = maximum developed isometric tension; $\dot{\tau}_{max}$ = maximum time derivative of τ; TPT = time to peak tension and ν = frequency of stimulation.

Results. As figure 1 shows, different substrates affected the contractile performance in both the control and experimental group. While this difference was evident for each muscle tested, the order of the substrate effect on contractility was not consistent in either group. In addition, for each muscle, the different substrates produced identical changes in the relation $\dot{\tau}_{max}$ vs ν as they did on τ vs ν. Although there was no consistent substrate effect on τ vs ν or $\dot{\tau}_{max}$ vs ν within either group, there was a reproducable effect upon the time to peak tension (TPT) - table.

In comparing figure 1, A and B, 2 points are evident. First, for the glycolytic substrates (G, P and L), the slope of both the τ vs ν and $\dot{\tau}_{max}$ vs ν curves were flatter for the experimental group than for the control group. Second, for the experimental group both curves monotonically decrease as a function of ν with butyrate as the substrate while they increase in the control group. This functional anomaly was observed for all 4 experimental muscles and was never present in the controls.

One other consistent observation is shown in figure 2. While most mammalian papillary muscles exhibit a treppe lasting 8-10 contractions, the muscles of both groups required more than 20 twitches to reach a steady state. In 4 of the control muscles, propranolol (0,5 µM) was added. As is evident from figure 2, B, this produced a slight decrease in developed force and, more importantly, a prolongation of the treppe.

Discussion. Although there is no coherent difference in

	Control	Experimental
Glucose	0.44±0.06	0.44±0.04
Pyruvate	0.61±0.07	0.52±0.06
Lactate	0.53±0.09	0.47±0.09
Butyrate	0.57±0.09	0.58±0.04

Mean time to peak tension (TPT) in sec±SEM as a function of the available exogenous substrate (10 mM). Control: n=6; Experimental: n=4.

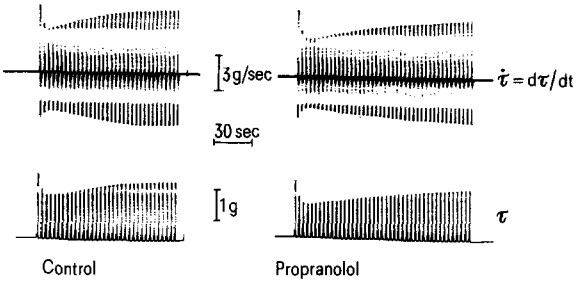

Fig.2. Mechanical response of control rhesus papillary muscle in the absence and presence of propranolol [0.5 µM]. Upper trace is time derivative of the twitch tension ($d\tau/dt$); lower trace is the isometric twitch tension (τ). Substrate=10 mM glucose; frequency of stimulation=0.6 Hz.

mechanical response elicited by the different substrates, the data clearly demonstrates that mechanical performance is affected by the available exogenous substrate in the primate papillary muscle. Not only is τ affected, but the basic parameters ($\dot{\tau}_{max}$ and TPT) which determine the developed tension are also sensitive to the available substrate. Although the method(s) by which substrates modulate mechanical performance remains unclear, the data presented here along with those of other studies[7] demonstrate that a functional dependence exists between mechanical performance and the available substrate.

A striking feature of the data presented in figure 1,B is the negative slope of the butyrate curves (τ vs v and $\dot{\tau}_{max}$ vs v). This occurred for all experimental muscles. Since butyrate was the 2nd or 3rd substrate presented, the negative slope cannot be ascribed to either premature testing during the initial equilibration period or excessive muscle fatigue at the end of the experiment. Instead it would appear that the cardiac reserve in the experimental animals is compromised when presented with a short chain fatty acid as the substrate.

The mammalian myocardium preferentially metabolizes long chain ($\geq$C16) fatty acids[2]. Since butyrate is a short chain fatty acid (C4), the results presented here may not be applicable to the in-vivo heart. However, both long and short chain fatty acids are catabolized via β oxidation and the TCA cycle with only the long chain carnitine transport system being different. This system may[16,17] or may not[18] be directly responsible for the dysfunction during ischemia. Since butyrate does not require this labile transport system and is catabolized via the same pathway as the long chain fatty acids, the data presented here should be applicable to the more physiological in situ situation. These data indicate that the chronic ingestion of an athrogenic diet fundamentally changes the relation between available substrate and mechanical performance.

2 points should be made about the data shown in figure 2. First, unlike most excised mammalian papillary muscle, the treppe continues well beyond the tenth contraction. The cause of the treppe response in the mammalian myocardium is not well understood but may be related to mobilization of sarcolemmal calcium[19,20]. Thus, the protracted treppe may indicate that rhesus monkey papillary muscles mobilize sarcolemmal calcium differently from most other mammals. The 2nd point is that in addition to its slight negative inotropic effect, β-adrenergic blockade by propranolol extends the treppe response even longer. This suggests that there is at least some adrenergic involvement in the protracted treppe.

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Is tubulin involved in the electrically-induced mechanical activity of the isolated rat sciatic nerve?[1]

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Summary. The electrically-induced mechanical activity of isolated segments of rat sciatic nerves remains unaffected following incubation with 10^{-4} M colchicine, vinblastine or melatonin. Vinblastine depressed tubulin levels in incubated nerves. These results suggest that microtubules are not involved in nerve mechanical activity in vitro.

In previous studies we documented a distinct electrically-induced mechanical activity in isolated segments of guinea-pig sciatric nerve[2]. Several reports have indicated the existence in nervous tissue of a relatively high concentration of tubulin, a microtubule protein, which accounts for 11-40% of the total soluble protein[3]. Since various types of intracellular movement, such as axoplasmic transport, have been attributed in part to the microtubules[4,5], it was decided to explore the possiblity of their role in the mechanical activity of isolated and electrically-stimulated nerve preparations.

Methods. Adult Wistar rats (250 g) were killed by decapitation and the distal part of the principal trunk of the sciatic nerve was dissected out. Segments 2 cm long were removed and manipulated as described earlier[2]. Nerve preparations were suspended in Krebs-Ringer bicarbonate solution (KRB) containing 11.0 mM glucose. The medium was bubbled with a mixture of 95% O_2^- 5% CO_2 at a constant pH (7.4) and temperature (37 °C).

Electrical stimuli (square waves of 40 V, 3 msec duration and frequency of 20 Hz) were applied at 10 sec intervals. These values were selected following the previous testing of