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Tolerance of Isolated Cardiac Muscle to Hypoxia: Force-Frequency Interrelationships (38566)[1]

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(Introduced by W. H. Abelmann)

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During hypoxia cardiac muscle must rely on the relatively inefficient process of anaerobic glycolysis to maintain function and viability. Since heart rate is a major determinant of myocardial energy requirements, it might be expected that during hypoxia at more rapid heart rates, energy demands may exceed substrate supply with subsequent deleterious effects. In the present investigation we have systematically examined the effects of stimulation rate on the tolerance of isolated cardiac muscle to hypoxia. In addition to evaluating the ability of heart muscle to develop tension during hypoxia, we have observed the development of contracture and assessed the recovery of cardiac muscle function during reoxygenation after a 60-min period of hypoxia. In order to evaluate the relative importance of heart rate to other factors determining myocardial energy requirements during hypoxia, studies were also carried out using cardiac muscle from two mammalian species; one with a positive, and the other with a negative force-frequency relationship over the range of stimulation rates examined.

Materials and Methods. Following decapitation, the hearts of male Charles River CD^R rats weighing 150–250 g and guinea pigs weighing 400–600 g were rapidly removed and placed in oxygenated Krebs–Henseleit solution (1) at 30°. Trabeculae carneae and papillary muscles were carefully dissected from the left ventricle, mounted between two spring clips and placed in a chamber containing Krebs-Henseleit solution with 5.5 m*M* glucose. The solution was bubbled through with 95% O_2 and 5% CO_2 (pH 7.4), and the temperature was kept at 28° by a Lauda model K-2 circulating pump. The lower spring clip attached to the muscle preparation was connected to a Statham G7B-0.75-350 force transducer by a hollow stainless steel section of 30 gauge needle tubing, which passed through a mercury seal at the bottom of the muscle chamber. The upper clip was connected by a thin gold chain to a lever arm, above which a micrometer stop was mounted for the adjustment of muscle length. The preparations were stimulated 12 times per min by a Grass model S88 stimulator which delivered 7 msec square wave pulses through parallel platinum electrodes at voltages which were approximately 10% greater than the minimum required to produce a maximum mechanical response. After a 30-min period, during which the preparations were permitted to shorten while carrying light loads, the muscles were carefully stretched to the apices of their length-tension curves. The muscles contracted isometrically at this length for a 15-min period, and the first control value (C1) was obtained. Stimulation rate was then either maintained at 12/min or changed to 1 or 60/min. Contractions took place at this rate for an additional 15-min period. A second control value (C11) was obtained, and hypoxia was then initiated. Hypoxia was induced by bubbling the solution through with 95% N_2 and 5% CO_2. During reoxygenation the original 95% O_2, 5% CO_2 gas mixture was used, and the stimulation rate was always returned to a rate of 12/min. The *in vitro* length of each muscle preparation was measured at the apex of its length-tension curve using a Gaertner cathetometer and telescope. At the end of each experiment, the muscle between the two clips was blotted and weighed and the cross sectional area of the

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muscle calculated, assuming cylindrical uniformity and a specific gravity of 1.000. Tension was normalized for muscle cross sectional area, and absolute values for tension were calculated. During hypoxia, changes in mechanical activity were expressed as percent of prehypoxia control (C11). During reoxygenation, since stimulation rate was always 12/min, changes in mechanical activity were expressed as percent of C1 values. Mechanical events were obtained at a speed of 100 mm/sec on a Sanborn model 7700 recorder. Data from irritable and poor preparations were discarded. The following measurements were obtained and expressed in absolute terms and/or percent of prehypoxia control values: (a) Peak isometric tension (T), (b) maximum rate of tension development (dT/dt), (c) time from the onset of tension to peak isometric tension (TPT) and (d) contracture tension, defined as an increase in resting tension above control values.

Results. Although the cross sectional area of the muscles studied was similar, the intrinsic mechanical properties of guinea pig and rat left ventricular preparations differed considerably (Table I). At all stimulation rates examined, developed tension was significantly increased in rat muscle preparations in comparison to the guinea pig. Rate of tension development (dT/dt) was markedly greater while time to peak tension (TPT) was shortened. These interspecies differences tended to diminish at increasing stimulation rates. In addition to differences in the magnitude and duration of contraction, the force-frequency response of the two species was opposite in direction at the stimulation rates examined. Developed tension in the rat increased with decreasing stimulation rates while in the guinea pig the reverse was true (Fig. 1).

In Fig. 2, results from the hypoxia experiments are presented. When developed tension is expressed as a percent of prehypoxia control (C11), the decline in developed tension during early hypoxia was similar at comparable stimulation rates in the two species. With prolonged hypoxia, some differences between the rat and the guinea pig were observed. In rat preparations, at a stimulation rate of 60/min, contractile activity ceased after 30–45 min of hypoxia. In the guinea pig, at the same time and stimulation rate, preparations developed 5–10% of C11 tension. A more striking difference between the two species is seen in the con-

TABLE I. EFFECTS OF STIMULATION FREQUENCY ON THE MECHANICAL PROPERTIES OF ISOLATED RAT AND GUINEA PIG LEFT VENTRICULAR MUSCLE PREPARATIONS UNDER OXYGENATED CONDITIONS

CII Stimulation Rate		Muscle cross sectional area (mm^2)	T[c] (g/mm^2)		TPT[d] (sec)		dT/dt[e] (g/mm^2/sec)	
			C1[a]	C11[b]	C1	C11	C1	C11
1/min	Rat	0.79 ± 0.12	4.96 ± 0.52	5.91 ± 0.66	0.14 ± 0.01	0.13 ± 0.01	57.1 ± 5.9	76.5 ± 6.0
	n = 6		$P < 0.05$		NS		$P < 0.02$	
	Guinea Pig	1.12 ± 0.12	0.60 ± 0.11	0.20 ± 0.04	0.30 ± 0.01	0.38 ± 0.01	3.1 ± 0.7	1.2 ± 0.3
	n = 7		$P < 0.01$		NS		$P < 0.01$	
	R/GP		8.3	29.6	0.46	0.34	18	63
12/min	Rat	1.22 ± 0.12	4.50 ± 0.37	4.50 ± 0.37	0.13 ± 0.01	0.13 ± 0.01	59.2 ± 6.0	59.2 ± 6.0
	n = 13		NS		NS		NS	
	Guinea Pig	1.35 ± 0.16	0.88 ± 0.21	0.83 ± 0.19	0.32 ± 0.01	0.32 ± 0.01	3.7 ± 0.3	9.1 ± 1.0
	n = 5		NS		NS		NS	
	R/GP		5.1	5.4	0.41	0.41	13.8	14.1
60/min	Rat	1.05 ± 0.11	4.7 ± 0.43	2.79 ± 0.28	0.14 ± 0.01	0.11 ± 0.0	56.3 ± 7.0	38.1 ± 4.6
	n = 6		$P < 0.001$		$P < 0.01$		$P < 0.001$	
	Guinea Pig	1.17 ± 0.24	0.67 ± 0.07	1.31 ± 0.17	0.28 ± 0.01	0.22 ± 0.01	3.7 ± 0.3	9.1 ± 1.0
	n = 6		$P < 0.01$		$P < 0.02$		$P < 0.01$	
	R/GP		7.0	2.1	0.50	0.50	15.2	4.2

[a] C1 = initial stimulation rate = 12/min.
[b] C11 = new stimulation rate = 1, 12 or 60/min.
[c] T = developed tension.
[d] TPT = time to peak tension.
[e] dT/dt = maximum rate of tension development.

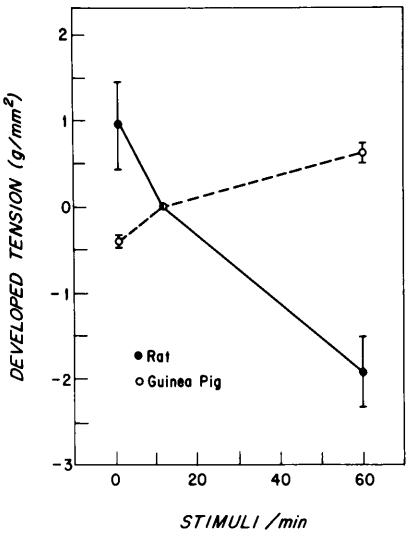

FIG. 1. Changes in isometric tension at different stimulation rates compared to the standard stimulation rate of 12/min. Studies are carried out in rat and guinea pig isolated left ventricular muscle preparations under well oxygenated (95% O_2, 5% CO_2) conditions. Brackets indicate mean ± SEM

tracture developed during hypoxia (Fig. 2, bottom). In the rat, contracture is seen at a stimulation rate of 1/min, and there is a progressive increase in contracture tension with increasing stimulation rate and with time. In the guinea pig, only the highest stimulation rates were associated with the development of contracture.

Following hypoxia, the rate of recovery of developed tension during the first 10 min of reoxygenation was more rapid in guinea pig than rat ventricular muscle (Fig. 3). At 20 min of reoxygenation, rat preparations at the three stimulation rates (during hy-hypoxia) had not recovered to 90% of control (C1) values. In the guinea pig, full recovery of developed tension upon reoxygenation took place following a stimulation rate of 1 and 12 but not 60/min during hypoxia ($P < .05$). Recovery from contracture was essentially complete by 30 min of reoxygenation in all muscle preparations from both the rat and guinea pig. This

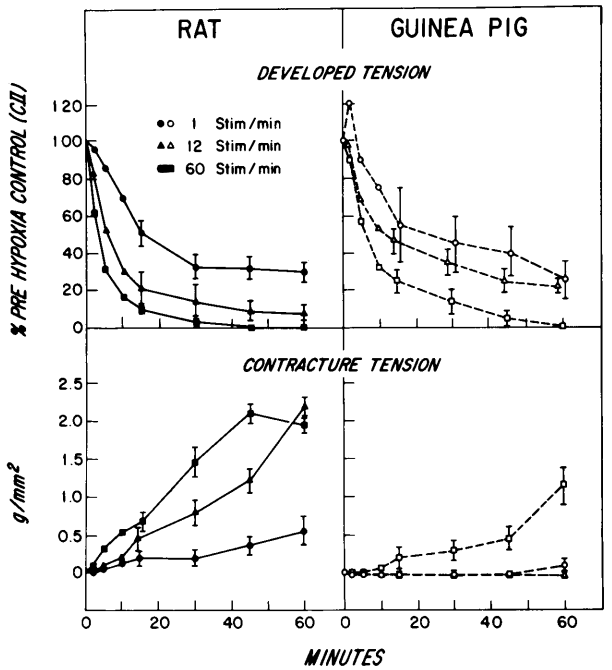

FIG. 2. Developed tension (top) and contracture tension (bottom) developed during 60 min of hypoxia at three different stimulation rates in isolated rat (left) and guinea pig (right) left ventricular muscle preparations. Brackets indicate mean ± SEM

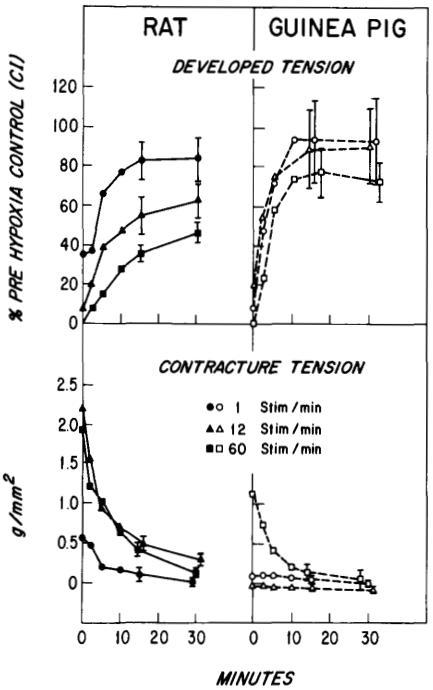

FIG. 3. Developed tension (top) and contracture tension (bottom) during reoxygenation following 60 min of hypoxia at three different stimulation rates in rat (left) and guinea pig (right) left ventricular preparations. Reoxygenation stimulation rate was 12/min in all experiments. Brackets indicate mean ± SEM

occurred irrespective of stimulation rate or severity of contracture during hypoxia.

Discussion. The primary objective of the present study was to examine the effects of heart rate on tolerance of cardiac muscle to hypoxia. An investigation of this question using the isolated muscle preparation offers the advantage of allowing direct control of most of the parameters influencing cardiac performance. A special problem is posed by a study of the effect of heart rate since it has been shown that frequency of stimulation will also alter the intrinsic contractile state of the myocardium (2). To help assess the roles of the two variables, heart rate and inotropic state, studies were carried out in two species with different force-frequency relationships over the range of heart rates studied. The importance of heart rate in determining tolerance to hypoxia is shown in these experiments by the more rapid decline in mechanical performance during hypoxia and the poorer recovery upon reoxygenation of rat ventricular preparations at increasing frequencies, despite the fact that inotropic state is diminished at more rapid stimulation rates.

The guinea pig is similar to most other mammalian species in that a positive force treppe is seen with increasing stimulation frequencies (Fig. 1). At a rate of 1, 12, and 60 contractions/min, left ventricular muscle preparations from that animal develop 3, 18, and 47% as much tension as the rat. The decreased level of tension developed by guinea pig preparations is primarily due to a decrease in the rate of tension development (Table I), suggesting that at relatively slower stimulation rates the intensity of the active state of guinea pig left ventricular myocardium is less than that of the rat. Since considerably greater tension is developed by rat ventricular muscle at slower stimulation rates, as might be expected, a more rapid deterioration takes place during hypoxia and poorer recovery is seen upon reoxygenation. At a rate of 60 min, the responses of the two species to hypoxia and reoxygenation appear more similar. Studies at higher stimulation rates were not carried out because of the possibility of core hypoxia in the control state at more rapid rates. In addition, at rapid stimulation rates the negative force-frequency response of rat ventricular muscle is no longer present (3).

Rat ventricular muscle is not only unusual in its force-frequency relationship. At a stimulation rate of 12/min, shortening velocity and rate of tension development are greater and contraction time is shorter than in other mammalian species such as the cat (4). Additional differences between the rat and other mammalian species include an abbreviated action potential plateau (5) and a reduced responsiveness to the digitalis glycosides (6).

The present study of two species clearly demonstrates that increased stimulation rates during hypoxia are associated with detrimental effects upon cardiac muscle function. Performance during hypoxia is diminished, contracture takes place more rapidly, and

recovery of mechanical activity upon reoxygenation is impaired. It is of interest to note that the contracture state which develops during hypoxia, although quite severe, is not irreversible. Despite the fact that contracture was present for 30–40 min during hypoxia at the more rapid stimulation rates and that contracture tension in some cases was greater than 50% of prehypoxia developed tension, a decline in contracture began almost immediately upon reoxygenation and was virtually complete after 30 min.

Summary. The performance of isolated left ventricular muscle preparations during 60 min of hypoxia (95% N_2, 5% CO_2) and 30 min of reoxygenation (95% O_2, 5% CO_2) was examined at differing stimulation rates (1, 12 and 60 stimuli/min). Since a change in inotropic state accompanies a change in cardiac rate, studies were carried out in two species with different force-frequency relationships over the heart rate range examined the rat and the guinea pig. Preparations were studied at the apices of their length tension curves while contracting isometrically 12 times a min at 28°.

At increasing stimulation rates, under oxygenated conditions, isometric tension fell in rat ventricular muscle while the reverse is true in the guinea pig. Despite the differing force-frequency responses, both species demonstrated progressively poor performance during hypoxia at increasing stimulation rates. This was manifested by a more rapid and greater decline in mechanical activity, more severe contracture during hypoxia and and slower recovery upon reoxygenation.

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