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RELAXING EFFECTS OF CATECHOLAMINES ON MAMMALIAN HEART

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SUMMARY

1. The effect of catecholamines on the time course and amplitude of contraction and on KCl-induced contractures has been studied in mammalian hearts.

2. Marked and reproducible contractures could be obtained in mammalian ventricular trabeculae and papillary muscles after β-adrenergic block with propanolol or if the hearts were depleted of their catecholamine stores by reserpine or by chemical denervation with 6-hydroxydopamine.

3. In neonatal hearts with lower endogenous catecholamine stores and poorly developed sarcoplasmic reticulum KCl contractures are easily produced.

4. Catecholamines potentiate twitch tension and relax the contracture tension under all of the above circumstances.

5. The relaxant effect of catecholamines is present during the time course of a twitch. This increased relaxation rate as well as the shortening of the time-to-peak of tension is independent of the variation in the duration of the action potential.

6. The shortened relaxation time is present when the action potential is shortened with anodal repolarization or prolonged with cathodal depolarization (voltage-clamp).

7. The relaxant effect of catecholamines on the twitch is temperature and rate dependent. The effect is observed in the presence of high or low concentrations of calcium.

8. The presence of catecholamines is necessary for full relaxation of mammalian heart muscle under high performance conditions or states of calcium overload.

9. It is proposed that catecholamines exert their relaxant effect independent of their positive inotropic effect by stimulating the sequestering system (sarcoplasmic reticulum, mitochondria or sarcolemma) for calcium.

INTRODUCTION

Prolongation of the action potential by voltage-clamping mammalian (sheep and cat) myocardium above -30 mV results in maintained tension (contracture), the amplitude of which is directly related to the level of depolarization (Morad & Trautwein, 1968; Morad, Mascher & Brady, 1968). Depolarization by high concentrations of KCl often fails to produce large maintained tension. In frog ventricle, in contrast to mammalian heart, both KCl and voltage-clamp induced depolarizations result in development of marked maintained tension (Niedergerke, 1956; Lamb & McGuigan, 1966; Morad & Orkand, 1971). It is reasonable to ask why KCl depolarizations fail to produce significant contractures in mammalian myocardium. Since high concentrations of KCl (50 mM) are known to release endogeneous noradrenaline from the cat heart (Haeusler, Thoenen, Haefely & Huerlimann, 1968), it is conceivable that KCl-induced contractures are suppressed by the well known relaxing effect of catecholamines (Kavaler & Morad, 1966; Graham & Lamb, 1968; Morad, 1969).

In these investigations, we shall define conditions necessary to produce KCl contractures in mammalian ventricular muscle. It is shown that these contractures are highly susceptible to the relaxing action of catecholamines. Furthermore, this action of catecholamines is not limited to the myocardium in contracture, but is also present in the time course of a single twitch. The results suggest that the relaxing properties of catecholamines may be quite independent of the positive inotropic action on the myocardium.

A preliminary report of this work has been presented (Morad, Rolett & McCouch, 1971).

METHODS

Preparation and animals. Thin papillary or trabecular muscles, ranging in diameter from 0·2 to 1·0 mm, were excised from the right ventricles of anaesthetized adult male cats, New Zealand white rabbits or neonatal (1–2 days old) kittens. Cardiac noradrenaline stores were depleted in a number of cats by pretreatment with reserpine 1 mg/kg I.P. for 3 consecutive days before sacrifice. Chemical sympathectomy was achieved with 6-hydroxydopamine in five additional cats and four rabbits (Thoenen & Tranzer, 1968). These animals were given 6-hydroxydopamine HBr I.V. in divided doses of 50 and 100 mg/kg 10 days and 4 days respectively preceding study. The compound was dissolved in cold 0·01 N-HCl containing ascorbic acid 20 mg/l. and gassed with N_2 to prevent oxidation before use. Anaesthesia was produced by sodium pentobarbitone 30 mg/kg I.P. in cats and 60 mg/kg I.V. in rabbits. Some rabbits were killed by a sharp blow to the base of the skull.

Solutions. Three isotonic solutions were used with the following composition in m-mole/l.: (1) normal Tyrode: NaCl 137, $NaHCO_3$ 11·9, $CaCl_2$ 1·8, KCl 2·7, $MgCl_2$ 0·7, NaH_2PO_4 0·4, and glucose 5 to 7; (2) KCl-Tyrode: KCl substituted for NaCl, other ions the same; (3) 'zero' calcium Tyrode: calcium omitted from normal Tyrode.

All solutions were prepared with double distilled, deionized water at room temperature (24–25° C) equilibrated with 98% O_2–2% CO_2 (pH 7·2–7·4). In some experiments, calcium concentration was varied by addition of calcium chloride to the 'zero' calcium Tyrode.

Experimental set-up. Muscles were suspended in a 0·2 ml. chamber and were attached to an isometric transducer and stimulated by large Ag/AgCl electrodes at rates between 6 and 38/min with pulses of 5 msec and voltage about 1·3 times threshold. Isometric tension and time derivative of tension (dp/dt) were recorded. Membrane potential was measured with 3 M-KCl-filled micro-electrodes in some studies using previously described techniques (Kuvaler & Morad, 1966; Morad & Orkand, 1971). Muscles were equilibrated for 1–2 hr in normal Tyrode solution before the study.

The experimental set-up permitted rapid exchange of one solution for another. Contractures were induced with KCl-Tyrode solution. In some experiments to test the effect of high potassium alone, the solution was made hypertonic by adding 137 mM-KCl to normal Tyrode. Such contractures were then compared with those produced by high KCl-Tyrode made hypertonic with 137 mM choline chloride. Four muscles from cats pretreated with reserpine were incubated in Tyrode containing 'zero', 0·45 or 1·8 mM calcium and then exposed to KCl-Tyrode.

In 6-hydroxydopamine pretreated cats the noradrenaline content of ventricular myocardium was assayed by the histochemical fluorescence method of Falck and Hillarp (Falck & Owman, 1965). Noradrenaline content in tissue samples obtained from untreated adult cats, 6-hydroxydopamine pre-treated cats, and 1 day old kittens was measured fluorimetrically following alumina column chromatography (Breese & Traylor, 1970).

In papillary muscles obtained from untreated normal cats the effects of adrenaline were compared at different calcium concentrations (0·45–14·4 mM), stimulus frequencies (12 and 120/min), and temperatures (26 and 36° C). The action potential was shortened in some experiments with application of anodal current (Morad, 1966) or with a voltage-clamp technique (Morad & Orkand, 1971).

Drugs used were (−)adrenaline HCl (Adrenalin, Park–Davis), (−)noradrenaline bitartrate hydrate (Arterenol, Calbiochem, Sigma), (±)normetanephrine (NMM) HCl (Calbiochem), reserpine (Serpasil, Ciba), (±)propranolol HCl (Inderal, Ayerst), and 6-hydroxydopamine (6-OH-DA) HBr (Regis).

RESULTS

The two well-known effects of catecholamines on cardiac muscle – the positive inotropic action and the early onset of relaxation – are shown in Fig. 1. Time-to-peak tension shortens markedly while the duration of the action potential changes little or is prolonged. Addition of catecholamines to ventricular muscle preparations causes only relaxation when the muscle is put in contracture with KCl depolarization. This action made it possible to study the relaxing effect of catecholamines independent of the positive inotropic effect.

Contracture and catecholamines

Mammalian ventricular myocardium, unlike frog cardiac and skeletal muscle, develops small and inconsistent contracture tensions (10–40%

of twitch tension) when bathed in high KCl-Tyrode. Fig. 2 shows the response of a right ventricular papillary muscle exposed sequentially to a low concentration of NaCl and a high concentration of KCl or the reverse sequence. Despite the fact that complete replacement of NaCl by KCl increases the $Ca/(Na)^2$ ratio which might be expected to potentiate contracture tension (Lüttgau & Niedergerke, 1958), the magnitude of contracture tension remained only a small fraction of the preceding twitch

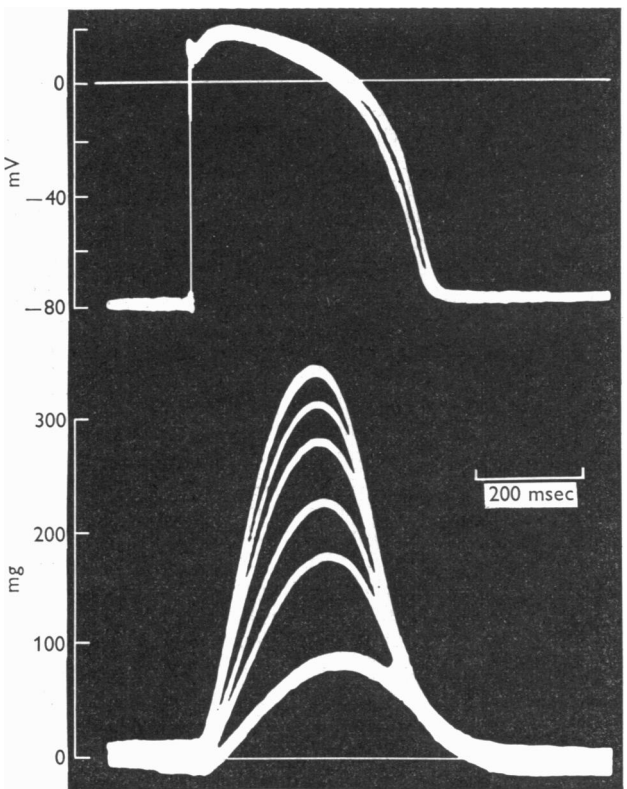

Fig. 1. Superimposed tracings of action potentials (above) and contractions (below) in cat papillary muscle on exposure to Tyrode solution containing adrenaline 2 μg/ml. Increase in force of twitch and shortening of time-to-peak tension occur with only a slight prolongation of action potential (temperature 28° C, diameter 0·5 mm).

tension. Twitch tension was consistently augmented and oscillatory contractions were often present following return to the normal Tyrode solution; this response was consistent with a KCl-induced noradrenaline release.

KCl-induced contractures in hearts from animals pretreated with reserpine. Ventricular trabeculae and papillary muscles obtained from the right heart of animals pretreated with reserpine gave normal twitch tension. In these

muscles, twitch duration was prolonged to 2·5–3·0 sec from the 1·2–1·5 sec normally observed at room temperature (Fig. 3, top panel). In contrast to untreated preparations, high levels of contracture tension (75–125 % of twitch) were observed when these muscles were exposed to high KCl concentrations (Fig. 3, middle panel). The contracture amplitude was reproducible with repeated exposures to high KCl over a period of 1–2 hr, but thereafter tended to decrease with further applications of high KCl. At the same time a shortening of the twitch duration, particularly of the

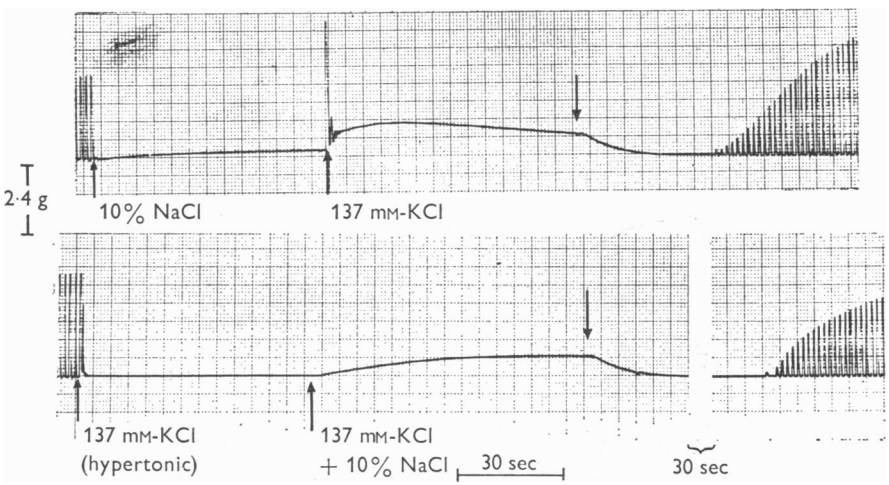

Fig. 2. Contractures produced by low sodium and high KCl in normal untreated cat ventricular trabeculum. *Upper panel* shows the contractile response of the myocardium when exposed to 10 % sodium chloride solution (NaCl replaced by choline chloride) followed by a high KCl solution in which sodium was reduced by 10 %. At the inverted arrow muscle is once again exposed to normal Tyrode solution. Post-contracture potentiation of tension is observed. *Lower panel* shows a contractile response of the same muscle when exposed to hypertonic KCl-Tyrode (KCl added to normal Tyrode) followed by an isosmotic solution of high KCl and low sodium (temperature 25° C, diameter 0·6 mm).

relaxation phase, was noted. Fig. 3 also shows the effect of adrenaline (2 μg/ml.) on both twitch and contracture tensions. Note, whereas twitch tension and dp/dt were potentiated and twitch duration abbreviated (interrupted lines, top panel), contracture tension was markedly reduced (bottom panel). Contracture tension in the presence of adrenaline seldom exceeded 10–20 % of the twitch tension.

KCl-induced contractures in muscles exposed to propranolol. Exposure of normal ventricular trabeculae or papillary muscles to propranolol (10^{-7}–10^{-6} M; eleven muscles) produced no change in twitch tension, whereas higher concentrations (10^{-5} M) tended to reduce twitch tension by 10–

25 %. Repeated applications of high KCl solutions to propranolol-treated muscles produced marked contracture tension (75–150 % of twitch) over periods of 2–3 hr. Unlike preparations from animals pre-treated with reserpine, the duration of the twitch in muscles treated with propranolol

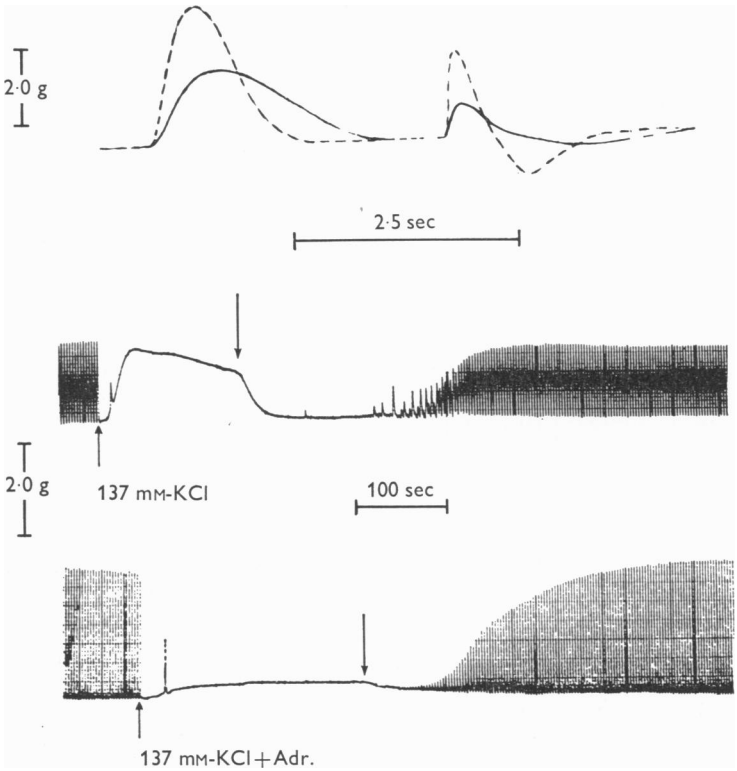

Fig. 3. *Upper panel* shows superimposed tracings of twitch tension of a papillary muscle obtained from a reserpinized cat and the effects of the addition of adrenaline (broken lines). The rate of rise and fall of tension as well as the contractile force are augmented by the presence of adrenaline (Adr.). The *middle panel* shows the contractile response of the same muscle to addition of KCl-Tyrode. Contracture is developed and maintained for the duration of exposure to KCl. Post-contracture potentiation of twitch is absent. *Lower panel* shows the contractile response of the same muscle exposed to adrenaline (2 μg/ml.). While the twitch tension is markedly potentiated, contracture is suppressed. The 2 g marking of tension calibration is equivalent to 11 g/sec of the dp/dt traces (temperature 25° C, diameter 1·0 mm).

remained constant despite repeated exposures to KCl. Fig. 4 suggests that the amplitude of contracture tension is related to the concentration of propranolol. The ability of propranolol to permit KCl contractures was inhibited by a higher concentration of adrenaline (5×10^{-5} M).

KCl-induced contractures in hearts from 6-hydroxydopamine pre-treated animals. In the two cats from this series, which were prepared for electron-microscopy and fluorescence studies, the ventricular myocardium revealed complete degeneration of sympathetic nerve endings and total absence of catecholamine fluorescence. These findings agree with those previously

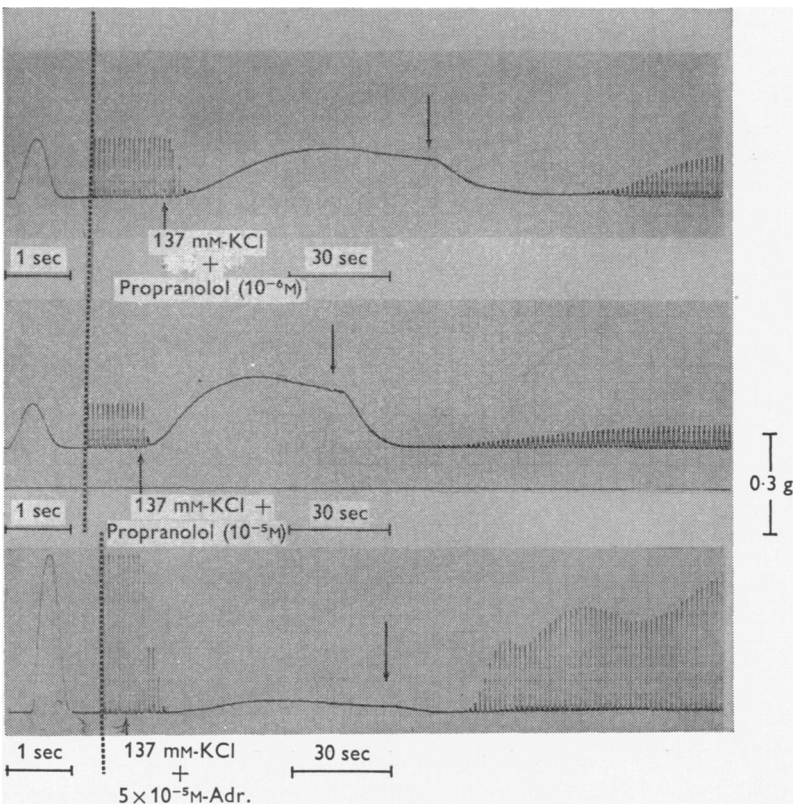

Fig. 4. Cat papillary muscle pretreated with propranolol. KCl solutions which induce contractures in the upper two panels also contained propranalol. More contracture tension is obtained with the higher concentration of propranolol. The effect of propranolol is overcome by addition of an even higher concentration of adrenaline (Adr.) which augments contraction tension and suppresses contracture tension (temperature, 26° C, diameter 0·4 mm).

reported by Tranzer & Thoenen (1968) and Malmfors & Sachs (1968). The noradrenaline content of myocardium in 6-OH-DA treated cats was markedly reduced when measured by the fluorimetric method (Table 1). Twitch tension in papillary muscles obtained from 6-OH-DA pre-treated rabbits and cats ranged from 0·5 to 1 kg/cm^2 and was not appreciably

different from that observed in control preparations. The duration of total twitch, time-to-peak tension and relaxation time were not changed by 6-OH-DA pre-treatment. However, exposure of such muscles to high KCl-Tyrode produced contracture tensions ranging from 85 to 150 % of twitch tension (Fig. 5, top panel). Contractures were reproducible upon repeated

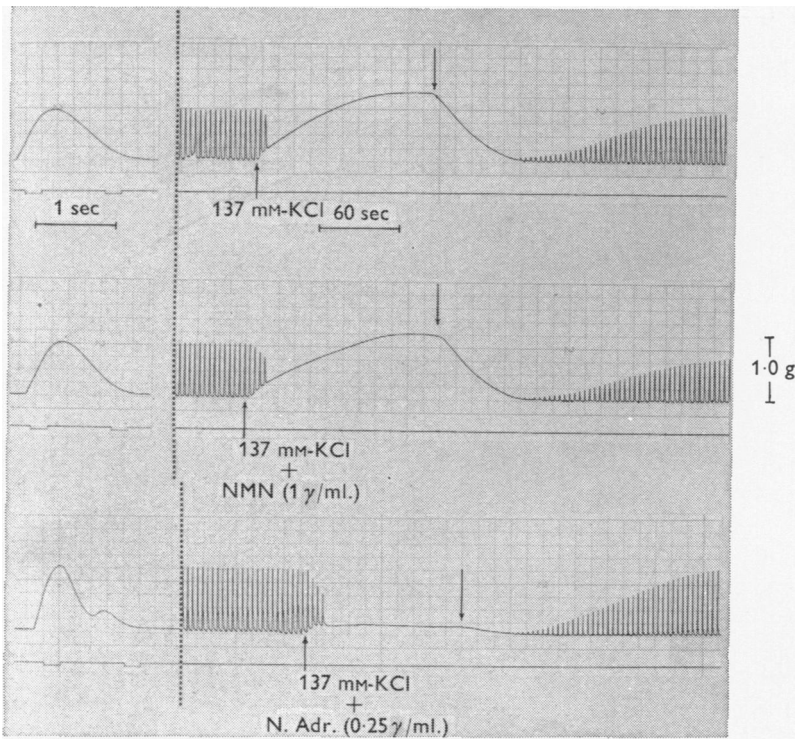

Fig. 5. KCl contractures in myocardium obtained from rabbit pre-treated with 6-hydroxydopamine. *Upper panel*, contractile response of the muscle on exposure to KCl-Tyrode. There is no post-contracture potentiation of the twitch tension. The same experimental procedure is repeated in the middle panel in the presence of normetanephrine (NMN, 1 μg/ml.) and a similar response is obtained. The *lower panel* shows the effect of noradrenaline (N.Adr.) both on potentiation of the twitch and enhancement of relaxation (twitch and contracture) (temperature 25° C, diameter 0·6 mm).

KCl exposures. Addition of noradrenaline (0·25 μg/ml.) diminished contracture amplitude markedly (Fig. 5, bottom panel). Since studies of [^{3}H]noradrenaline uptake by rabbit heart muscle in this laboratory (unpublished observation, 1970) showed that more than 50 % of the ^{3}H label was present as NMN, it was important to know whether NMN mediates the relaxant effect of noradrenaline. Fig. 5 (middle panel) shows

that incubation of a muscle with NMN (1 μg/ml.) had no effect on the duration or amplitude of contraction or on contracture tension.

The four rabbit and two cat preparations studied by micro-electrode impalement showed no abnormality of resting membrane potential or action potential amplitude. Exposure of a muscle to 137 mM-KCl depolarized the membrane from a resting potential of -85 to -80 mV to about -8 to -4 mV during KCl contracture. The addition of noradrenaline during KCl contracture had no effect on membrane potential in treated or non-treated preparations.

KCl-induced contractures in hearts from neonatal kittens. The noradrenaline content of ventricular myocardium from 1-day old kittens was measured and found to be 30% of the normal adult value (Table 1). Exposure of papillary muscles from these hearts to high concentrations of KCl produced marked contracture tensions ranging from 150 to 400% of

TABLE 1. Mean values ($\pm$ 1 S.E.) for noradrenaline content of ventricular myocardium in adult, one-day old, and 6-hydroxydopamine (6-OH-DA) pre-treated cats. Noradrenaline was separated by column chromatography and analysed by fluorimetry

Group	No. of animals	Noradrenaline content (μg/g)
Adult cats	3	$1{\cdot}00 \pm 0{\cdot}12$
1-day old kittens	4	$0{\cdot}30 \pm 0{\cdot}04$
6-OH-DA treated cats	2	$0{\cdot}003 \pm 0{\cdot}003$

twitch tension. With long intervals between contractures (15–20 min), the contracture tension was reproducible over a narrow range for at least 2–3 hr. With short intervals between KCl exposure, contracture tension was suppressed despite a marked accentuation of twitch tension post-contracture. Fig. 6 (upper panel) shows the response of a neonatal muscle exposed to KCl-Tyrode with adrenaline added at the peak of the contracture. Contracture tension decreased markedly. This muscle was then bathed in normal Tyrode solution containing adrenaline (2 μg/ml.) before re-exposure to KCl-Tyrode. The lower panel of Fig. 6 shows the positive inotropic effect of adrenaline on the twitch and its inhibitory effect on contracture tension in the same muscle.

Factors influencing the strength of contracture. The four experimental preparations described in the preceding sections share in common the ability to develop high levels of contracture tension upon repeated exposures to high KCl solutions. These muscles are highly susceptible to addition of catecholamines. Adrenaline or noradrenaline, while potentiating twitch tension, diminish markedly twitch duration and amplitude of contracture.

Of interest in the catecholamine-depleted preparations is whether the

depolarization of the muscle by KCl itself is sufficient to produce the contracture tension or whether the reduction of sodium (which is equivalent to increasing the external calcium concentration) is necessary for the development of high contracture tension. Fig. 7 represents an experiment in which a muscle from a cat pre-treated with reserpine was sequentially exposed to high potassium or low sodium followed by the contracture solution. Such muscles required both the reduction of external sodium and depolarization with KCl for the development of high contracture tension.

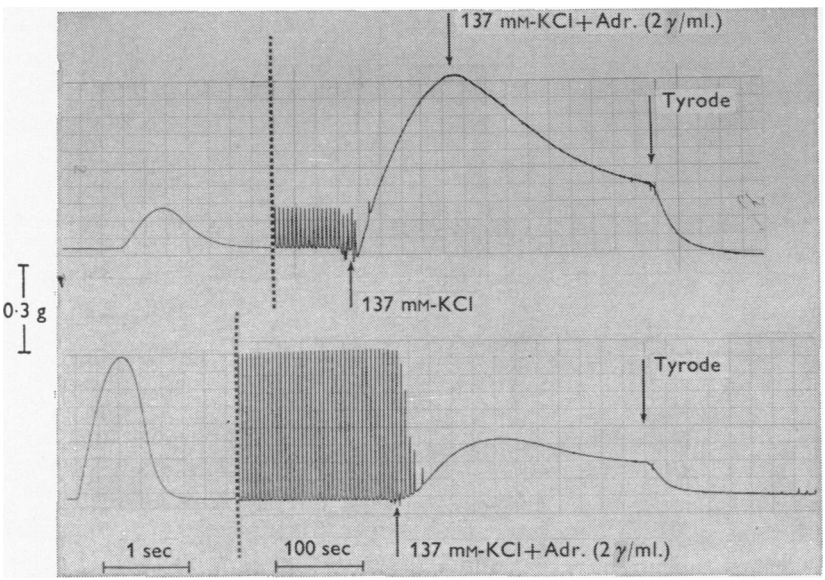

Fig. 6. KCl contractures in neonatal cat heart. Upper panel shows the KCl-Tyrode induced contracture. At the peak of the contracture (first downward arrow), 2 μg/ml. adrenaline (Adr.) is administered. Contracture tension relaxes. Readmission of Tyrode (second downward arrow) relaxes the muscle completely. The same experimental procedure is repeated in the presence of 2 μg/ml. adrenaline. Contracture tension is suppressed (temperature 26° C, diameter 0·3 mm).

The order of exposure to high potassium and low sodium affected the time course but not the amplitude of contracture development. Essentially similar responses, but with much lower contracture tension, were observed in control muscles (Fig. 2).

Increasing the external calcium concentration threefold, although augmenting twitch tension, had little or no effect on contracture tension. On the other hand, incubating the muscle in low calcium Tyrode solution ('zero' or 0·45 mM calcium) for at least 1 hr before exposure to contracture solutions (containing 'zero' to 5·4 mM calcium) markedly influenced the

contracture tension (Fig. 8). Such experiments indicate that at a given calcium concentration in the contracture solution the incubating calcium concentration influences the subsequent contracture tension.

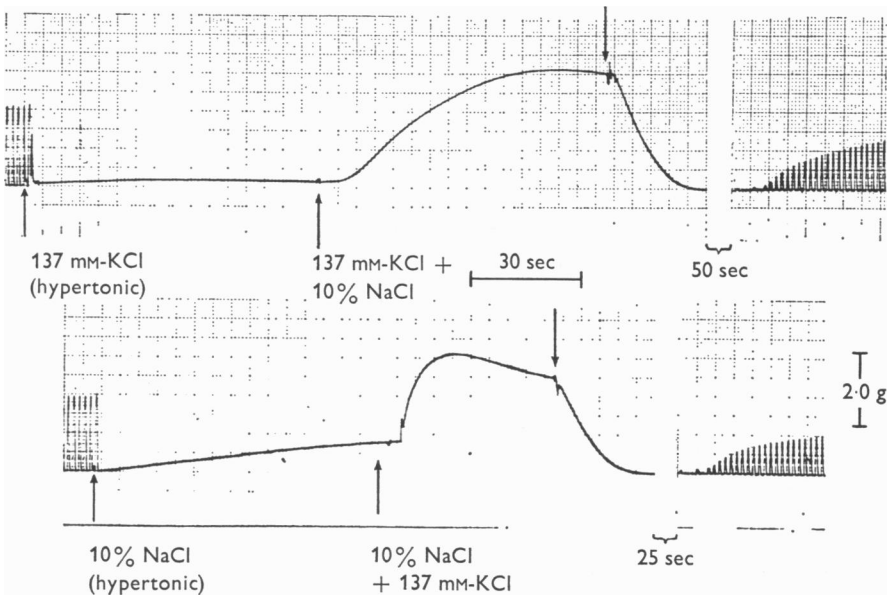

Fig. 7. Contracture responses of a cat ventricular trabeculum obtained from a cat pre-treated with reserpine in response to low sodium and high KCl-Tyrode. *Upper panel*, contractile response of the muscle to sequential exposure of hypertonic KCl and isotonic KCl-Tyrode. The downward arrow indicates readmission of normal Tyrode. *Lower panel*, the same maximum contracture tension is obtained when the sequence of exposure of muscle to the contracture solutions was reversed (temperature 25° C, diameter 0·33 mm).

Contraction and catecholamines

In the KCl-induced contracture studies, the relaxing effect of catecholamines was examined independently of the duration of depolarization. In the following section the relaxing effect of catecholamines on tension is examined during the time course of an action potential under conditions which alter the duration of the action potential and the amplitude of the twitch.

The effect of temperature and stimulus frequency on the relaxing effect of adrenaline. In nine cat papillary muscles simultaneous recording of isometric tension and transmembrane potential permitted study of the time course of the action potential, contraction cycle, and rest interval in the presence and absence of adrenaline (2 μg/ml.) at two temperatures (26 and 36° C) and two stimulus frequencies (12 and 120/min). Under the appropriate conditions the effect of adrenaline was to prolong action potential

duration and increase the force of contraction while shortening relaxation time. The effects on action potential and relaxation time were most evident at the lower frequency and temperature (Fig. 9*A*). At 12/min and 26° C the total twitch duration in the absence of adrenaline was 738 ± 21 msec (S.E. of mean); twitch duration in the presence of adrenaline was 594 ± 12 msec ($P < 0.001$). Adrenaline shortened relaxation time from 430 ± 25 msec to 364 ± 7 msec ($P < 0.05$) and simultaneously lengthened action

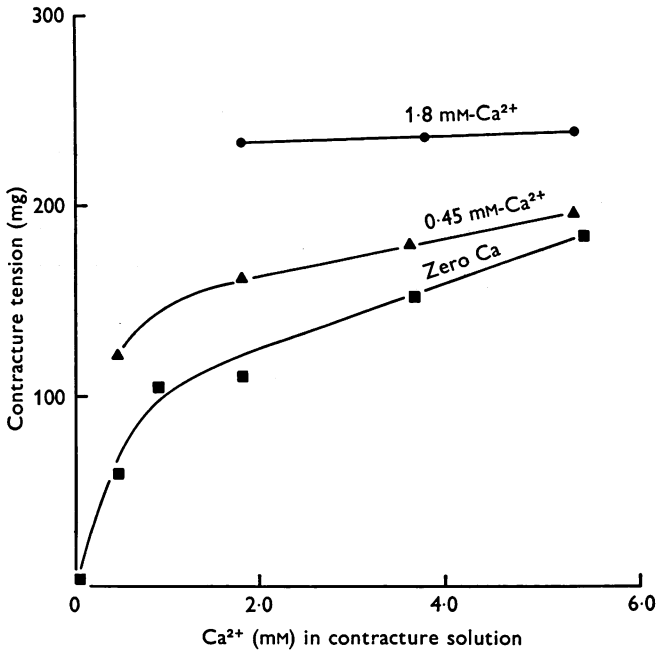

Fig. 8. Influence of $[Ca]_o$ on contracture tension in papillary muscle from a cat pre-treated with reserpine. Following incubation of the muscle in 1·8 (●), 0·45 mM (▲), or 'zero' (■) $[Ca]_o$, the concentration of calcium in the contracture solution was varied. More tension is obtained with higher $[Ca]_o$. At higher incubating calcium concentrations, the effect of increasing $[Ca]_o$ in the contracture solutions on developed tension is minimal (temperature 24° C, diameter 0·4 mm).

potential duration from 488 ± 27 to 610 ± 32 msec ($P < 0.001$). An increase in temperature (Fig. 9*B*) served to shorten the twitch by shortening the action potential so that adrenaline had little or no further effect on relaxation time. As at the lower temperature, adrenaline was observed to prolong the action potential. At the higher stimulus frequency and lower temperature the muscle was unable to relax fully before the next beat despite complete membrane repolarization (Fig. 10*A*). Although there was slight prolongation of the plateau of the action potential with the addition

of adrenaline, the muscle relaxed completely. This effect was observed consistently at low temperature and stimulus frequencies above 96 beats/min. At both high frequency of stimulation and increased temperature the effect of adrenaline on relaxation was manifested by the lengthening of the interval between contractions (increase in diastolic time) despite an unchanged interval between action potentials (Fig. 10*B* and *C*).

The effect of extracellular calcium concentration on the relaxing effects of adrenaline. The effects of adrenaline over a wide range of $[Ca]_o$ were examined in eight cat papillary muscles. At low frequency of stimulation (12/min) and 28° C, maximum twitch tension was achieved by 14·4 mM

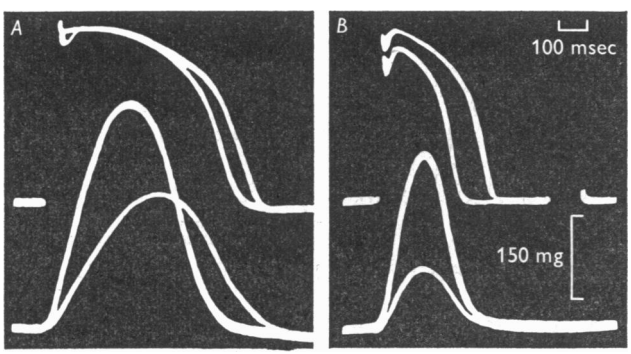

Fig. 9. Effect of adrenaline on contraction (lower trace) and action potential (upper trace) at two different temperatures. *Panel A*, the positive inotropic effect, the shortening of the time-to-peak tension, the prolongation of the action potential and the increase of rate of relaxation are seen with addition of adrenaline at 25° C. *Panel B*, the same muscle is subjected to 35° C in presence and absence of adrenaline. Although there is a marked positive inotropic effect with addition of adrenaline (the bigger tension trace), the time-to-peak of tension and rate of relaxation do not seem to be substantially augmented. A 100 mV square signal for 100 msec is indicated at the end of the membrane potential traces (frequency of stimulation 12/min, diameter 0·5 mm).

$[Ca]_o$. The addition of adrenaline to this high $[Ca]_o$ not only failed to augment twitch tension, but often reduced the peak developed tension by initiating relaxation earlier. Fig. 11*A* illustrates this point with superimposed tracings of action potentials and twitches obtained in the presence and absence of adrenaline at 14·4 mM-$[Ca]_o$. Fig. 11*B* shows the effect of two calcium concentrations (0·45 and 14·4 mM) on action potential and contraction in the presence of adrenaline. The lower calcium concentration prolonged the action potential and reduced twitch amplitude. Oscillatory contractions often appeared in low calcium solutions when the action potential was further prolonged by the addition of adrenaline. Comparable oscillatory contractions were observed when the sarcolemmal

membrane was voltage-clamped at +20 or +50 mV in the presence of adrenaline (M. Morad, unpublished observations). If the voltage–clamp was maintained for 3–5 sec, the oscillations tended to dampen to a steady tension which was markedly below the maintained (contracture) tension achieved in the absence of adrenaline.

The state of membrane polarization and adrenaline-augmented relaxation. In the preceding experiments adrenaline appeared to cause a dissociation between relaxation of the twitch and repolarization of the action potential.

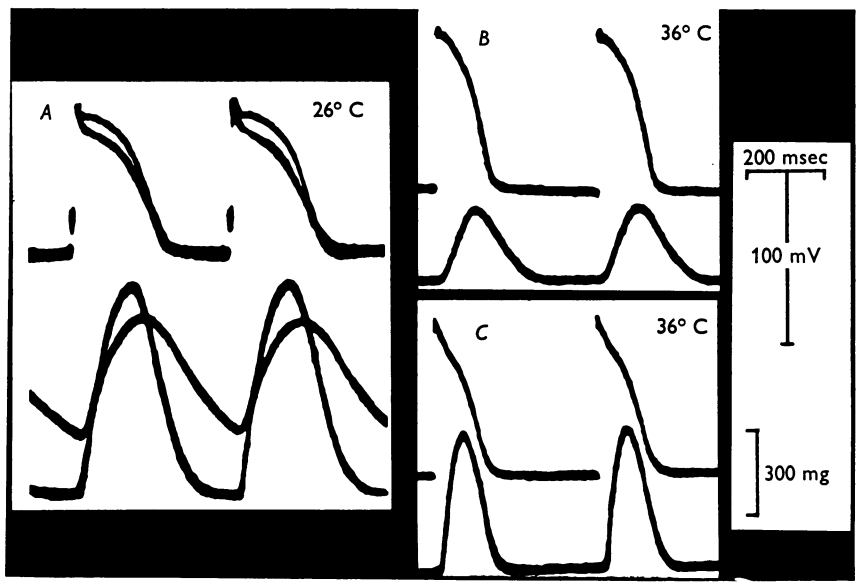

Fig. 10. The effect of adrenaline on contraction (lower trace) and action potential (upper trace) at variable rates and temperatures. At a frequency of stimulation of 120 beats/min and 26° C muscle fails to relax in spite of complete repolarization of the action potential. Addition of adrenaline augments the relaxation so that the muscle relaxes fully (*panel A*). At these frequencies adrenaline, while failing to prolong the action potential, slightly augments the plateau. *Panel B* shows that the relaxation is augmented in presence of high temperature (36° C), so that the muscle completely relaxes before the occurrence of the next action potential. Nevertheless, adrenaline further accelerates the relaxation (*panel C*) (diameter, 0·7 mm).

A series of experiments was carried out to confirm that the shortening of relaxation time by adrenaline is independent of the state of the membrane potential. In six cat papillary muscles following control measurements, the action potential was terminated abruptly at the 'apparent onset of contraction' by anodal repolarization or by clamping the membrane potential to the rest potential (Fig. 12). The twitches accompanying such shortened action potentials were examined in the presence and absence of adrenaline

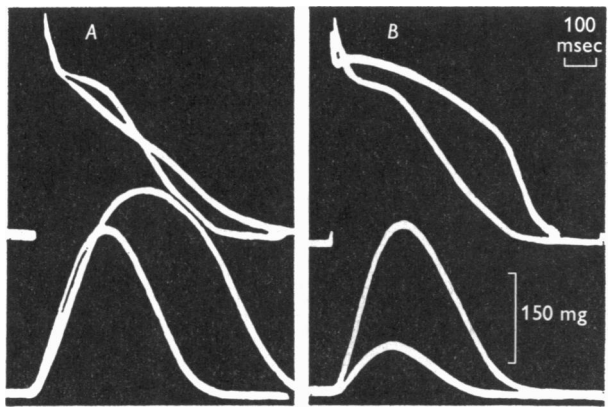

Fig. 11. Effect of adrenaline on cat ventricular trabeculum at high and low calcium concentrations. In *panel A* muscle is equilibrated in 14·4 mM calcium-containing Tyrode solution. Addition of adrenaline fails to augment twitch tension (lower trace), but shortens the relaxation time. Adrenaline shortens the action potential at this calcium concentration while the plateau is consistently augmented. *Panel B*, the effect of reduction of the calcium concentration from 14·4 to 0·45 mM in the presence of adrenaline 2 μg/ml. Although the action potential is prolonged and twitch tension diminished with a decrease in external calcium concentration, the time-to-peak of tension is shortened (temperature 28° C, diameter 0·30 mm).

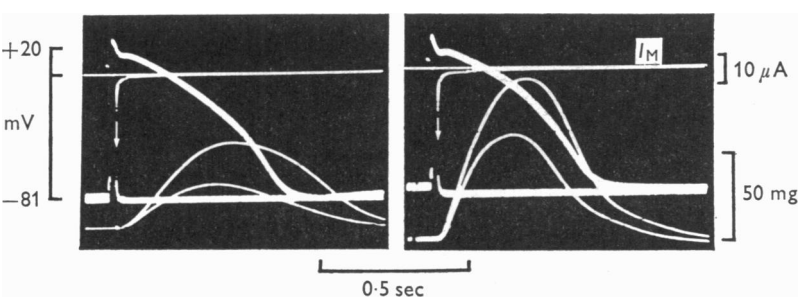

Fig. 12. State of membrane polarizations and the enhancement of relaxation by adrenaline. Left panel shows oscilloscopically superimposed tracings of a control action potential and contraction and a shortened action potential (at the downward arrow) and its accompanying contraction obtained in a cat papillary muscle bathed in normal Tyrode. In right panel, the action potential and the terminated action potentials (at the downward arrow) and their accompanying contractions in the presence of adrenaline 2 μg/ml. are shown in the same preparation. Note that adrenaline shortens the time-to-peak or relaxation time whether the plateau is allowed to have its normal time course or is prematurely terminated to the resting potential. Inward current I_m (plotted downward) caused by clamping the membrane potential to the resting potential are included (temperature 25° C, frequency of stimulation 12 beats/min, diameter 0·4 mm).

(1 μg/ml.). Fig. 12 (left panel) shows the superimposed tracing of a normal action potential and contraction and a shortened action potential (approximately to 40 msec) and its accompanying contraction in Normal Tyrode solution (see also Morad & Trautwein, 1968). Fig. 12 (right panel) shows the results of repeating the same experimental procedure in the presence of adrenaline, 2 γ/ml. The positive inotropic and the potentiated dp/dt effects of adrenaline are well apparent, both in the control and the manipulated beats. Fig. 12 also illustrates that the contraction accompanying the 'shortened' action potential in the presence of adrenaline has a shorter time-to-peak (by about 100 msec) and relaxation time than the shortened contraction in the absence of adrenaline. Thus the augmentation of contraction and relaxation by adrenaline was seen despite the abrupt repolarization of the membrane at the onset of the plateau. The shortening of the relaxation time or the time-to-peak caused by adrenaline were approximately the same whether the plateau was allowed to have its normal time course or whether the action potential was shortened prematurely by clamping it to the resting potential.

DISCUSSION

The main conclusion from these studies is that catecholamines (adrenaline and noradrenaline) enhance or stimulate the relaxation processes of mammalian myocardium. The presence and release of endogenous catecholamines protects the myocardium from going into contracture (state of calcium overload) when exposed to high KCl concentrations. The relaxation-enhancing property of catecholamines on the myocardium seems to be independent of their well known positive inotropic effect and of their effect on the action potential. The relaxant action of catecholamines manifests itself regardless of the state of membrane polarization.

The relaxation effect of catecholamines on contraction and contracture in mammalian myocardium is compatible with a model in which the mechanisms whereby catecholamines enhance relaxation and exert a positive inotropic action are independent of one another. The major features of the model ('independent relaxation hypothesis') are as follows: (1) catecholamines increase the influx of activator substance during the action potential, causing an increase in positive dp/dt and twitch (Grossman & Furchgott, 1964; Reuter, 1965; Vassort, Rougier *et al.* 1969); (2) catecholamines stimulate the calcium sequestering system (sarcoplasmic reticulum, mitochondria, and sarcolemmal membrane) independently of the inotropic action (see also Graham & Lamb, 1968); (3) the extent of the development of sarcoplasmic reticulum and other components of the relaxation system is the final determinant of the rate of

sequestering of calcium; (4) the effect of catecholamines on the sequestering rate of calcium is not mediated by the increased calcium influx; (5) both the positive inotropic and relaxing effects of catecholamines are dependent on intact β-adrenergic receptor sites. The model does not necessarily require a common binding site for these two properties.

This hypothesis is supported by the following observations: (1) adrenaline and noradrenaline prevent or relax KCl-induced contractures in a number of species: in frog, Kavaler & Morad (1966), Graham & Lamb (1968); in cat, Morad (1969) (see also Figs. 3, 4, 5 and 6) and in slow avian skeletal muscle (Somlyo & Somlyo, 1969); (2) KCl causes release of endogenous catecholamines (Haeusler *et al.* 1968; Kirpekar & Wakade, 1968); (3) catecholamine depletion (reserpine), sympathetic denervation (6-OH-DA), or adrenergic blockade (propranolol) enhance contracture development on exposure of adult myocardium to KCl (Figs. 3, 4, 5); (4) neonatal cat myocardium with low noradrenaline content (30% of adult level) is susceptible to KCl-induced contracture (Fig. 6); (5) in maximally enhanced twitch (by increasing $[Ca]_o$), adrenaline exhibits its marked relaxant effect only (Fig. 11); (6) the relaxant effect is present whether the membrane potential is held at the plateau or returned to the resting level by anodal repolarization (Fig. 12) and when the action potential is altered by variations in temperature (Fig. 9), frequency of stimulation (Fig. 10), and calcium concentration (Fig. 11).

Calcium sequestering system and catecholamines. The ability of adult mammalian myocardium to resist contracture and the ease with which contractures develop and are maintained in frog and neonatal mammalian ventricular muscle correlate well with the amount and degree of organization of sarcoplasmic reticulum and the development of the T-tubular system (frog: Staley & Benson, 1968; Sommer & Johnson, 1969; adult cat: Fawcett & McNutt, 1969; neonatal mammal: Orkand, 1964). *In vitro* studies of calcium binding by skeletal and cardiac muscle microsomes have suggested that the sarcoplasmic reticulum is the major component of the muscle relaxation system (Hasselbach & Makinose, 1961; Ebashi & Lipmann, 1962; Carsten, 1964). Although our model predicts that the mechanism of the catecholamine relaxing effect is the enhancement of calcium sequestration, the evidence for a direct effect of catecholamines on microsomal calcium uptake in *in vitro* studies is variable and largely negative (Chimoskey & Gergely, 1968; Hess, Briggs, Shinebourne & Hamer, 1968; Dhalla, Sulakhe, Khandelwal & Hamilton, 1970). The enhancement of calcium turnover by catecholamines in guinea-pig atrium (Grossman & Furchgott, 1964; Reuter & Wollert, 1967) is more consistent with the model. The present studies show convincingly that catecholamines promote relaxation in a depolarized ventricular muscle preparation

where the calcium sequestering system has been overloaded by increasing the 'effective' calcium activity (reduction of $[Na]_o$, Figs. 3, 4, 5) or where the calcium sequestering system is poorly developed (e.g. neonatal cat, Fig. 6; frog: Niedergerke, 1956).

The contracture experiments are also consistent with the observed enhancement of relaxation by the catecholamines during the time course of the single twitch. At high frequencies of stimulation and low temperatures, partly because of high influx of calcium (Langer, 1965), the developed tension is large. Since low temperatures, at the same time, reduce the rate of sequestration of the sarcoplasmic reticulum (Harigaya & Schwartz, 1969) the rate of relaxation is slowed and the myocardium often fails to relax completely and develops 'diastolic contractures' (Fig. 10, panel *A*), i.e. a state of calcium overload. The addition of adrenaline under these circumstances causes complete relaxation of the muscle as a result of enhanced rate of relaxation (Fig. 10, panel *A*). At higher temperatures the sequestering system takes up calcium more rapidly (Harigaya & Schwartz, 1969) and the myocardium handles the calcium load more effectively. Consequently, the effect of adrenaline on relaxation time, though unmistakable, is less pronounced at higher than at lower temperatures (Figs. 9 and 10).

Membrane potentials and the relaxant action of adrenaline. The effect of adrenaline on the action potential does not seem to be related to its effect on relaxation. In fact, the ability of catecholamines to enhance myocardial relaxation appears to be independent of whether the action potential is prolonged or shortened by adrenaline (Fig. 11) or whether the action potential is terminated prematurely or prolonged by anodal or cathodal pulses (Fig. 12 and M. Morad, unpublished observations). Since the action potential duration appears to determine the amplitude and the duration of contraction in mammalian heart (Morad & Trautwein, 1968), the fact that the relaxant action of catecholamines is present whether the action potential is long or short supports assertion 2 of the 'independent relaxation hypothesis'.

The prolongation of action potential plateau by adrenaline in normal or low calcium solutions (Figs. 9 and 11 and Carmeliet & Vereecke, 1969) could bring about an increase in developed tension by a more complete replenishment of the calcium stores during the action potential (Wood, Heppner & Weidmann, 1969; Morad & Orkand, 1971). Although this hypothesis could offer a mechanism for the positive inotropic action of catecholamines, it does not provide for the dissociation of the time-to-peak of contraction from the onset of the fast repolarization phase of the action potential.

Catecholamine induced stimulation of 'energetics' and enhancement of

relaxation. The exact mechanism by which catecholamines enhance relaxation remains still unknown. However, the experiments discussed above seem to suggest that the relaxation-enhancing properties of catecholamines are not mediated through changes in the action potential duration or the state of membrane polarization or the availability of the extracellular calcium. These experiments further suggest that the relaxant enhancing properties are highly temperature dependent (Fig. 9 and 10). These findings led us to believe that the relaxant enhancing characteristics of catecholamines may be tightly linked to the rate of energetic turnover in the myocardium. There is considerable evidence that calcium accumulation by the sarcoplasmic reticulum and mitochondria is associated with a stoicheiometric uptake of oxygen (Chance, 1965) and ATP hydrolysis (Hasselbach & Makinose, 1961) and is inhibited by 2,4-dinitrophenol and oligomycin (Lehninger, Carafoli & Rossi, 1967). Anoxia readily induces contractures in cardiac muscle (Walker & Weatherall, 1964). This finding was consistent with our own observations that cardiac muscle in poor physiological condition (inadequate oxygenation, etc.) always showed a slow rate of relaxation and produced contractures upon challenging it with KCl solutions.

Since the sarcoplasmic reticulum is highly implicated in the processes of muscular relaxation (Hasselbach, 1964), its stimulation by catecholamines to increase calcium uptake (in the *in vitro* studies) could provide an explanation for enhanced relaxation processes seen in the intact myocardium. Although catecholamines stimulate a number of cellular enzymatic reactions (e.g. adenyl cylase: Murad *et al.* 1962; phosphorylase: Hess & Haugaard, 1958; phosphorylase kinase: Namm & Mayer, 1968), they have little or no direct effect on microsomal ATP-ase activity (Scales & McIntosh 1968) or on microsomal calcium uptake (Chimoskey & Gergely, 1968; Hess *et al.* 1968; Dhalla *et al.* 1970). Such negative findings on the isolated microsomal preparation may suggest that the relaxant action of catecholamines requires membrane binding sites or intermediate steps which are only present in intact tissue. However, there is additional evidence in the intact myocardium which suggests that the catecholamine induced relaxation of the KCl contracture is not mediated by adenyl cyclase stimulation. For instance, Namm, Mayer & Maltbie (1968) showed that adrenaline failed to increase cyclic 3′,5′-AMP when $[K]_o$ was elevated to 56 mM in a perfused rat heart. Further, that the administration of 10^{-3} M dibutyryl, 3′,5′-cyclic AMP (Sigma), while potentiating twitch tension, fails to enhance the rate of relaxation of contraction or depress contracture-tension in the frog ventricular strips (M. Morad, unpublished observations).

It may well be that cardiac muscle *in situ*, in responding to conditions

which demand a high performance, is as dependent on the adrenergic stimuli and circulating adrenaline to increase the speed of the relaxing system as to augment contraction.

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