

THE EFFECTS OF ADRENALINE ON THE WORK- AND POWER-GENERATING CAPACITY OF RAT PAPILLARY MUSCLE *IN VITRO*

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Summary

The work loop technique was used to examine the effects of adrenaline on the mechanics of cardiac muscle contraction *in vitro*. The length for maximum active force (L_{\max}) and net work production (L_{opt}) for rat papillary muscles was determined under control conditions (without adrenaline). The concentration of adrenaline producing the maximum inotropic effect was determined. This concentration was used in the remainder of the experiments. Sinusoidal strain cycles about L_{opt} were performed over a physiologically relevant range of cycle frequencies (4–11 Hz). Maximum work and the frequency for maximum work increased from 1.91 J kg⁻¹ at 3 Hz in

controls to 2.97 J kg⁻¹ at 6 Hz with adrenaline. Similarly, maximum power output and the frequency for maximum power output (f_{opt}) increased from 8.62 W kg⁻¹ at 6 Hz in controls to 19.95 W kg⁻¹ at 8 Hz with adrenaline.

We suggest that the power–frequency relationship, derived using the work loop technique, represents a useful index with which to assess the effects of pharmacological interventions on cardiac muscle contractility.

Key words: adrenaline, inotropic, chronotropic, papillary muscles, power output, work loops, rat.

Introduction

At rest, the heart operates at submaximal contractile potential. Consequently, it has considerable contractile reserves with which to meet increasing circulatory demands by changing the force and frequency of contraction (Katz, 1992). Cardiac rate is controlled primarily by the interaction of sympathetic (releasing noradrenaline to increase the heart rate) and parasympathetic, vagal (releasing acetylcholine to decrease heart rate) innervation. At rest, the moderate, tonic discharge in the sympathetic nerves is limited by the relatively greater effect of tonic vagal discharge (vagal tone). During conditions of physiological stress or exercise, there is an increase in sympathetic discharge with the liberation of noradrenaline at the cardiac nerve terminals. In addition, the adrenal medulla is stimulated to release adrenaline directly into the bloodstream. The resultant elevated levels of circulating catecholamines (noradrenaline and adrenaline) increase the heart rate (chronotropic effect) and contractile force (inotropic effect).

Adrenergic stimulation of isolated cardiac muscle preparations produces an increase in developed force, rate of force development, rate of relaxation (lusitropic effect) and shortening velocity (e.g. Sonnenblick, 1962; Endoh and Blinks, 1988). These effects are mediated primarily through stimulation of the β -adrenergic receptors at the surface of the cardiac cells, which, *via* a cascade of reactions involving cyclic AMP and protein phosphorylations, results in profound alterations in the

dynamics of Ca²⁺ handling (for details see Endoh and Blinks, 1988). In addition, there is evidence that changes in the maximum unloaded shortening velocity (V_{\max}) and in activation and relaxation rates may be mediated by a direct effect of adrenaline on crossbridge cycling independently of its action on Ca²⁺ metabolism (e.g. Hoh *et al.* 1988; Saeki *et al.* 1990; Strang *et al.* 1994). Adrenaline may therefore act by increasing both crossbridge recruitment (by increasing the available Ca²⁺) and the rate of crossbridge cycling.

The effects of inotropic agents on the mechanical performance of isolated cardiac preparations have commonly been assessed using indices derived from isometric or isotonic contractions. However, ‘contractility’ so estimated can be strongly dependent on the index chosen (Chiu *et al.* 1989). Furthermore, determination of V_{\max} is complicated by the significant internal load of cardiac preparations (Chiu *et al.* 1989; de Tombe and ter Keurs, 1991) and the level of activation (de Tombe and ter Keurs, 1991).

Maximum power has been proposed as a more desirable index with which to demonstrate the effects of inotropic interventions since it is sensitive to changes in both the force-generating ability and the shortening capability of the muscle and, as it is derived by interpolation of the data, it is subject to less error than the extrapolated value of V_{\max} (Ford, 1991). Furthermore, maximum power output is a physiologically relevant index of contractility

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since it is assessed under the conditions for which cardiac muscle was designed to operate (Ford, 1991).

Maximum power output, derived from the force–velocity curve, is distinct from the maximum sustainable power output of the muscle since it corresponds to a situation when the muscle is shortening at a constant velocity and is maximally activated (Josephson, 1985, 1993). As an alternative, the work loop technique (Josephson, 1985) was developed to measure mechanical power output of isolated muscles during more realistic cyclical contractions. This technique allows for changes in the shortening velocity and level of activation that are likely to occur during physiological contractions and takes into account both the work done by the muscle during shortening and the work required to re-extend the muscle during lengthening (reviewed by Josephson, 1993).

In a previous study (Layland *et al.* 1995a), the work loop technique was used to assess the work- and power-generating capacity of isolated rat papillary muscles for a physiological range of frequencies. The same technique was used in the present study to examine the work- and power–frequency relationships of rat papillary muscles during adrenergic stimulation. The results of these two studies are compared.

Materials and methods

Dissection procedure

Wistar laboratory rats (both sexes; mass 269 ± 25 g, mean \pm S.E.M., $N=7$) were killed by cervical dislocation and the heart was rapidly removed and rinsed in oxygenated (100% oxygen, Hepes-buffered) mammalian cardiac Ringer at room temperature (composition in mmol l^{-1} : NaCl, 144; sodium pyruvate, 10; KCl, 6; MgCl_2 , 1; CaCl_2 , 2; NaH_2PO_4 , 1; MgSO_4 , 1; Hepes, 10; pH 7.4 at room temperature (24 °C); Daut and Elzinga, 1989). The presence of Hepes in the Ringer prevented pH changes when temperature was elevated to 37 °C. The heart was mounted in a Petri dish on a Sylgard 184 base (Dow Corning) and immersed in fresh oxygenated Ringer. Papillary muscles were prepared using the method of Layland *et al.* (1995a,b). Briefly, an incision was made through the wall of the right ventricle, which was folded back. Papillary muscles were removed by cutting through the chordae tendinae and the ventricular wall, leaving a small piece of the myocardium attached. Ringer was replaced at frequent intervals during dissection. Small aluminium foil clips were attached to the tendon and the ventricular wall at each end of the papillary muscle. These clipped preparations were pinned out immersed in Ringer and bubbled with oxygen until required.

For experiments, the preparation was mounted and placed between a force transducer (AME 801, SensoNor, Horten, Norway) and a servomotor in a flow-through chamber containing circulating oxygenated Ringer at 37 ± 0.5 °C. Stimuli were delivered *via* two parallel platinum wire electrodes on either side of the muscle. The muscle preparation was allowed to recover for 30 min before experimentation.

Optimising muscle length

As described previously (Layland *et al.* 1995a,b), the muscle

length for maximum active isometric force generation (L_{max}) was established. The length for maximum net work production (L_{opt}) was derived using the work loop technique (Josephson, 1985). Muscle length was increased in 0.1 mm intervals within the physiological length range, from 12.5% below L_{max} to L_{max} (Brutsaert and Paulus, 1977). The muscle was subjected to four sinusoidal length change (strain) cycles about each starting length and was stimulated to contract at a particular phase (phase shift) during each cycle. The cycle frequency (of strain cycles), strain ($\pm\%L_{\text{opt}}$) and stimulation phase shift (relative to strain cycle, where 0° corresponds to L_{opt} during muscle lengthening, full cycle=360°) used were those previously found to produce maximal work and power output (Layland *et al.* 1995a,b). A plot of force *versus* length over one cycle produced a work loop (see Fig. 1), the area of which represents net work; the difference between shortening and lengthening work (Josephson, 1985). A clockwise loop indicated that more work was being done to stretch the muscle than was produced during shortening (negative net work). Muscles were allowed 5 min to recover between experimental runs. In each case, the first cycle of the four gave the highest net work. In preliminary experiments using larger numbers of cycles, work was shown to decline after the first cycle in a series. The muscle then recovered steadily until, at approximately cycle 30, work was the same as for the first cycle. Work was maintained for at least a further 20 cycles. Therefore, the net work performed during the first cycle was used as an estimate of maximum work output. L_{opt} was derived from a plot of starting length against net work and, for the remainder of the experiment, cyclical contractions were performed about this length.

Establishing the dose–response relationship for adrenaline

Adrenaline in the form of commercial pharmaceutical preparations (1 mg ml⁻¹ adrenaline tartrate in sterile physiological saline, stabilised with sodium metabisulphite; Antigen Pharmaceuticals Ltd, Roscrea, Ireland) was used as a stock solution. To minimise the oxidation of adrenaline, EDTA (0.02 mmol l⁻¹) and ascorbic acid (0.027 mmol l⁻¹) were added to the Ringer's solution (Endoh and Blinks, 1988).

An isometric twitch was elicited at L_{opt} prior to any addition of adrenaline. The active and passive force were measured together with the twitch kinetics: the time from the application of the stimulus until half-peak force ($t_{1/2,a}$), the time from the stimulus to peak force (t_{t}), the time taken for relaxation from peak to half-peak force ($t_{1/2,r}$) and the time from stimulus application to 90% relaxation (t_{90}). Comparison of these control twitches (no adrenaline) with those of a previous study (Layland *et al.* 1995a) demonstrated that the concentrations of EDTA and ascorbic acid used in the present study had no significant effect on isometric twitch force and kinetics.

The dose–response curve was established by cumulative addition of adrenaline (Endoh and Blinks, 1988). The volume of stock solution required to increase the final adrenaline concentration of the circulating Ringer by 1 log unit was calculated. The appropriate volume of stock solution required to produce an adrenaline concentration of 10^{-8} mol l⁻¹ in the

circulating Ringer was added to the Ringer reservoir, and the solutions were mixed by gentle shaking. A period of 5–10 min was allowed for the adrenaline to exert its effects on muscle contractility (Chiu *et al.* 1989; de Tombe and ter Keurs, 1991). An isometric twitch was elicited and active force, passive force and twitch kinetics ($t_{1/2,a}$, t_{rt} , $t_{1/2,r}$ and t_{90}) were measured. This routine was repeated with each increase in the concentration of adrenaline (i.e. 10^{-7} mol l $^{-1}$, 10^{-6} mol l $^{-1}$, 10^{-5} mol l $^{-1}$ and 10^{-4} mol l $^{-1}$). This range is similar to those used in previous studies (e.g. 10^{-8} to 3×10^{-5} mol l $^{-1}$, Endoh and Blinks, 1988; 10^{-7} to 10^{-4} mol l $^{-1}$, Hoh *et al.* 1988). The force produced as a percentage of the maximum force was plotted against adrenaline concentration (on a logarithmic scale) to produce a characteristic sigmoidal dose–response curve. From this, the adrenaline concentrations producing the maximum effect and 50% of the maximum effect (EC $_{50}$) could be determined. Maximal response was achieved at a concentration of 10^{-4} mol l $^{-1}$ adrenaline. This concentration was used for the remainder of the experiments.

Deriving the work–frequency and power–frequency relationships under adrenergic stimulation

The work loop technique was used to determine net work and power output for adrenergically stimulated papillary muscles over a range of cycle frequencies (4–11 Hz). Strain ($\pm L_{opt}$) and phase shift (degrees) were manipulated to maximise the net work at each cycle frequency. The average power output was calculated as the product of net work and cycle frequency. To reduce muscle deterioration, 5 min was allowed between experimental runs. Control runs were carried out after every third experimental run using the parameters yielding maximum power output, to monitor and correct for any decline in muscle performance over the course of the experiment (Layland *et al.* 1995a,b). Comparable methods of correcting for the decline in muscle performance are in common use (e.g. Kentish *et al.* 1986; Marsh, 1990; Stevenson and Josephson, 1990). Muscle preparations were abandoned on the rare occasions that net work performed during a control run declined to less than 80% of its initial value. There was no indication that the typically small decline in force during an experiment influenced the relationships under study; the same results were obtained for

Table 1. Mean values for muscle dimensions, isometric stress and passive:active force ratio at L_{max} prior to the addition of adrenaline

	Mean	S.E.M.	N
Rat mass (g)	268.57	24.73	7
Muscle wet mass (mg)	0.57	0.06	7
Muscle cross-sectional area (mm 2)	0.18	0.01	7
Muscle length at L_{max} (mm)	2.94	0.25	7
Muscle length at L_{opt} (mm)	2.80	0.24	7
Stress at L_{max} (kN m $^{-2}$)	37.57	3.04	7
Passive:active force ratio at L_{max}	0.49	0.04	7

muscles to which correction factors had been applied and for those that showed no significant deterioration.

Data collection

At the end of each experiment, the papillary muscle was weighed (wet mass to the nearest 0.01 mg) following removal of the aluminium clips, tendon and ventricular wall. Muscle mass and length were used to calculate mean cross-sectional area (assuming a muscle density of 1060 kg m $^{-3}$). Power output (W kg $^{-1}$) and isometric stress, i.e. force per unit cross-sectional area (kN m $^{-2}$), were calculated.

The experiments were controlled and the data collected and analysed on-line via a microcomputer, using in-house software. Paired Student's *t*-tests were used to compare muscle force and twitch kinetics before and after the addition of adrenaline. A paired non-parametric Wilcoxon signed-rank test was used to compare the passive:active force ratios of preparations before and after the addition of adrenaline. Unpaired Student's *t*-tests were used to compare net work and power output of adrenergically stimulated muscles with 'controls' (Layland *et al.* 1995a). All results are expressed as mean \pm S.E.M. (number of observations).

Results

Isometric experiments

Table 1 summarizes the average values of muscle dimensions, active isometric stress and the passive:active force

Table 2. Comparison of the force-generating capacity and twitch kinetics of rat papillary muscles before and after the application of adrenaline

	No adrenaline	10^{-4} mol l $^{-1}$ adrenaline	Significance
Active stress at L_{opt} (kN m $^{-2}$)	34.21 \pm 2.89 (7)	49.64 \pm 3.70 (7)	$P < 0.01$
Passive stress at L_{opt} (kN m $^{-2}$)	9.60 \pm 1.27 (7)	7.28 \pm 1.61 (7)	NS
Passive:active force ratio at L_{opt}	0.29 \pm 0.03 (7)	0.15 \pm 0.03 (7)	$P < 0.05^*$
Time to half-activation (ms)	28.17 \pm 1.49 (6)	23.40 \pm 0.94 (6)	$P < 0.05$
Twitch rise time (ms)	65.17 \pm 2.45 (6)	53.50 \pm 2.26 (6)	$P < 0.01$
Time to half-relaxation (ms)	37.08 \pm 1.28 (6)	30.92 \pm 2.05 (6)	$P < 0.01$
Time to 90% relaxation (ms)	132.83 \pm 3.10 (6)	107.33 \pm 4.93 (6)	$P < 0.01$

Stress and twitch kinetics were derived isometrically at L_{opt} (the length for maximum work production).

* indicates Wilcoxon signed-rank test (all other tests are paired *t*-tests); NS, not significant.

Values are mean \pm S.E.M. (N).

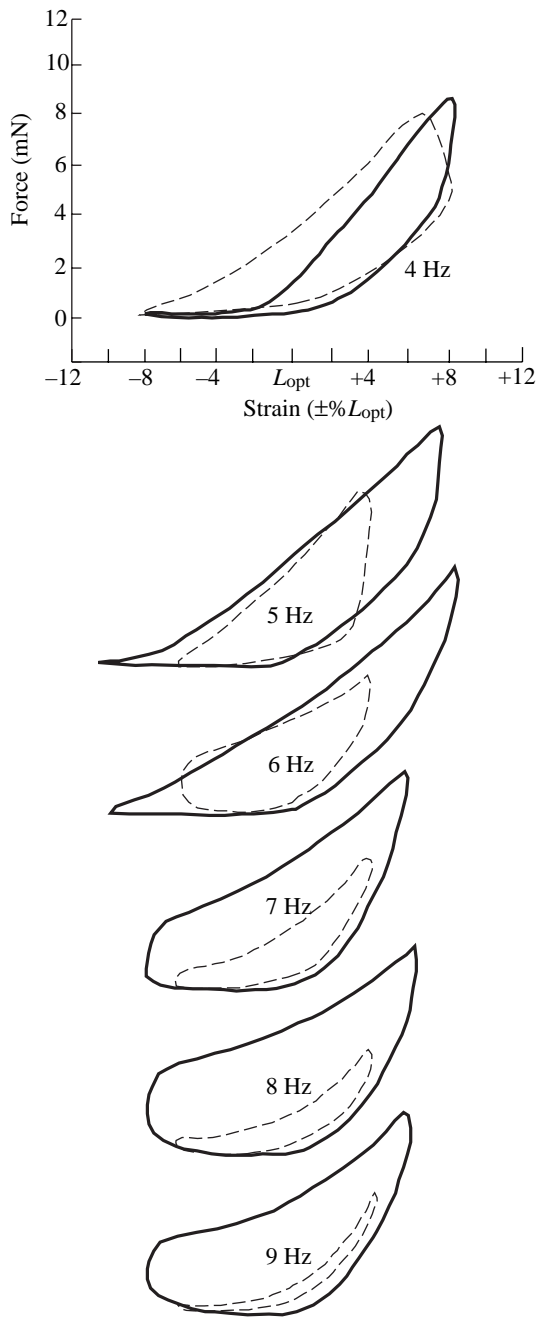


Fig. 1. Comparison of the maximal work loops at each cycle frequency for rat papillary muscles operating in normal Ringer's solution (broken lines) and under adrenergic ($10^{-4} \text{ mol l}^{-1}$) stimulation (solid lines). The cycle frequency (Hz) is indicated with each loop. All loops are anti-clockwise, indicating positive net work.

ratio, all measured at L_{max} . The values for active isometric stress, passive:active force ratio and twitch kinetics measured at L_{opt} for the same muscles operating in normal Ringer's solution and during stimulation with $10^{-4} \text{ mol l}^{-1}$ adrenaline are summarised in Table 2. Maximum active isometric force was achieved at adrenaline concentrations between 10^{-5} and $10^{-4} \text{ mol l}^{-1}$ and $\log EC_{50}$ was calculated to be -5.99 ± 0.21 ($N=7$). These concentrations are comparable to those found to

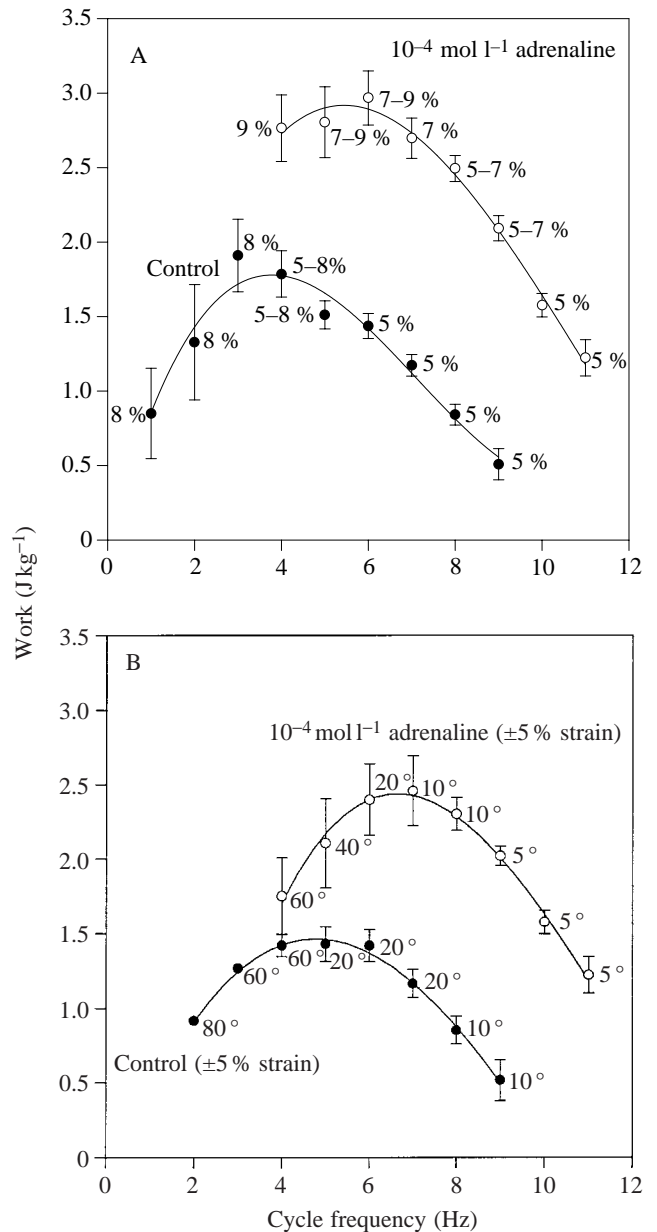


Fig. 2. (A) The work–frequency relationships for control (filled symbols; $N=5-9$) and adrenergically stimulated (open symbols; $N=6$) muscles. All values are mean \pm S.E.M. Optimal strain amplitudes ($\pm\% L_{\text{opt}}$) are given next to the appropriate symbol. Curves were fitted using third-order regressions. (B) The work–frequency relationships for control (filled symbols; when no error bar given $N=1$; for all other points $N=3-7$) and adrenergically stimulated (open symbols; $N=6$) muscles when strain is maintained at $\pm 5\% L_{\text{opt}}$. Optimal stimulation phase shifts (degrees) are given next to the appropriate symbol. All values are mean \pm S.E.M. Curves were fitted using third-order regressions.

elicit a maximal response ($10^{-5} \text{ mol l}^{-1}$) and 50% maximal response ($\log EC_{50} = -6.09 \pm 0.12$, $N=6$) in a previous study on rabbit papillary muscles under similar conditions (Endoh and Blinks, 1988).

Under adrenergic stimulation, active isometric stress at L_{opt}

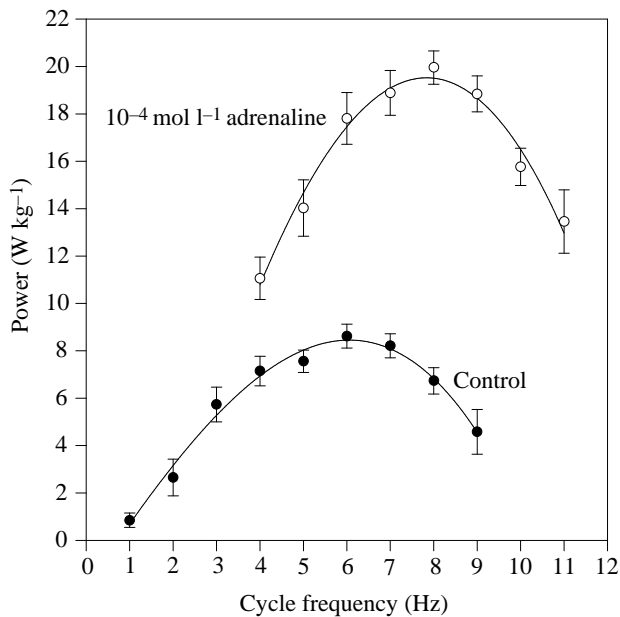


Fig. 3. Comparison of the power–frequency relationships for rat papillary muscles operating in normal Ringer’s solution (filled symbols; $N=4-9$) and under adrenergic stimulation with 10^{-4} mol l $^{-1}$ adrenaline (open symbols; $N=6$). All values are mean \pm S.E.M. Curves were fitted using third-order regressions. Tests for significant differences between the power outputs of control and adrenergically stimulated muscles at each frequency are given in Table 3. Strain is as in Fig. 2A and stimulation phase as in Fig. 2B.

was increased significantly by 45% and the passive:active force ratio decreased significantly by 48%. The decrease in passive:active force ratio can be largely attributed to the increased developed force, since there was no significant change in the passive force in the presence of adrenaline. Adrenaline induced significant 17–20% decreases in isometric activation and relaxation times, in agreement with previous studies (Endoh and Blinks, 1988).

Work loop experiments

A preliminary work loop study established L_{opt} as approximately 95% of L_{max} . For the remainder of the

Table 3. Comparison of the mean power output measured at each cycle frequency in control and adrenergically stimulated muscles

Cycle frequency (Hz)	Power output (W kg $^{-1}$)		Factorial increase	Significance
	No adrenaline	10^{-4} mol l $^{-1}$ adrenaline		
4	7.15 \pm 0.62 (7)	11.06 \pm 0.90 (6)	1.55	$P<0.01$
5	7.56 \pm 0.47 (9)	14.03 \pm 1.19 (6)	1.86	$P<0.01$
6	8.62 \pm 0.50 (9)	17.81 \pm 1.09 (6)	2.06	$P<0.01$
7	8.21 \pm 0.51 (9)	18.89 \pm 0.95 (6)	2.31	$P<0.01$
8	6.73 \pm 0.56 (8)	19.95 \pm 0.70 (6)	2.98	$P<0.01$
9	4.58 \pm 0.94 (5)	18.84 \pm 0.76 (6)	4.10	$P<0.01$

Values are means \pm S.E.M. (N).

experiments, muscle length was cycled around this length (L_{opt}). Fig. 1 compares maximal work loops for each cycle frequency in normal Ringer’s solution (without adrenaline) with maximal work loops obtained with adrenergic stimulation. Fig. 2A compares the work–frequency relationship in the control experiment (Layland *et al.* 1995a) with that derived under adrenergic stimulation. The maximum net work increased by 55% from 1.91 ± 0.24 J kg $^{-1}$ ($N=6$) in normal Ringer to 2.97 ± 0.18 J kg $^{-1}$ ($N=6$) in the presence of 10^{-4} mol l $^{-1}$ adrenaline. Furthermore, there was a shift in the optimal frequency for work production from 3 Hz to 6 Hz. The optimal strain amplitude is sometimes greater in the presence of adrenaline than it is in control Ringer’s solution for comparable cycle frequencies (Fig. 2A) but there is little difference in optimal phase (see Fig. 2B). The increase in strain would contribute to an increase in net work. However, even when work at a single strain is compared (Fig. 2B), the work-generating capacity of rat papillary muscles is still greatly increased by adrenaline.

With adrenergic stimulation, the maximum power produced at 37°C is more than double that produced in control conditions: 19.95 W kg $^{-1}$ and 8.62 W kg $^{-1}$ respectively (Fig. 3; Table 3). In addition, the cycle frequency for maximum power output (f_{opt}) increased from 6 Hz in the control experiments to 8 Hz under adrenergic stimulation, although power output is within 10–20% of this maximum over the range 6–9.5 Hz. At the higher cycle frequencies, the power output with adrenergic stimulation can be as much as four times greater than at the same frequency in normal Ringer (e.g. 4.58 ± 0.94 W kg $^{-1}$ for control and 18.84 ± 0.76 W kg $^{-1}$ with adrenaline at 9 Hz, see Table 3).

Discussion

Rat papillary muscle preparations were subjected to stimulation applied during sinusoidal length-change (strain) cycles simulating physiological conditions (Josephson, 1985). The net work- and power-generating capacity of the muscles were assessed at 37°C, over a physiologically relevant range of cycle frequencies. The results from muscle preparations with adrenergic stimulation were compared with those found for muscles operating in the absence of adrenaline (Layland *et al.* 1995a). This is the first study to use the work loop technique to examine the effects of adrenergic stimulation (10^{-4} mol l $^{-1}$) on cardiac contractility. The main finding is that maximal adrenergic stimulation shifted the power–frequency relationship upwards and to the right, increasing the power-generating capacity and frequency for maximum power output respectively.

Effects of adrenaline on the isometric properties

Maximal adrenergic stimulation (10^{-4} mol l $^{-1}$) induced a 45% increase in maximum isometric stress and a 17–20% decrease in twitch activation and relaxation times (Table 2). These results agree with the well-documented effects of adrenaline on isolated cardiac muscle (e.g. Endoh and Blinks,

1988). The increase in active stress can be attributed to an increase in the number of active crossbridges formed in response to an adrenaline-induced increase in the intracellular Ca^{2+} transient. The increased rate of relaxation is generally explained by increases in the rate of Ca^{2+} dissociation from troponin-C and in the rate of Ca^{2+} uptake by the sarcoplasmic reticulum. For a precise review of the mechanisms involved in β -adrenergic responses, see Endoh and Blinks (1988).

Effects of adrenaline on the work–frequency relationship

Adrenergic stimulation caused a 55% increase in the maximum work output (Fig. 2A). This can be attributed largely to the effects of adrenaline on force generation suggested by the 45% increase in maximum isometric stress. However, work was further increased by increasing strain (compare Fig. 2A with Fig. 2B). Adrenergically stimulated muscles can operate at higher strains than control muscles since adrenergic stimulation shifts the force–velocity relationship to higher values of both force and velocity (Sonnenblick, 1962). Consequently, for a given cycle frequency, adrenergically stimulated muscles, operating at larger strain amplitudes, could still generate higher forces than control muscles.

The cycle frequency for maximum work output is determined largely by the fraction of the shortening period over which force is generated (Josephson, 1993; Syme, 1993; Layland *et al.* 1995a). Maximal adrenergic stimulation decreased twitch duration (assessed isometrically as t_{90} , the time from stimulus to 90% relaxation) by 19%. This would produce an increase in the optimal cycle frequency for work output. Contraction duration may also be further abbreviated by the effects of shortening deactivation (Edman and Nilsson, 1971), which may be more pronounced in adrenergically stimulated muscles operating at higher frequencies and strains since shortening deactivation may depend on both velocity (Colomo *et al.* 1988) and length (Cooper, 1990). In addition, a direct effect of adrenaline on the crossbridge cycling rate (e.g. Hoh *et al.* 1988; Saeki *et al.* 1990) would allow the muscles to operate at higher frequencies by increasing the rate of crossbridge detachment.

Effects of adrenaline on the power–frequency relationship

Adrenergically stimulated muscles produced a maximum power output of 19.95 W kg^{-1} at 8 Hz compared with a maximum power output of 8.62 W kg^{-1} generated by the control muscles at 6 Hz (Layland *et al.* 1995a). Hence, adrenergic stimulation increased the maximum power-generating capacity of rat papillary muscles by a factor of 2.3.

Maximal power output is achieved by a muscle when it contracts at a shortening velocity (V) of approximately $0.3V_{\text{max}}$ or against a load (P) of approximately $0.3P_0$, where V_{max} is the maximum unloaded shortening velocity and P_0 is the maximum isometric force (reviewed by Josephson, 1993). It would be expected, therefore, that the value of V/V_{max} yielding maximum power output should be similar in both control and adrenergically stimulated muscles. Maximum power output was produced at a greater cycle frequency and strain, and thus

higher velocity, in adrenergically stimulated muscles than in control muscles. Consequently, a similar value of V/V_{max} would only be maintained if adrenaline also induced an increase in V_{max} . This implied increase in V_{max} could be attributed to the effects of adrenaline on Ca^{2+} dynamics and crossbridge recruitment (de Tombe and ter Keurs, 1991) or its direct effect on the rate of crossbridge interaction (Hoh *et al.* 1988; Saeki *et al.* 1990), or both.

The increase in power output becomes more pronounced as the cycle frequency is increased (from a factor of 1.5 at 4 Hz to 4.1 at 9 Hz) (Fig. 3). This is because the adrenergically stimulated muscles are able to operate effectively at higher cycle frequencies than control muscles, which are at the limits of their operating frequency. The dramatic increase in the maximum power output can be attributed both to the increase in maximum net work and to the increased cycle frequency. The shift in the power–frequency relationship produced by adrenaline reflects the combined effects of adrenaline on force production, shortening velocity and the rates of activation and relaxation, and probably reflects changes in both the number of crossbridges recruited and the rate at which they cycle.

Physiological significance

This investigation presents a new approach to the assessment of changes in cardiac muscle contractility induced by pharmacological intervention. Using the work loop technique, the muscles are studied under conditions which are more representative of the normal cardiac cycle. The effects of maximal adrenergic stimulation are compared with the situation in the absence of adrenergic stimulation. Under these conditions, adrenaline caused a dramatic increase in the force, net work- and power-generating capacity and increased the cycle frequency and strain for maximum power output. The increases in force production and net work in isolated muscle are analogous, respectively, to increases in the systolic pressure and stroke work of the heart observed *in vivo* during exercise or adrenergic stimulation (Irlbeck and Zimmer, 1993). The increase in optimal strain is analogous to an increase in stroke volume. The increase in the cycle frequency for maximal power output from 6 to 8 Hz is analogous to an increase in heart rate *in vivo* and is close to the increase in heart rate observed in rats treated with the β -agonist isoproterenol (5.5 Hz to 7 Hz; Irlbeck and Zimmer, 1993). It should be noted, however, that the observed responses may represent a more dramatic response than is physiologically relevant since catecholamines are present at low concentrations (of the order of $10^{-10} \text{ mol l}^{-1}$) under normal circumstances and, even during stress or exercise, it is unlikely that their concentrations would approach those required for maximal stimulation.

The present study clearly demonstrates that the work– and power–frequency relationships derived using the work loop technique are sensitive to changes in force, velocity and activation and relaxation rates. As a result, this technique may represent a useful new approach for assessing the effects of pharmacological intervention on cardiac contractility, and one

that could be used to examine the effects of more physiologically relevant changes in catecholamine levels.

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