J. Physiol. (1968), **197**, *pp.* 479–509
With 11 *text-figures*
Printed in Great Britain

THE EFFECT OF ADRENALINE ON THE TENSION DEVELOPED IN CONTRACTURES AND TWITCHES OF THE VENTRICLE OF THE FROG

BY J. A. GRAHAM* AND J. F. LAMB
From the Institute of Physiology, The University, Glasgow, W. 2

(*Received* 13 *March* 1968)

SUMMARY

1. The effect of adrenaline on contracture and twitch tension in frog's ventricle has been examined, using the superfused preparation.

2. In 1 mM-Ca Ringer, contractures induced with excess KCl concentrations from 50 to 200 mM, are reduced by 1×10^{-6} g/ml. adrenaline to an average of 0·62 of control values, in marked contrast to the well known positive inotropic effect of adrenaline on the heart twitch. This effect of adrenaline is directly dose dependent. Increasing $[Ca]_o$ diminishes the effect of adrenaline on contracture tension, and on the twitch tension.

3. Adrenaline has a significantly greater effect on the KCl contracture tension than noradrenaline or isoprenaline.

4. In 1 mM-Ca Ringer, Na-free contractures are reduced to 0·72 of controls by 1×10^{-6} g/ml. adrenaline. Adrenaline also significantly reduces tension in contractures induced by 50 c/s alternating current.

5. The action of adrenaline on contracture tension is largely complete in 1–2 min at various rates of stimulation and calcium concentrations. A similar time course has been found for the effect of adrenaline on membrane potential.

6. Pronethalol blocks the action of adrenaline on both twitch and contracture. The action on the contracture can also be blocked by ouabain (1×10^{-5} M), and exposure of the tissue to K-free or Na-free Ringer solution.

7. Adrenaline hyperpolarizes the membrane potential with a range of $[K]_o$ from 0 to 200 mM. This effect is blocked by pronethalol and ouabain. After exposure to ouabain, adrenaline causes a significant decrease in the membrane potential. This may be due to an increase in the sodium permeability.

8. At low values of the $[Ca]/[Na]^2$ ratio, adrenaline takes a relatively constant number of beats for full action, but at high values of the ratio the development of full effect is largely time dependent.

* Present address: Department of Surgery, Western Infirmary, Glasgow, W. 2.

9. The time course of the effect on the twitch of changing from 0·5 to 2 mM-Ca Ringer has been studied at various rates of stimulation. The equilibration time has been found to depend on the heart rate.

10. The effect on the contracture suggests that adrenaline decreases the calcium permeability. It is further suggested that the development of twitch tension is not due to direct Ca entry but is due to the release of Ca from a local store within or between the cells. The inotropic action of adrenaline is explained in terms of this store.

INTRODUCTION

In frog ventricle tension is normally produced via an action potential. Tension may also be produced by depolarization with excess KCl (Burridge, 1911) or by removal of sodium from the external medium (Lüttgau & Niedergerke, 1958). Generally twitches and potassium contractures react in a similar way to various agents. Thus both increase during the stair-case phenomenon (Niedergerke, 1956*a*, *b*); changes in the ratio [Ca]/[Na]^2 of the bathing medium affect the twitch (Wilbrandt & Koller, 1948) and the contracture similarly (Lüttgau & Niedergerke, 1958); and ouabain increases both twitch (Gold & Cattell, 1940) and contracture tension (Otsuka & Nonomura, 1963). Measurements of Ca influx in these various conditions show that the flux alters in the same direction during development of tension in twitch and in contracture (Winegrad & Shanes, 1962; Niedergerke, 1963*a*, *b*; Holland & Sekul, 1959). On this and other evidence it has been suggested that tension development in cardiac muscle is due to the direct entry of Ca into the cells (Winegrad & Shanes, 1962; Niedergerke, 1963*a*, *b*).

Stimulation of the sympathetic nerves to the heart or addition of adrenaline greatly increases the force of contraction (Gaskell, 1884; Oliver & Schäfer, 1895; Elliot, 1905); the metabolism is also increased (Barcroft & Dixon, 1907; Evans & Ogawa, 1914). Recent extensions of these early observations show that adrenaline increases the force of contraction of heart muscle at all velocities on the force–velocity graph (Sonnenblick, 1962) and causes large changes in cellular biochemical mechanisms (Haugaard & Hess, 1965; Øye, 1965; Williamson, 1966). However, the mechanism of the inotropic action of adrenaline is still obscure.

The present experiments started with the observation that adrenaline decreased the contracture tension in frog ventricle, although it increased the twitch tension as usual (Graham & Lamb, 1966; Graham, 1966). Since then a similar observation has been reported for both frog and cat heart muscle by Kavaler & Morad (1966), which they have called the 'paradoxical' action of adrenaline on the heart. This paper is largely concerned

with the characterization of this new phenomenon, and its relation to the other effects of adrenaline. It is suggested that this effect occurs because adrenaline decreases the calcium permeability (P_{Ca}) of the cells.

METHODS

Frogs (*Rana temporaria* or *Rana pipiens*) were brought from the cold room, decapitated, pithed and the hearts exposed. The ventricles were either used as superfused preparations to measure tension, or as strips for measuring membrane and action potentials. The experiments were carried out at room temperature (about 21° C) throughout the year. The superfused preparations were driven electrically at 30/min unless otherwise stated.

Tension recording. Half ventricles were pinned out cut side uppermost by two entomological pins and irrigated with a jet of Ringer solution (Lamb & McGuigan, 1966). The third 'corner' of the ventricle was connected by a short length of Arbrasilk 4/0 thread to a Grass FTO 3 transducer used on its most sensitive range. This has a natural resonance of 85 c/s. The output was displayed on a Devices Single Channel Recorder; the over-all response was flat from 0 to 60 c/s. The ventricle was stimulated via two small Ag–AgCl electrodes with a Devices Isolated Stimulator driven by a Digitimer, using 10 V pulses of 1 msec duration, roughly 3 times the threshold value. At the end of each experiment the wet weight of the ventricle was measured and the results expressed as developed tension per wet weight.

Electrical contractures. In a few experiments tension was elicited by passing 50 c/s alternating current through the ventricle. This was achieved by pinning the ventricle on to a grid made of silver wire and laying a similar grid across the cut surface of the ventricle. Superfusion was then carried out through this top grid. Current was applied from the secondary winding of a heater transformer (nominally 6 V), and was controlled by a Variac between the primary coil and the mains supply. The voltage applied to the heart was switched on and off manually. For some experiments this electrode arrangement was used to drive the ventricle in the manner described above; the results obtained were similar to those found using small electrodes. It was thought that with these large electrodes all the cells were being activated both during the twitch and the contracture, both in hypodynamic ventricles and in the presence of adrenaline.

Potential measurements. These were measured by intracellular micro-electrodes inserted into cells in strips of ventricle held in a wax bath by light glass clips. The micro-electrodes were held in a Prior manipulator and connected to a cathode follower 1 in. (2·54 cm) away. The output was displayed on a Tektronix oscilloscope. Usually potentials were measured directly by 'backing off' the trace with a known potential; occasionally they were recorded on a Siemens Oscillominx pen recorder (frequency response 0-500 c/s) and measured later. Stimulation was as described for tension recording. As washing of these strips was not as effective as in the superfused preparation only subpericardial cells were penetrated. It was thought that oxygenation and perfusion by the various solutions should be adequate at this superficial site. Micro-electrodes were pulled from Pyrex glass on a Micro-pipette Puller Ml (Industrial Sciences Associates, Inc.), and they were filled by boiling in 3 M-KCl. Resistances ranged from 5 to 30 MΩ; no tip potentials were measured but the other criteria of Adrian (1956) were observed.

Solutions. These are shown in Table 1. The 200 mM-KCl actually contained 202·5 mM of KCl. The osmotic pressure of the sucrose solutions was calculated from the equation of Dydyńska & Wilkie (1963). Calcium was varied within the range 0–10 mM, with no compensating variations in the other constituents. The normal concentration was 1 mM.

Drugs. These were made as stock concentrated solutions and stored at 7° C. They were discarded after 3–4 weeks. Final dilutions were made immediately before use. The following stocks were used:

Adrenaline; Evans' Medical 1:1000 multi-dose vial. Noradrenaline; 1×10^{-3} g/ml. from solid (−)-noradrenaline bitartrate (Koch-light Laboratories). Isoprenaline; 1×10^{-3} g/ml. as the hydrochloride from solid DL-N-isopropylnoradrenaline (Aldrich Chemical Co., Inc.). Pronethalol; 1×10^{-2} g/ml. from solid 'Alderlin' (I.C.I.) pronethalol HCl. Ouabain; 1×10^{-3}M from oubain glucoside (Laboratory Nativelle, Ltd.). The concentrations of the catecholamines refer to the base, those of pronethalol and ouabain to the salt.

TABLE 1. Solutions used, figures are in mM. Calcium was varied within the rage of 0–10 mM by adding molar $CaCl_2$; the normal was 1 mM. As shown, Ringer + 200 mM contained 202·5. mM-KCl

Solution	Title	Na	K	Cl	HPO_4	H_2PO_4	Glucose	Sucrose g/l.	Relative tonicity
A	Normal Ringer	120·15	2·5	117·5	2·15	0·85	5·55	—	1·00
B	K for Na Ringer	5·15	117·5	117·5	2·15	0·85	5·55	—	1·00
C	Ringer + 200·00 mM-KCl	120·15	202·5	317·5	2·15	0·85	5·55	—	2·53
D	Ringer + excess sucrose	120·15	2·5	117·5	2·15	0·85	5·55	123·0	2·53
E	Na-free Ringer	5·15	2·5	2·5	2·15	0·85	5·55	73·1	1·00

RESULTS

Experiments to characterize the negative inotropic action of adrenaline on the contracture

The effect of adrenaline on contractures induced by excess KCl. Figure 1 shows a typical experiment. The upper record shows the twitch tension developed in a superfused preparation driven at 30/min in 1 mM-Ca Ringer. At the first bar stimulation was stopped and the ventricle was exposed to Ringer + 200 mM-KCl. This gave rapid onset of tension reaching a peak and then decaying with a $T_{\frac{1}{2}}$ of about 60 sec. Replacing the high KCl Ringer with normal Ringer solution at the second bar caused a fairly rapid return of tension back to the resting level. After 5 min recovery, adrenaline 1×10^{-6} g/ml. was added (lower record) and allowed to produce its full inotropic effect on the twitch. Another KCl contracture was then induced as before. The following points can be made: (i) adrenaline has increased the twitch tension but has decreased the maximum contracture tension, (ii) the twitch tension after adrenaline now exceeds the tension of the adrenaline affected contracture, (iii) in the presence of adrenaline the contracture is preceded by a well developed 'twitch', whereas tension develops smoothly and uniformly to a peak in the control contracture.

In order for adrenaline to have its full effect on the contracture induced by excess KCl, the ventricle must be exposed to adrenaline until the full inotropic effect on the twitch is obtained. The presence or absence of adrenaline in the contracture fluid then used made no difference to the effect, and for convenience was usually omitted. The speed with which the

contracture tension decayed in the presence of excess KCl was somewhat increased by adrenaline. This means that adrenaline has a continuing effect on reducing contracture tension. Contractures induced with slow application of KCl are affected more by the rate of decay of tension in the presence of KCl than in a superfused preparation (Lamb & McGuigan,

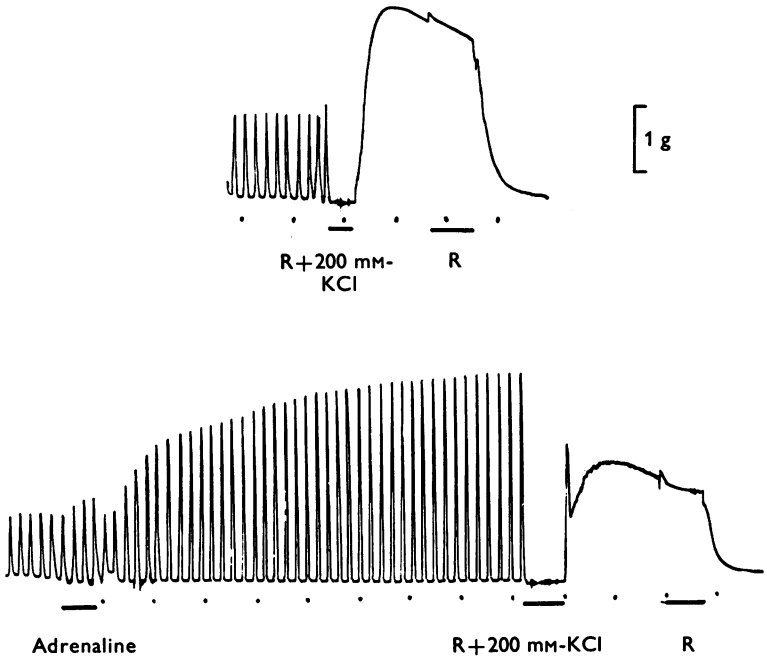

Fig. 1. The effect of adrenaline (10^{-6} g/ml.) on twitch and contracture tension. Ordinate, tension; abscissa, time in 10 sec intervals. Upper record, control. Lower record, effect of adrenaline developing on the twitch and subsequent contracture. Note initial 'twitch' before sustained tension on adrenaline contracture record. Contractures initiated by replacing normal Ringer (R) with Ringer + 200 mM-KCl. Adrenaline added where indicated by bar. $[Ca]_o$ = 1 mM. Driven at 30/min. In this and subsequent records the distance of 1 g scale above the base line indicates the resting tension used; all records were traced and redrawn.

1966) and therefore provide another way of testing this point. In one experiment in 1 mM $[Ca]_o$ adrenaline reduced the contracture tension to 61 % in a superfused preparation and to 44 % with slow application of KCl.

The initial twitch in KCl contractures in the presence of adrenaline. This was tentatively assumed to be due to the increased excitability of the tissues under the influence of adrenaline, so that the high KCl produced an action potential, and therefore a twitch before the slower steady depolarization was complete. This twitch prolongs the time to peak con-

tracture tension by a variable but small amount. As the peak tension declines relatively slowly with a $T_{\frac{1}{2}}$ of 40–60 sec (Lamb & McGuigan, 1966) this should not greatly affect the tension developed, and comparison of equivalent points on contracture records before and after adrenaline confirmed that in fact this had less than a 5% effect on peak twitch tension. It might be supposed however that the presence of this spike could be reducing the contracture tension in some other way, and experiments were carried out in situations where this initial twitch could be abolished. This was carried out in the three ways described below. In general all these techniques gave similar results in that adrenaline still reduced the contracture tension when the initial spike was absent.

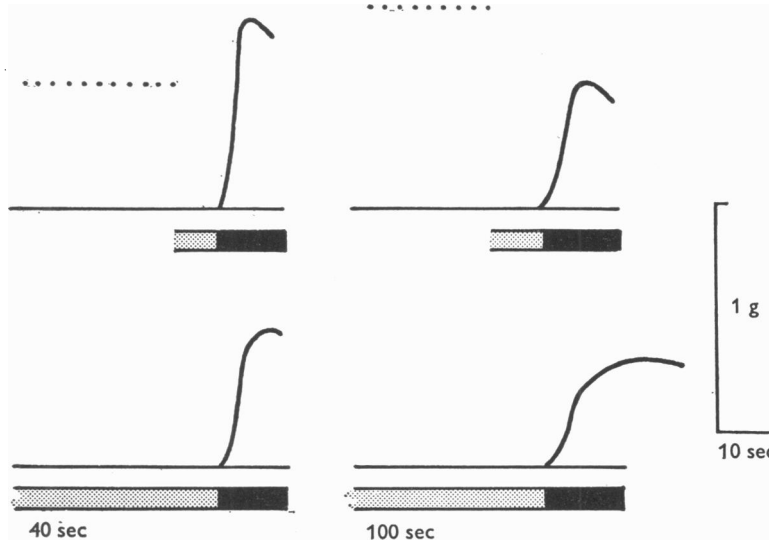

Fig. 2. The effect of pretreatment with 25 mM-KCl on contractures. Contractures induced with Ringer + 200 mM-KCl as in Fig. 1, indicated by the solid bar; pretreatment with Ringer + 25 mM-KCl indicated by the stippled bar. Recording stopped once tension started to decline. Dots indicate tops of twitches. Upper traces left, control; right, after adrenaline (10^{-6} g/ml.) pretreatment. In each case ventricle treated with Ringer + 25 mM-KCl for 5 sec before addition of Ringer + 200 mM-KCl. Note adrenaline contracture still smaller, and has a slower onset, with no initial 'twitch'. Lower traces; effect of 40 and 100 sec in Ringer + 25 mM-KCl before adding contracture fluid; control trace, upper left. $[Ca]_o$ = 1 mM.

Firstly, the twitch could be abolished by a preliminary exposure of the ventricle to an increased external potassium concentration. Figure 2 (upper traces) shows an experiment in which the twitch was abolished by exposure to 25 mM-KCl for 5 sec before the contracture was induced with

Ringer + 200 mM-KCl. It can be seen that the contracture is still greatly decreased by adrenaline. An additional finding of these experiments was that pretreatment of the ventricle with Ringer + 25 mM-KCl reduced the subsequent contracture response to 200 mM-KCl. The lower traces of Fig. 2 show that exposing the ventricle to 25 mM-KCl for 40 sec reduced the subsequent contracture to 0·71 and exposure for 100 sec reduced it to 0·54 compared to the control (upper left). The 5 sec necessary to abolish the twitch in the adrenaline experiments had no effect on contracture tension. Leaving the ventricle quiescent for 100 sec did not reduce the KCl contracture. The explanation for this effect of 25 mM-KCl is not clear but suggests that inactivation occurs at these levels of KCl although no tension is developed. It partly explains the observation made by Lamb & McGuigan (1966) that contractures with KCl applied slowly were usually appreciably smaller than those after fast KCl application.

Secondly, in several experiments, the osmotic pressure of the Ringer solution was increased by adding excess sucrose (to the same tonicity as Ringer + 200 mM-KCl). This abolished the twitches, but enhanced the tension of the control contractures, and after adrenaline the contracture tension was again reduced in the absence of the initial twitch.

Thirdly, in three experiments ACh was used to abolish the initial twitch. It was shown that ACh itself had no, or only a slight, potentiating effect on the contracture tension, and also that after exposure of the ventricle to ACh (1×10^{-5} g/ml.) for 5 sec (1×10^{-6} g/ml.) adrenaline still reduced contracture tension although the initial twitch was abolished. The reduction of tension was less, however, than that found without ACh, and further experiments showed that more prolonged exposure of the ventricle to ACh could completely abolish the effect of adrenaline on the contracture. This is similar to observations by Vincent & Ellis (1959) who showed that ACh blocked the effect of adrenaline on twitch tension and glycogenolysis in guinea-pig atria. This is possibly due to the known effect of ACh in counteracting the biochemical action of adrenaline (Murad, Chi, Rall & Sutherland, 1962; Hess, Shanfeld & Haugaard, 1962; Haugaard & Hess, 1966).

The conclusion from all these experiments is therefore that the initial twitch seen before the contracture in the presence of adrenaline is not responsible for the subsequent reduction in the developed tension.

A comparison of various procedures on the positive and negative inotropic actions of adrenaline. At this stage we had established that these two actions of adrenaline were related in that both required some time for full action after exposure to adrenaline, and we were now concerned to establish how closely parallel these two opposing actions were. For this purpose three kinds of experiments were done, (1) the actions of the blocking agent,

pronethalol, (2) dose–response curves of adrenaline and (3) variation in $[Ca]_o$ on these actions.

(1) In two experiments pronethalol (10^{-5} g/ml.; Ahlquist, 1948) blocked the effect of adrenaline on the twitch and the contracture in a similar time. This evidence is interpreted to mean that both actions result from an action of adrenaline at the same site on the cell.

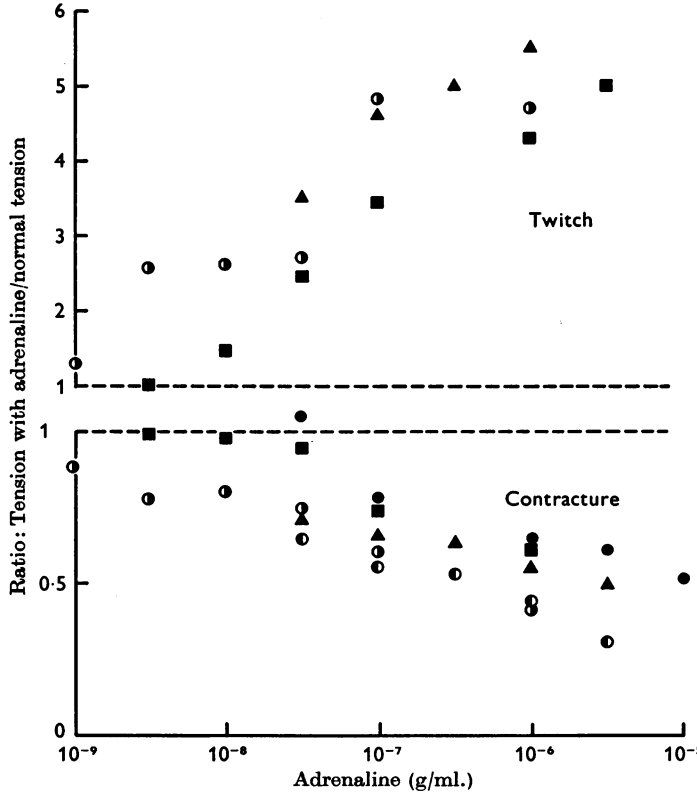

Fig. 3. Effect of different concentrations of adrenaline on the twitch and contracture. Abscissa; adrenaline (g/ml.). Ordinate; ratio of tension with adrenaline/control tension. Each experiment indicated by the same symbol. Contracture fluid for two experiments (◑, ◐) Ringer + 100 mM-KCl; for other three, Ringer + 200 mM-KCl. Increasing concentrations of adrenaline increase the twitch and decrease the contracture. (Correlation coefficient −0·448, $P = 0{\cdot}01$.) $[Ca]_o = 1$ mM. Rate, 30/min.

(2) The effect of adrenaline on contractures and twitches was examined in five experiments over the range 1×10^{-9} to 1×10^{-5} g/ml. The results are shown in Fig. 3. The lower graph shows the effect of adrenaline on the contractures in all the experiments and the upper the effect on twitch tension in only three of the experiments (chosen because the ventricles in

these three experiments were hypodynamic and therefore adrenaline has a greater effect on the twitch (Oliver & Shäfer, 1895)). It is clear that increasing concentrations of adrenaline increase the twitch and diminish contracture tension. In the three experiments shown completely in Fig. 3, the correlation coefficient of the effect of adrenaline on twitches and contractures was $-0{\cdot}448$ ($P = 0{\cdot}01$). There was also a significant correlation in the other two experiments, but the slope of the regression line was less, owing to the smaller effect on the twitches. Adrenaline at a concentration of 10^{-5} g/ml. produced no appreciable difference from 10^{-6} g/ml. in these five and in two other experiments and therefore 10^{6} g/ml. was used throughout these experiments. This evidence shows that although there is a correlation between these two actions of adrenaline, this is not very marked. This suggests that these two actions of adrenaline are not closely related.

(3) The effect of $[Ca]_o$ variation on the positive and negative inotropic actions of adrenaline was examined in various experiments over the range of $[Ca]_o$ of 0·5–10 mM. The ventricle was generally perfused with 1 mM-Ca Ringer in between test periods in other Ca solutions in order to avoid loading or depleting the tissue. The ventricle was exposed to the test Ca solution for a time sufficient to allow the twitch to settle to a new steady level.

The results of these experiments are shown in Fig. 4 in the same form as for Fig. 3. Each point is an average value with the number of experiments beside it. It is clear from Fig. 4 that increasing $[Ca]_o$ decreases the magnitude of both effects, although the effects do not occur at exactly the same $[Ca]_o$ values.

The conclusion at this stage is that the negative inotropic action of adrenaline on the contracture and the positive inotropic action of adrenaline on the twitch are correlated but not very strongly.

The effect of adrenaline on contractures induced with different concentrations of KCl. The tension developed in KCl contractures is related to log $[KCl]_o$ or membrane potential (E_m) along a sigmoid curve (Burridge, 1911; Lüttgau & Niedergerke, 1958; Lamb & McGuigan, 1966). Table 2 shows the effect of adrenaline on KCl contractures induced with varying values of $[KCl]_o$, but at constant osmolarity corresponding to Ringer + 200 mM-KCl. It can be seen that 1×10^{-6} g/ml. adrenaline produces a similar reduction of tension for all KCl contractures, averaging 0·62 of the tension developed without adrenaline.

The fact that tension becomes less sensitive to external KCl is expected for the negative inotropic action of adrenaline on the contracture and for a lower calcium permeability (Lamb & Lindsay, 1968); for in conditions inducing a positive inotropic action (increase in $[Ca]_o$) tension becomes

more sensitive to external KCl and the calcium entry increases (Lüttgan & Niedergerke, 1958; Niedergerke, 1963*a*, *b*).

Comparison of adrenaline, isoprenaline and noradrenaline. In four experiments it was found that 1×10^{-6} g/ml. of each of these catecholamines reduced the KCl contracture on average to 0·52, 0·64 and 0·72 respectively of the control. The effect of adrenaline was significantly greater than either of the others. No conclusions on the relative effect on the twitch could be reached because of the variable initial twitch tensions.

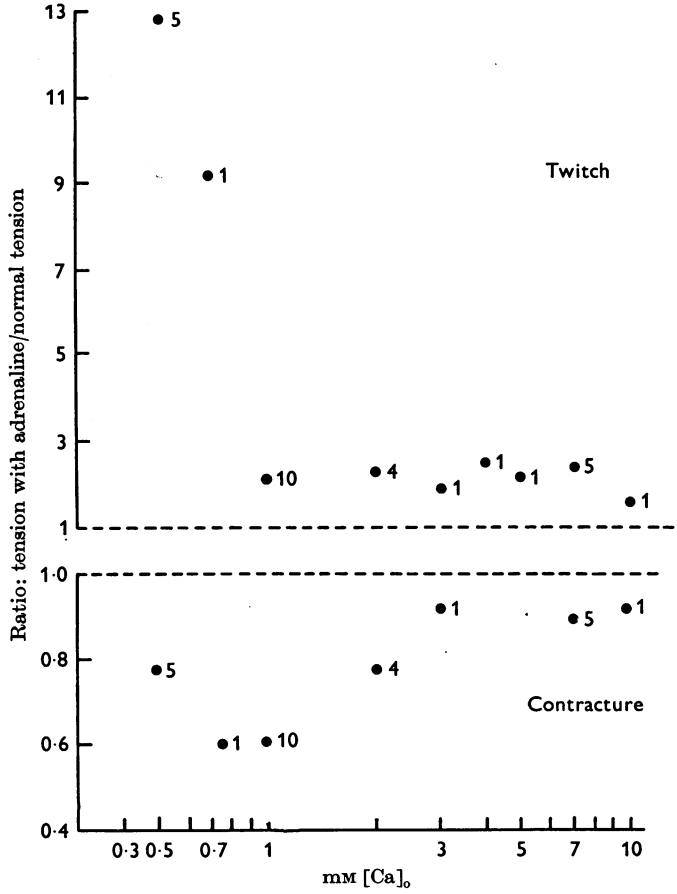

Fig. 4. The effects of adrenaline at various calcium concentrations. Abscissa; $[Ca]_o$ on a log scale. Ordinates; as Fig. 3. Each point is an average value with the number of experiments given beside it. As [Ca] rises then adrenaline has less effect on the twitch and the contracture. Driven at 30/min. The reduction in contracture at 1 mM $[Ca]_o$ is significantly greater than that at 7 mM $[Ca]_o$ [$P < 0{\cdot}001$]. The reduction in contracture at 0·5 mM $[Ca]_o$ is not significantly different from that at 1 mM $[Ca]_o$. The twitch increase at 0·5 mM is also significantly greater than that at 7 mM $[Ca]_o$ [$P < 0{\cdot}001$].

Contractions may be induced in frog's ventricle by other means, e.g. Na removal or by electrical depolarization. These methods enabled us to determine where this negative inotropic action of adrenaline was due to some specific interaction between KCl and adrenaline or was due to an action on the contracture producing mechanism itself.

TABLE 2. Effect of adrenaline on contractures produced by various KCl concentrations. ± denotes 1 S.E. of the mean. All reductions significant ($P < 0.001$). By analysis of variance the different ratios at the various KCl concentrations are not significant at P of 0·2

External KCl concn. (mM)	No. of observations	Normal contracture tension (*a*)	Contracture tension with adrenaline (*b*)	Ratio of *b*/*a*
50·0	4	13·6 ± 2·2	8·0 ± 0·9	0·59
70·0	3	25·4 ± 6·1	17·0 ± 4·3	0·67
80·0	4	33·2 ± 6·5	16·9 ± 3·3	0·51
100·0	8	40·1 ± 3·3	25·8 ± 2·6	0·64
150·0	4	64·1 ± 7·6	43·1 ± 2·4	0·67
200·0	7	80·5 ± 12·8	49·8 ± 7·9	0·62

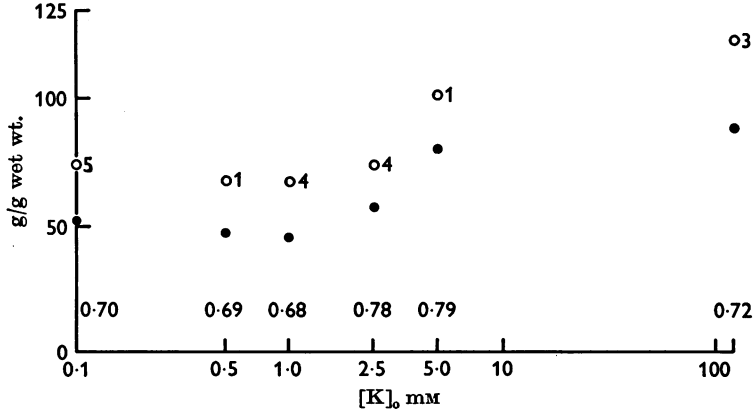

Fig. 5. The effect of adrenaline on Na-free contractures at various $[K]_o$ values. Abscissa; $[K]_o$ in Ringer solution on log scale. Ordinate; developed tension, g/g wet wt. Open circle, control. Filled circles, with adrenaline (10^{-6} g/ml.). Average values with number of experiments beside it. Figure below each set of values is ratio of tension with adrenaline/control tension. Absolute tension developed only slightly sensitive to $[K]_o$. Effect of adrenaline relatively constant. Ringer solution made isosmotic with sucrose. $[Ca]_o$ = 1 mM. Analysis of variance of these results showed no significant differences between them.

The effect of adrenaline on contractures induced by Na-free Ringer

Frog ventricle develops a contracture in Na-free Ringer (Lüttgau & Niedergerke, 1958) owing to a large rise in P_{Ca} (Niedergerke, 1963*a*). Figure 5 shows the average reduction in tension of contractures after adrenaline in Na-free Ringer with $[K]_o$ as shown. It can be seen that the $[K]_o$ makes much less difference to the tension developed in the absence

of Na (first shown by Lüttgau & Niedergerke, 1958). Adrenaline reduces the contracture tension to 0·72 on average in these experiments which is significantly less than the reduction in 1 mM-Ca Ringer + 200 mM-KCl (0·62, $P < 0·001$) but significantly greater than the reduction in 7 mM-Ca Ringer + 200 mM-KCl (0·89, $P < 0·001$). It has been shown that this difference in the effect of adrenaline on contractures induced by Na-free Ringer compared to those induced by Ringer + excess KCl is not due to the increased osmotic pressure of the latter solutions, for when Na-free Ringer is made iso-osmotic with Ringer + 200 mM-KCl by addition of sucrose, adrenaline still has a smaller effect on the contracture lesion.

It will be noted from the results presented so far that increases in $[Ca]_o$ greatly reduce the effect of adrenaline on the contractures whereas removal of $[Na]_o$ only causes a small reduction in the effect. This result was somewhat unexpected in that generally Ca and Na act synergistically on tension development according to the ratio $[Ca]/[Na]^2$ (Wilbrandt & Koller, 1948, Lüttgau & Niedergerke, 1958). In a further experiment to check this point in the same preparation the effect of adrenaline was tested in Ringer solutions containing 1 mM-Ca + 120 mM-Na, 4 mM-Ca + 120 mM-Na and 1 mM-Ca + 60 mM-Na (i.e. relative $[Ca]/[Na]^2$ ratios of 1, 4 and 4 respectively). In these solutions adrenaline 1×10^{-6} g/ml. gave reductions of contracture tension to 0·65, 0·87 and 0·58 respectively. This confirms that for this action of adrenaline the effects of changing $[Ca]_o$ and $[Na]_o$ do not follow the ratio $[Ca]/[Na]^2$. A possible explanation for this unexpected result will be discussed later.

The effect of adrenaline on electrical contractures

Figure 6 shows a typical result. The upper record shows control submaximal contractures produced by 1 and 1·5 sec periods of alternating current. The lower record shows contractures after adrenaline application, produced by the same alternating current strength for 1·5 and 4 sec periods. After washing away the adrenaline the contracture returned to its previous value. The contracture was reduced to 0·42 in the presence of adrenaline; a previous KCl contracture in the same experiment was reduced to 0·84 by adrenaline. Similar results were obtained in several other experiments.

Several other points arising from these experiments should be mentioned here. (1) The maximum electrical contracture was similar in amplitude to the contracture produced by 200 mM-KCl. (2) These contractures were not mediated through the action potential mechanism, for if this was abolished by 20 mM-KCl or by ACh normal electrical contractures were still obtained. (3) The depression of the twitch after the contracture was abolished by atropine (10^{-7} g/ml.) and so was probably due to ACh release. (4) Large d.c. pulses (60 V at 20 msec) applied across the ventricle did not alter the hypodynamic or adrenaline treated twitch very much, therefore hypodynamia is not due to conduction failure and adrenaline action to more cells being activated.

These experiments show that adrenaline produces a negative inotropic action on contractures produced by (1) excess KCl, (2) Na removal or (3) alternating current. It seems probable then that adrenaline is producing this effect by a basic action on the contracture producing mechanism.

Up to this stage in the investigation it appeared that these two actions of adrenaline were significantly if rather poorly related. It became apparent however that under certain conditions these two actions could become dissociated to a varying extent. These results will now be described.

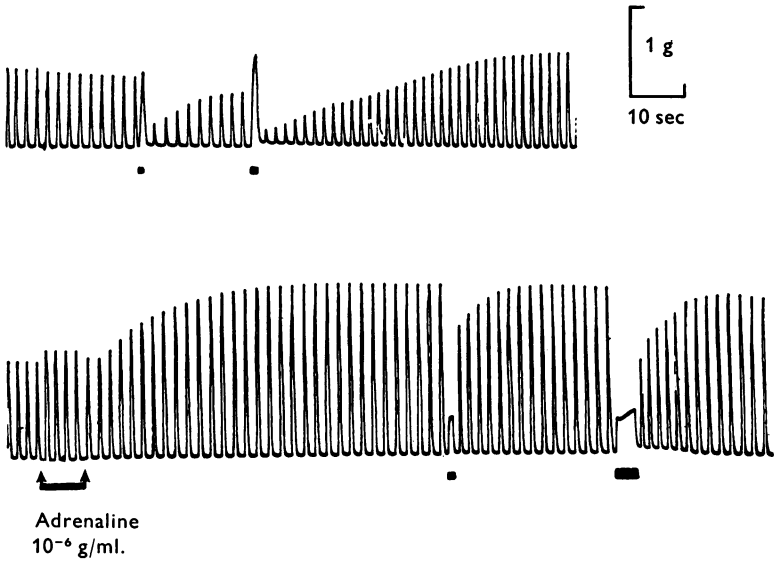

Fig. 6. Effect of adrenaline on electrically induced contractures. Continuous record showing; above, two control electrical contractures of 1 and 1·5 sec duration; below, two contractures of 1·5 and 4 sec duration after addition of adrenaline (10^{-6} g/ml.). Applied current indicated by bars, adrenaline changeover period by bar with arrows. Alternating current of 50 c/s at 3·9 V applied by grid electrodes above and below the ventricle. Preparation beating spontaneously at about 30/min. Note that the current pulses depress the subsequent twitches during the control and adrenaline treated periods, and then cause a late potentiation of the control twitch.

(1) *Factors affecting the time course of the negative and positive inotropic actions of adrenaline*

In the initial experiments in this work carried out in 1 mM-Ca Ringer at a stimulation rate of 30/min, it was found that the time taken for the inotropic action of adrenaline on the twitch to develop was roughly the same as the time for the effect on the contracture to become maximal. Later it was found that in other conditions, e.g. low-Ca Ringer and slow stimulation rates the time courses could be quite dissimilar. Figure 7

shows the result of one complete experiment carried out on 0·5 mM-Ca Ringer to show this point. It shows the rate of development of the inotropic effect on the twitch at rates of stimulation of 15 and 30/min, and of the contracture effect at rates of 3–10, 15, 30 and 75/min. It can be seen that

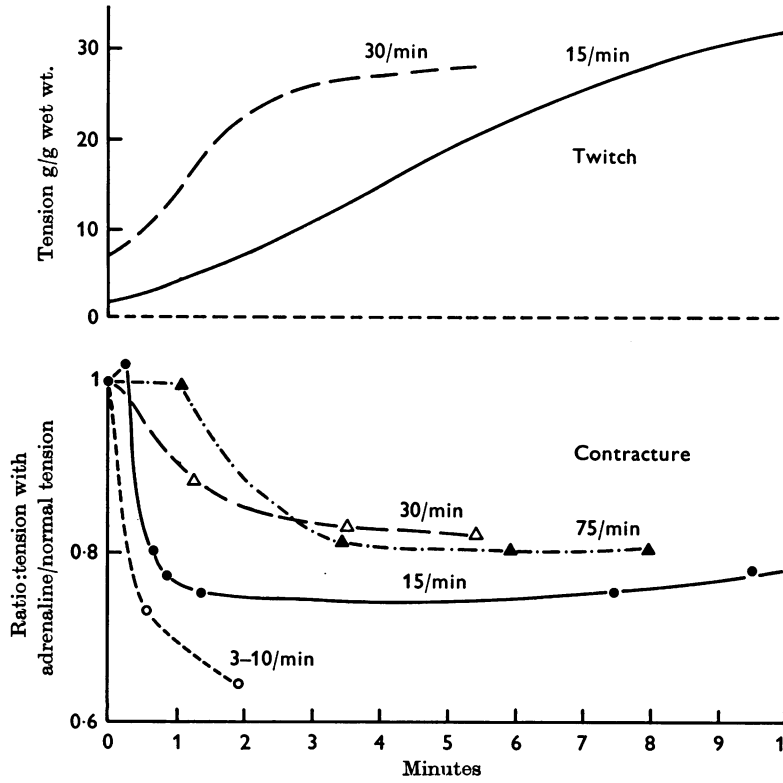

Fig. 7. Effect of adrenaline on twitch and contracture tensions at various stimulation rates. Ordinate; upper, tension g/g wet wt.; lower, ratio tension with adrenaline/normal tension. Abscissa, elapsed time (min) from addition of adrenaline (10^{-6} g/ml.). Contracture curves drawn by eye through the experimental points obtained. Twitch curves drawn through the twitch tensions and then points removed for simplicity. Contractures induced by KCl for NaCl Ringer. $[Ca]_o$ = 0·5 mM, all results on the same ventricle. The inotropic effect was slower in developing than usual.

reducing the rate of stimulation gives a marked slowing of the development of the full positive inotropic effect on the twitch (in this experiment $T_{\frac{1}{2}}$ = 1·4 and 4·5 min for rates 30 and 15/min respectively), but tends to increase the rate of the negative inotropic effect on the contracture tension. Therefore although the time course of the two adrenaline effects is quite similar at a stimulation rate of 30/min, there is marked variation

at other rates of stimulation due largely to an alteration in the time course of the positive inotropic action of adrenaline. Similar but less complete results were obtained in another four experiments.

In several experiments the quantitative interaction of the ratio of $[Ca]/[Na]^2$ and rate of beating on the time for the full positive inotropic action of adrenaline was examined. A typical result is shown in Table 3.

TABLE 3. Effect of heart rate on time course of adrenaline action at two values of the ratio of $[Ca]/[Na]^2$. All results from the same experiment. Number of twitches and time are from the addition of adrenaline (10^{-6} g/ml.) to completion of action

		6/min	15/min	60/min
0·5 mM-Ca	No. of twitches	69	55	55
100% Na	Time (sec)	690	200	55
2 mM-Ca	No. of twitches	5	—	25
100% Na	Time (sec)	50	—	25
0·5 mM-Ca	No. of twitches	6	—	23
50% Na	Time (sec)	60	—	23

At low values of $[Ca]/[Na]^2$ (0·5 mM-Ca; 120 mM-Na) the number of twitches for maximum effect is almost constant for the rates tested, whereas the time required decreases from 690 to 55 sec. When the $[Ca]_o/[Na]_o$ ratio is increased by altering either $[Ca]_o$ or $[Na]_o$, rate of stimulation still has an effect but time is now more important. The observation that the number of impulses to reach the maximum inotropic effect is constant at low $[Ca]/[Na]^2$ is very similar to observations on the time of action of ouabain (Wilbrandt, Brawand & Witt, 1953); although the effect of $[Ca]/[Na]^2$ ratio was not examined in these ouabain experiments.

(2) *Procedures which block the negative inotropic action of adrenaline without affecting the positive inotropic action*

The initial observation was that ouabain could block this negative inotropic action but still leave a positive inotropic action of adrenaline. As ouabain is known to block the Na-K pump, we then tried two other (but quite different) methods of doing this and found that these also blocked the negative inotropic action of adrenaline on the contracture. These results will now be presented.

The effect of ouabain. Cardiac glycosides have an inotropic effect on the hypodynamic twitch (Gold & Cattell, 1940), and they also block the Na pump (Schatzman, 1953). In frog ventricle 10^{-5} M ouabain blocks K uptake (and therefore presumably Na extrusion) within 1 min (Hannan, 1967). Figure 8 shows the effect of 10^{-5} M ouabain on the action of 1×10^{-6} g/ml. adrenaline. Initially adrenaline reduced the contracture in the usual way (Fig. 1 is from the same experiment); on adding 10^{-5} M ouabain to the preparation upper trace of Fig. 8 the twitch became bigger and the contracture was also 10% greater than the control. On continued exposure

of the ventricle to ouabain the twitch declined. On adding adrenaline the twitch increased again (Fig. 8, lower record), but the contracture was not now reduced from the previous tension. In three similar experiments (using 1 mM-Ca Ringer and 30/min stimulation), 10^{-5} M ouabain increased a 200 mM-KCl contracture by 7% from the control and reversed the adrenaline effect on contracture from a decrease of 37% to an increase of 4%. Addition of ouabain for only short periods (10–30 sec) or in lesser concentrations had only a reduced or no effect on the action of adrenaline.

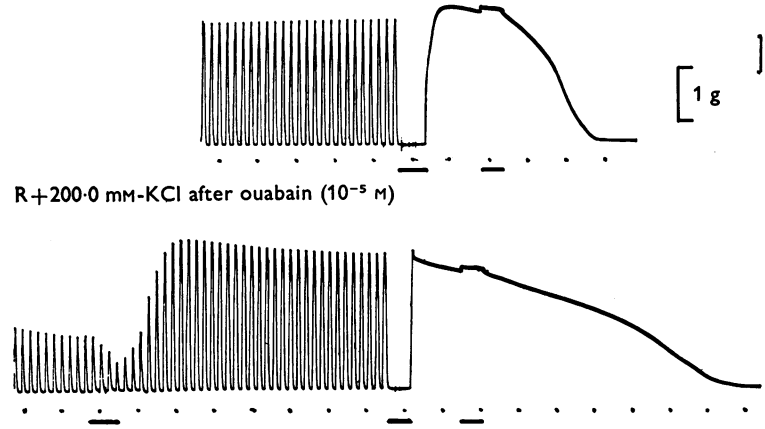

Fig. 8. The interaction of ouabain and adrenaline on the contracture. Ordinate, tension; abscissa, time in 10 sec intervals. Upper record shows maximum inotropic effect of ouabain (10^{-5} M; treatment for 4½ min) with a contracture to Ringer + 200 mM-KCl. 6 min later (lower record) the inotropic effect of ouabain has lessened; adrenaline still causes an inotropic effect. The KCl contracture is now not reduced by adrenaline. Note the slow decline of tension in normal Ringer solution again. Same experiment as Fig. 1, which shows the control contractures.

As shown in Fig. 8 (lower record) ouabain causes a progressive steepening of the onset of contracture tension; this phenomenon occurs in the absence of adrenaline and is not affected by it.

The effect of K-free Ringer. In four experiments it was found that after perfusing the ventricles with K-free Ringer for 10 min the contractures were no longer reduced by adrenaline, although the positive inotropic effect on the twitches remained.

The effect of prolonged treatment with Na-free Ringer. In two experiments ventricles were perfused with Na and Ca-free Ringer for 10 min (Na replaced by sucrose) by which time little Na is left in the tissues (unpublished experiments). Contractures were then induced by adding Na-free Ringer + 1 mM-Ca. In these conditions adrenaline produced a slight increase in contracture tension instead of the usual decrease.

The conclusion from these experiments is that if the Na pump is stopped (the result of all the methods described) then the negative inotropic action of adrenaline is abolished. These experiments are not, however, wholly satisfactory as all the procedures used cause a great increase in resting tension and so the developed tension becomes a smaller fraction of the resting tension than usual.

The effect of adrenaline on the resting potential of frog ventricle

It is well known that adrenaline causes a hyperpolarization of the resting potential of various muscle fibres (skeletal, Brown, Goffart & Vianna Dias, 1950; guinea-pig taenia, Bülbring, 1960; dog auricle, Dudel & Trautwein, 1957). Evidence has been presented suggesting that this is

TABLE 4. The effect of adrenaline on resting potentials (*a*) in Ringer + excess KCl and (*b*) in Na-free Ringer with various $[KCl]_0$ values (made isosmotic with sucrose). Errors are ±1 S.E. of the mean. All differences are significant ($P < 0.001$)

	External KCl concn. (mM)	No. of expts.	E_m Control	E_m after adrenaline	Increase in E_m after adrenaline
(*a*)	2·5	26	79·5 ± 1·7	86·5 ± 1·6	7·3 ± 0·8
	10·0	4	55·8 ± 1·0	63·2 ± 0·5	7·4 ± 0·6
	25·0	4	38·9 ± 0·9	43·9 ± 1·8	5·0 ± 1·2
	40·0	4	30·4 ± 0·5	35·2 ± 1·3	4·8 ± 0·7
	50·0	5	26·8 ± 0·9	29·7 ± 1·0	2·5 ± 0·3
	70·0	4	20·6 ± 0·6	23·8 ± 1·3	3·2 ± 0·8
	100·0	4	15·4 ± 0·5	18·9 ± 0·4	3·5 ± 0·3
	150·0	4	12·6 ± 0·3	17·0 ± 0·6	4·4 ± 0·4
	200·0	4	8·4 ± 0·8	13·5 ± 1·1	5·1 ± 0·7
(*b*)	2·5 KCl Na-free	4	84·6 ± 2·2	94·7 ± 2·2	10·2 ± 1·5
	1·0 KCl Na-free	4	91·1 ± 3·7	102·3 ± 4·0	11·2 ± 0·7
	0·0 KCl Na-free	4	97·6 ± 1·5	113·1 ± 3·2	15·5 ± 2·1

due to an effect on the Na pump (Trautwein & Schmidt, 1960; Haas & Trautwein, 1963). During our experiments numerous results were obtained on the effect of adrenaline and various inhibitors on the membrane potential. These will now be described.

In excess KCl solutions. Table 4*a* shows the results of several experiments to measure the effect of adrenaline on the resting potential of quiescent ventricles at different $[KCl]_0$ values. Between recordings the ventricles were washed with 1 mM-Ca Ringer and stimulated at 15/min. The test solutions were made iso-osmotic with Ringer + 200 mM-KCl by addition of sucrose, as before. In each experiment the membrane potential was measured in normal Ringer solution and in the high KCl Ringer with and without addition of 1×10^{-6} g/ml. adrenaline. In each situation ten impalements were made and the results averaged. It can be seen that adrenaline produced a hyperpolarization of the membrane potential at all KCl concentrations; which was significant ($P < 0.001$) for individual experiments and also for each group mean. Controls showed that adrena-

line had no effect on the base line potential with the micro-electrode outside the cell and therefore was not having an effect on the liquid junction potential. On three occasions 'blind' experiments were carried out with unknown solutions in order to test observer bias. On each occasion the adrenaline solution was identified correctly. The hyperpolarization was complete in under 1 min.

Because the relationship between E_m and tension is so steep it is possible to explain the negative inotropic action of adrenaline on the basis of the above hyperpolarization, at all levels of KCl (Graham & Lamb, 1966). To test this hypothesis we measured the negative inotropic action and potential changes in a situation where changes in E_m do not produce a large change in tension. This condition occurs in Na-free contractures (Lüttgau & Niedergerke, 1958; see also Fig. 5). As already shown (Fig. 5) adrenaline still reduces the contracture tension and in fact also produces an even greater degree of hyperpolarization in the Na-free Ringer (Fig. 4*b*). It appears therefore that the negative inotropic action of adrenaline and the hyperpolarization are both consequences of the addition of adrenaline, but that the tension change is not a consequence of the membrane potential change.

The interaction of adrenaline with pronethalol and ouabain on the membrane potential. Pronethalol blocks the action of adrenaline on the heart (Black & Stevenson, 1962) and therefore ought to stop the hyperpolarizing actions on the membrane potential. Table 5, line 3, shows that this is so in Ringer + 100 mM-KCl. Ouabain blocks Na-K exchanges in cells generally (Schatzman, 1953) and therefore it might be expected to alter the actions of adrenaline on the membrane potential. Table 5, line 1, shows that by itself ouabain causes a significant decrease in the membrane potential of 9 mV. On addition of adrenaline to ventricles treated with ouabain there is a further significant drop in the E_m of 8 mV. These results were unexpected but may be explained by supposing that (1) the activity of the sodium pump makes some direct contribution to the membrane potential either by keeping $[K]_o$ near the membrane low or by an electrogenic action (Adrian & Slayman, 1966) and (2) that ouabain blocks the Na pump effects of adrenaline but not its other effects; once the effects of adrenaline on the Na pump are blocked other actions of adrenaline on the membrane potential become apparent. The effect observed may be due to an increase in sodium permeability (P_{Na}) by adrenaline (e.g. Woodbury, 1962). If this were so then an increase in P_{Na} of 70 % would account for this effect (taking $[K]_i$ as 115 and $[Na]_i$ as 25 m-moles/l. fibre water, Anderson, 1966) as calculated from the Goldman equation.

The rate of action of changes in the concentration of $[Ca]_o$ on the twitch tension

Niedergerke (1957 and 1963*b*) noted that the time course of the change in twitch tension on changing $[Ca]_o$ was consistent with the idea that the rate limiting process was extracellular diffusion. This was expected on the

hypothesis that direct calcium entry activated the contractile mechanism. We shall argue later that the twitch is unlikely to be due to direct calcium entry. Part of the evidence for this view was obtained in experiments based on those of Niedergerke but using the superfused preparation driven at various rates. To do so we have made use of the observation (Niedergerke, 1957, 1963*b*; Lamb & McGuigan, 1966) that twitch tension is proportional to $[Ca]_o$ over the range from 0·5 to 2 mM-Ca. (Note that this relationship is established for steady-state conditions; in the present and previous experiments it is assumed to hold for transient conditions also).

TABLE 5. Interaction of adrenaline with ouabain or pronethalol on resting potential. Errors are ±1 S.E. of the mean. All changes are significant ($P < 0·01$)

Solutions	Control	Adrenaline 10^{-6} g/ml.	Ouabain 10^{-5} M	Adrenaline + ouabain	Pronethalol 10^{-5} g/ml.	Adrenaline + + Pronethalol
2·5 mM-KCl	84·5 ± 5·3	94·0 ± 2·1	75·5 ± 2·1	67·3 ± 3·0	—	—
100 mM-KCl	14·6 ± 0·3	17·8 ± 0·3	15·2 ± 0·6	14·6 ± 0·5	—	—
100 mM-KCl	14·6 ± 0·3	17·3 ± 0·3	—	—	15·2 ± 0·2	15·1 ± 0·3

Figure 9 shows a typical experiment in which the effect of changing from 0·5 to 2 mM-Ca Ringer was examined. The records show this at a heart rate of 3/min (upper record), at 30/min (lower left), and during a contracture. The contracture was elicited after a period of stimulation at 3/min and had a $T_{\frac{1}{2}}$ of 2 sec. A similar contracture after a period of stimulation at 30/min had a $T_{\frac{1}{2}}$ of 0·9 sec. It is clear that the twitch takes a longer time to equilibrate at 3/min than at 30/min; the contracture equilibrates most quickly.

These and other results from this experiment are plotted on Fig. 10 as % increment in tension against time. The symbols represent the experimental points for the twitches, the curves are computed from the model discussed below. The contracture results would lie between the 30/min curve and the ordinate and have been omitted to avoid overcrowding. These results show that (1) the ventricle equilibrates fastest during a contracture, then at 30/min and then at 3/min and (2) that with time of perfusion the rate of equilibration becomes slower (a point also noted by Niedergerke, 1963*b*).

The tentative hypothesis which we suggest for this phenomenon is as follows. We suppose that the twitch is dependent on the calcium concentration held in some activator region within or between the cells and that this region equilibrates with the outside medium mainly during a depolarization. (It is clear, however, that some equilibration occurs under quiescent conditions, for a twitch 2 sec after the change-over from $\frac{1}{2}$ to 2 mM is smaller than one 18 sec after the changeover (Fig. 13); note, if left quiescent for 60 sec in 2 mM the first twitch is still no bigger than after 18 sec.)

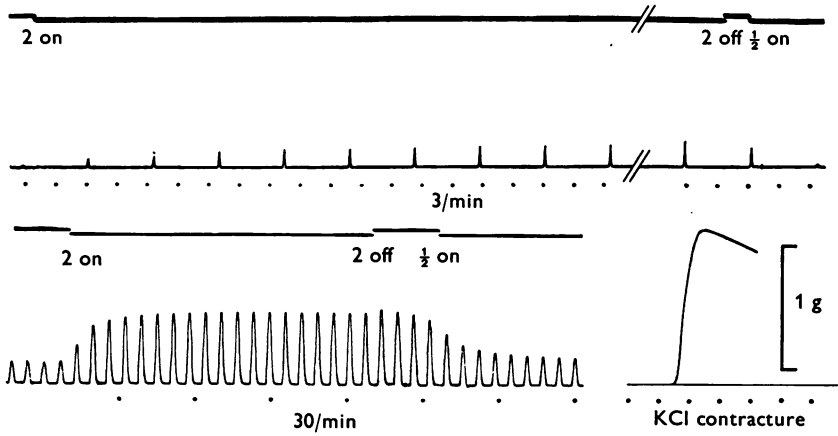

Fig. 9. The effects of changing from 0·5 to 2 mM-Ca at two rates of stimulation. Ordinate, tension; abscissa, time in 10 sec intervals (note expansion of time scale during 30/min stimulation). All records part of the same experiment. Contracture induced by changing from a 2 mM-Ca Ringer + 200 mM-KCl to a 0·5 mM-Ca Ringer + 200 mM-KCl in a ventricle previously stimulated at 3/min, other changeovers indicated on the record. In upper record 4 twitches omitted where indicated. Record of 30/min taken early in the experiment, others at the end.

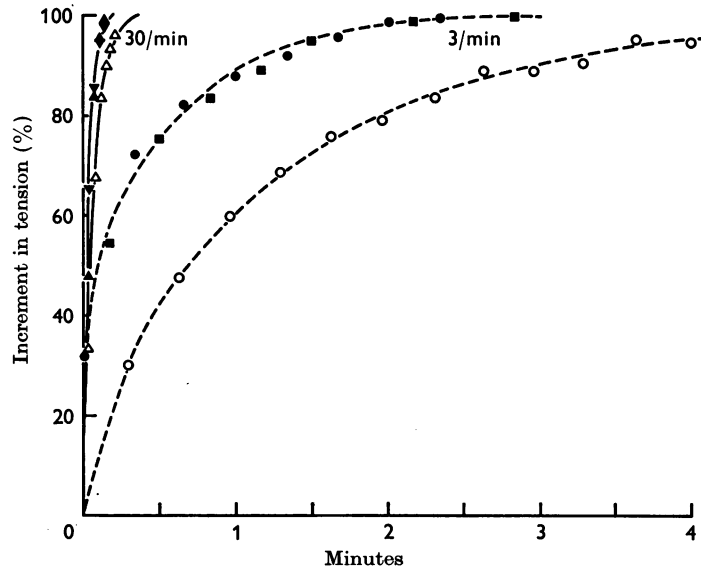

Fig. 10. Rate of equilibration in 2 mM-Ca Ringer at two heart rates and at different times during a single experiment. Ordinate, increment from steady tensions in 0·5 to 2 mM $[Ca]_o$ as a percentage. Abscissa, elapsed time in 2 mM-Ca (min). Same experiment as Fig. 9. Experimental points; filled, early in the experiment; open, late in the experiment; a different symbol is given for each 'run'. Note that at 3/min in the fresh preparation the changeover from 0·5 to 2 mM occurred early (■) or late (●) in the 20 sec interval between beats. The curves were computed according to the model discussed later in the text.

This model was studied quantitatively by building an electrical analogue (see the Appendix) and fitting the experimental points with curves. These curves (Fig. 10) were determined by the following parameters (1) the ratios of the resting P_{Ca} to the P_{Ca} during an action potential or K contracture, taken as 0·25 from the flux experiments (Lamb & Lindsay, 1968); (2) the action potential durations at 30/min and 3/min in 2 mM $[Ca]_o$; taken as 500 and 630 msec respectively, the mean values determined experimentally (Graham, 1966) in similar experiments; using these values the basic equilibration rate of the calcium store was determined (i.e. the $T_{\frac{1}{2}}$ which would be found if a prolonged depolarization was employed). For Fig. 10 these computed $T_{\frac{1}{2}}$s (for the end of the experiment) were 1·8 and 0·8 sec for 3 and 30/min respectively. The measured $T_{\frac{1}{2}}$s of the K contractures (also at the end of the experiment) were 2 and 0·9 sec for 3 and 30/min respectively, which shows quite a good fit with the calculated values. The calculated $T_{\frac{1}{2}}$s for the initial results in this experiment are roughly twice as fast, which then means that during a contracture following stimulation at 30/min equilibration is about at the rate expected for free diffusion in this preparation (see fig. 10, Lamb & McGuigan, 1968). The simplest explanation for the difference between fresh and old experiments is to suppose that the $T_{\frac{1}{2}}$ for the 'store' taking up calcium is the variable but there may be a more complex explanation.

It appears that this simple model does provide a good quantitative fit for the observations. If correct it does bring the heart into line with skeletal muscle, in suggesting that the twitch in both tissues uses a local store of calcium (see Sandow, 1965). The main difference is that the store in the heart, with its much smaller cells, is able to equilibrate with the bathing fluid more rapidly.

This model was computed on the basis that calcium entered the 'store' mainly during depolarization but partly at rest, but did not leave it again either during or between action potentials. The evidence that the 'store' did not decline rapidly between twitches in 2 mM $[Ca]_o$ was that omission of a few twitches did not much decrease the next twitch. On the other hand, the 'store' emptied very rapidly if replaced in 0·5 mM $[Ca]_o$, when the ventricle was quiescent or beating slowly (Fig. 12). No information about changes in the store during an action potential was available in these experiments, once equilibration at 2 mM-Ca was achieved. To take account of this in the analogue experiments the store was given various discharge rates (from the normal time (T) of entry of about 1 sec to zero discharge) and the rates of equilibration computed. It was found that over this range of values the equilibration time was not much affected by the discharge rate, so that it was concluded that this parameter would not affect the computations.

In this experiment (Fig. 9) it is clear that the range of tensions covered at 3 and 30/min and during the contracture are rather dissimilar. Some doubt might, therefore, be felt about comparisons based on such data. It was found, however, that in another of the six experiments carried out in this way, the twitch tension at 3 and 30/min in 0·5 and 2 mM $[Ca]_o$ were approximately equal. Detailed analysis of this experiment gave similar results to that in Fig. 10, so that we concluded that the different ranges of tension were not important. In the

experiment shown in Figs. 9 and 10 the $T_{\frac{1}{2}}$ of the curves fitted to the points in the 'fresh' preparation are 1·5 and 6 sec at 30 and 3/min respectively and are 2 and 40 sec at 30 and 3/min respectively in the 'older' preparation. In the other experiment analysed completely the $T_{\frac{1}{2}}$s were 1·5 and 16 sec at 30 and 3/min respectively. In one unusual preparation isolated for 6 hr the $T_{\frac{1}{2}}$s at 3, 15 and 45/min were very slow at 240, 37 and 12 sec. The theoretical T of diffusion in this preparation is less than 0·5 sec (Lamb & McGuigan, 1968, fig. 10), a value approached during contractures with high $[Ca]_0$ present. Therefore diffusional delays should not greatly affect the results shown in Figs. 9 and 10. Niedergerke (1963*b*) estimated an equilibration time of 5–10 sec in his preparation compared to observed $T_{\frac{1}{2}}$s of 10–20 sec (at stimulation rates of 10/min at 6–8° C). It would seem therefore that the main differences between his and our results is due to a more rapid perfusion system in our experiments, which enables these fairly rapid equilibrations to be distinguished from extracellular diffusion.

It will be noted in Fig. 9 that on returning from 2 to 0·5 mM [Ca] at 30/min the response is quite slow but that at 3/min it is very fast. These results are the opposite of changing from 0·5 to 2 mM at these rates. We presumed that on returning from high to low $[Ca]_0$ the rate of equilibration was largely determined by an active calcium extrusion, whereas a passive process is expected on going from low to high $[Ca]_0$. On this basis some difference between the on and off rates for high calcium might be expected. This problem was not studied further.

Changing from 0·5 to 2 mM $[Ca]_0$ therefore causes an alteration in the twitch tension which depends more on the number of elapsed twitches than on the elapsed time. Rather similar observations have recently been reported by Heppner, Weidmann & Wood (1966) on sheep and calf ventricular muscle following lengthening or shortening of the action potential by voltage clamping at a constant $[Ca]_0$.

DISCUSSION

The effect of adrenaline on contractures. These results show that adrenaline decreases the contracture tension produced by (1) excess KCl, (2) Na removal and (3) alternating current. This negative inotropic effect on the contracture is unexpected but appears to exist simultaneously with the more usual positive inotropic effect on the twitch. We propose to discuss the mechanism of this effect first and then outline a tentative hypothesis of these dual actions of adrenaline.

In parallel experiments with distended perfused ventricles it was found that adrenaline caused a decrease in the calcium influx with no effect on the efflux (Lamb & Lindsay, 1968), both during contractures and when beating. This evidence suggests that adrenaline decreases the calcium permeability and as a consequence the contracture tension is less. In these flux experiments it was also found that exposure to ouabain for 10 min stopped this action of adrenaline and itself led to a rise in P_{Ca}, a result fitting with the present observation that ouabain stops the negative inotropic effect of adrenaline on the contracture. These contracture and P_{Ca} measurements therefore fit with the hypothesis proposed by Nieder-

gerke (1963*a*) that contracture tension is due to the direct entry of calcium into the cardiac cell.

The mechanism of this decrease in P_{Ca} is not clear. We wish to discuss two possible ways it might occur. These are based on (1) the well known antagonism between Na and Ca at the cardiac cell membrane and (2) the recent observation that in squid axon changes in $[Na]_i$ produced changes in P_{Ca} (Baker, Baustein, Hodgkin & Steinhardt, 1967).

(1) The calcium–sodium interaction in frog ventricle (Wilbrandt & Koller, 1948) has been explained as a competition for anionic sites, R, at the cell surface (Lüttgau & Niedergerke, 1958) according to the relationship

$$2Na^+ + CaR \rightleftharpoons Na_2R + Ca^{2+}. \qquad \text{(i)}$$

If the total number of sites R remains constant and either Ca or Na occupies them, then by the law of mass action

$$k = \frac{[Ca][Na_2R]}{[Na]^2[CaR]} \qquad \text{(ii)}$$

where k, the equilibrium constant of the reaction, is an index of the affinity of Na, relative to Ca, for R. There is good evidence that P_{Ca} is proportional to CaR (see Niedergerke, 1963*a*, *b*) and some evidence that P_{Na} is proportional to Na_2R in frog's ventricle (Orkand & Niedergerke, 1964). If these relationships are correct then

$$k = \text{constant}\ \frac{P_{Na}}{P_{Ca}}. \qquad \text{(iii)}$$

Changes in P_{Na} should then produce changes in P_{Ca} according to eqn. (iii).

For some years it has been suggested that some of the actions of adrenaline (e.g. on the prepotential) could be explained by an increase in P_{Na} (Woodbury, 1962). We have obtained some evidence (Table 5) which can be interpreted as showing an increase in P_{Na} of 70 % by adrenaline. The contracture experiments under similar conditions suggest a decrease in P_{Ca} of 40 %. Qualitatively this data would fit with the hypothesis that adrenaline causes an increase in k in eqns. (ii) and (iii), favouring the formation of Na_2R at the membrane. Quantitatively this cannot be calculated as insufficient data are available. By assuming total R as unity and assigning a series of values to CaR (and so determining Na_2R) it can be shown that an increase in P_{Na} of 70 % and a decrease of P_{Ca} of 40 % would occur if k increased by a factor of 3 at an initial CaR/Na_2R ratio of 0·62. Therefore these observations are compatible with the hypothesis that adrenaline causes a change at the cell membrane favouring Na entry at the expense of calcium entry.

(2) It has been shown recently (Baker *et al.* 1967) that changes in $[Na]_i$

produce changes in P_{Ca} in the same direction in squid axon. In frog ventricle ouabain and K-free Ringer both cause an increase in P_{Ca} (Holland & Sekul, 1959; Thomas, 1960) and both cause a rise in $[Na]_i$ (Keenan & Niedergerke, 1967; Lamb & McGuigan, 1968). In the absence of more direct evidence it seems reasonable to suppose that frog ventricular membrane and squid axon behave similarly to changes both in $[Na]_o$ and $[Na]_i$. For some years it has been suggested that adrenaline increases sodium pumping (Bülbring, 1960) and there is some direct experimental evidence for this in frog's ventricle (Haas & Trautwein, 1963). This would be expected to decrease $[Na]_i$ and as a consequence cause a decrease in P_{Ca}. We could find no evidence in the literature that $[Na]_i$ does decrease and have so far been unable to demonstrate it directly, for the amount of sodium in the cells appears to be normally quite low (Keenan & Niedergerke, 1967) and not easy to detect.

The evidence from the blocking experiments shows that ouabain (10^{-5} M), K-free Ringer and Na-free Ringer when applied for about 10 min all stop this action of adrenaline on the contracture. The P_{Ca} change produced by adrenaline is also blocked by ouabain. These procedures all lead to a blocking in the sodium pump, although with different ionic consequences. Thus ouabain and K-free Ringer leave the cells with a raised $[Na]_i$ and normal $[Na]_o$, Na-free Ringer leaves the cells with low internal and external sodium. It may be thought that this evidence favours the Na pump hypothesis just discussed. However, ouabain does not immediately stop the contracture effect of adrenaline, but takes some 10 min to do so, although the K influx is reduced to 20% within the first minute of treatment (Hannan, 1967). Thus if K influx is a measure of sodium pumping, then the sodium pumping is blocked some time before the contracture effect of adrenaline is blocked. The other two ways of stopping the Na pump are slower in their effects and therefore cannot be used as evidence for the time course of the block. It seems probable therefore that these blocking agents act indirectly. A possible way would be that the rise in $[Na]_i$ consequent on stopping the Na pump would increase P_{Ca} to such an extent that any subsequent Na–Ca interaction would be ineffective.

A comparison of the negative and positive inotropic actions of adrenaline

We have shown that the relationship between the two actions of adrenaline varies greatly depending on the particular treatment applied. We propose to discuss this relationship in conjunction with a tentative outline of the way we think adrenaline acts on the twitch and contracture (Fig. 11).

(1) Pronethalol blocks both actions completely. This is taken to mean that both actions arise from adrenaline combining with the same receptor on the cell membrane.

(2) Adrenaline requires a certain minimum time to produce its positive and negative inotropic actions; about 30 sec in a ventricle stimulated at 30/min in 1 mM-Ringer. Also ACh antagonizes the action of adrenaline on the twitch (Vincent & Ellis, 1959), on the biochemical changes in the cell (see Haugaard & Hess, 1966) and also on the contracture. The evidence is taken to mean that both actions depend on some biochemical changes occurring in the cells, probably the formation of 3′,5′-cyclic adenosine monophosphate (AMP) (Williamson, 1966).

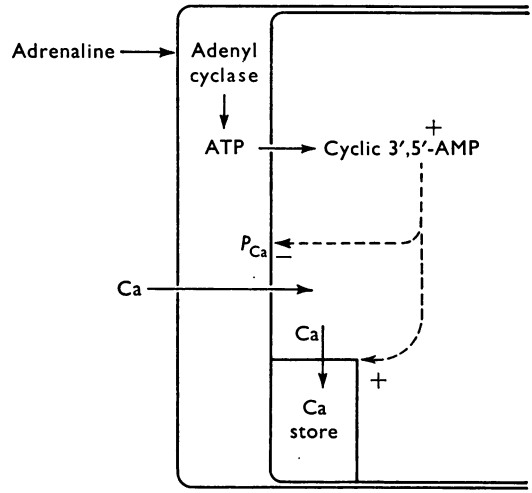

Fig. 11. Tentative mechanism for the action of adrenaline on twitch and contracture tension in frog's heart. Adrenaline applied to the cell causes the formation of cyclic 3′,5′-AMP ('the second transmitter') which then causes (1) a decrease in P_{Ca} and (2) an increase in the Ca storage capacity of the cell. The twitch tension is considered to be proportional to the Ca held in the Ca store, but the contracture proportional to P_{Ca}. The store is in equilibrium with the Ca entering during depolarization (i.e. P_{Ca} and time depolarized). After depolarization the free intracellular Ca is removed actively into a non-specific binding region and partly into the store shown. The store is considered to be associated with the cell membranes rather than with intracellular tubules. (Upper part of Fig. based on that of Sutherland & Robison, 1966).

(3) Increasing the adrenaline concentration causes a rise in twitch tension and a fall in contracture tension, effects which are significantly but poorly correlated. Similarly, increasing external calcium concentration diminishes both effects, though again the effects are not quite 'in step'. These results are taken to mean that although the two actions of adrenaline have a common initial pathway of action (Fig. 11), their final modes of action are different and under certain circumstances they may be separated.

(4) Thus at slow rates of stimulation in low calcium, normal sodium

Ringer, the positive inotropic action is very slow in developing (at 3/min in 0·5 mM-Ca Ringer it takes 15 min), whereas the negative inotropic action is large and fast. Alternatively, in the presence of blocking concentrations of ouabain or K-free Ringer the negative inotropic action of adrenaline is abolished, but the positive inotropic action persists.

As already discussed, it is proposed that the negative inotropic action is due to a decrease in P_{Ca} brought about as a result of the biochemical changes in the cell. This could either be a reorientation of the membrane material to favour Na entry rather than calcium entry, or as an end result of increasing the sodium pumping. Blocking this mechanism would occur near or at the penultimate stage and so would not affect the positive inotropic action. If this is correct then the positive inotropic effect on the twitch is occurring at a time when the P_{Ca} is less than normal. This seems to us to be incompatible with the hypothesis that the twitch is due to direct calcium entry (Winegrad & Shanes, 1962; Niedergerke, 1963*b*). We therefore favour the view that the twitch is the result of Ca released from some site between or within the cells and not due to direct calcium entry. The evidence presented earlier shows that this site equilibrates mainly during a depolarization and has a rate constant of filling similar to that of a contracture under the same conditions. In the presence of adrenaline we suppose that the capacity of this store is greatly increased but that the rate of filling is decreased due to the observed decrease in P_{Ca}. Qualitatively this would explain the observations that the rate of onset of the positive inotropic action of adrenaline depends on the amount of calcium entry and on the rate of stimulation, whereas the other actions of adrenaline are not affected by these factors. At a high rate of stimulation with a large Ca entry a rapid action would result; at a low stimulation rate with a low Ca entry a slow equilibration would result. Quantitatively an extension of our proposed model fits the observed results. Thus with an action potential duration of 1 sec (Graham, 1966) at a heart rate of 3/min in 0·5 mM-Ca Ringer and a store equilibration $T_{\frac{1}{2}}$ of 3 sec (taken from an adrenaline contracture) the twitch would take 15 min to reach its maximum value. With a rate of 30/min, at 2 mM-Ca it would take only a few beats, partly because the twitch mechanism saturates at high $[Ca]_o$. This kind of mechanism would again be relatively independent of the negative inotropic action of adrenaline (apart from the change in P_{Ca}) so that changes in rate of stimulation would affect the positive but not the negative inotropic action.

This model therefore provides a reasonable explanation of the results. It can only be considered to be very tentative at this stage. It would, for example, be necessary to demonstrate such a 'store' of calcium and show that it does increase during the action of adrenaline. This model also raises

some difficulties. Thus it is suggesting that the twitch and the contractures are different, in that the twitch depends on Ca in a 'store' whereas the contracture is adequately explained by Ca entry, although both are triggered by a depolarization. Various possibilities could be proposed to account for this, e.g. access to the store might be time dependent or voltage dependent so that only the fast reversal of the action potential would have access to it whereas the slower, smaller depolarization would not. This possibility is mentioned for there is some evidence (Kavaler, 1959; Morad, M., personal communication) that there is a biphasic tension response on rapidly depolarizing cardiac cells with applied current. We considered the possibility that the biphasic response (Fig. 1) obtained during a contracture with adrenaline might be of a similar nature, but rejected it because if the action potential mechanism is inoperative then the initial 'twitch' disappears. The experiments of Heppner *et al.* (1966) already discussed fit this hypothesis very well. In their experiments prolongation of the action potential by applied current would have the effect of increasing the Ca stored. The store would then require several action potentials to equilibrate back to its initial level.

On the direct calcium entry hypothesis, the relationship between the twitch and the contracture ought to be constant under various conditions, provided that the depolarizations during the action potential and the contracture are constant. On the store hypothesis the twitch and contracture need not maintain a fixed relationship. This latter hypothesis, therefore, fits better with the finding that in hypodynamia the twitch declines progressively (Clark, 1913) although the action potential duration and contracture remain relatively constant (Lamb & McGuigan, 1966). (On our hypothesis this would be due to a decrease in the Ca in the store.) It also allows the twitch in adrenaline to exceed the contracture tension, as found with 10^{-6} g/ml. adrenaline in 0·5 mM $[Ca]_o$ Ringer. It would also allow the time to peak tension to decline, although the action potential duration would be increasing, as occurs with adrenaline in high calcium concentrations (Graham, 1966; Graham & Lamb, 1967). The store hypothesis might explain this latter effect if the discharge rate from the store was dependent on the concentration held within it.

Although there is little evidence for Ca storage tubules in frog's ventricle (which seems to have a simple structure, Keenan & Niedergerke, 1967), it is clear that mammalian heart muscle contains structures which accumulate Ca (see Lee, Ladinsky, Choi & Kasuya, 1966; Gertz, Hess, Lain & Briggs, 1967; Carsten, 1967) for these can readily be obtained as microsomes *in vitro*. Therefore these hearts contain a similar, although probably less active (Palmer & Posey, 1967), mechanism for Ca storage to that existing in skeletal muscle (see review by Sandow, 1965). There is also

some evidence that in hypodynamia the Ca uptake is depressed and may be restored by ouabain (Gertz *et al.* 1967). It has also been noted that if the microsomal Ca uptake from skeletal muscle is depressed by caffeine, 3′5′-AMP will restore it (Carsten & Mommaerts, 1964). We think it possible that our store is similar to these sarcotubular elements, i.e. its capacity is decreased in hypodynamic states and restored by adrenaline, or 3′,5′-cyclic AMP, but clearly more direct evidence is required before this can be considered as established.

These experiments show that adrenaline decreases contracture tension (and P_{Ca}) but increases twitch tension. This effect is thought to be due either (1) to Ca–Na competition or (2) to an effect of adrenaline on the internal sodium concentration. This effect of adrenaline, and the results of studying the time course of $[Ca]_o$ changes on the twitch, make it unlikely that the twitch is due to direct calcium entry. An alternative hypothesis involving a calcium store is postulated. The inotropic effect of adrenaline can be explained by an action on this store. Quantitatively this model fits the observed data satisfactorily.

We are indebted to Mrs I. Thomson and Mrs A. Hume for skilled technical aid. We wish to thank Professor J. S. Gillespie for supplies of adrenaline and other drugs and for helpful discussions. Professor R. Miledi suggested the use of alternating current as a contracture producing agent.

APPENDIX

As already shown, the contracture tension equilibrated at different rates, depending on the previous stimulation rate. The analogue for this was a single compartment consisting of a condenser and preset resistor (see e.g. Sheppard, 1962, p. 93) which could be set to the appropriate time constant (0·5–4 sec). The quiescent calcium influx into the store was represented by a small continuous current flow, the extra calcium influx during depolarization by a pulse of current from a Digitimer of length appropriate to an action potential. These were set up as voltages of approximately -3 and -12 V respectively into an adding circuit. The next stage incorporated a voltage dividing network to alter the input by a factor of 4 (to represent a change from 2 to 0·5 mM-Ca). The voltage was then divided by a factor of 10 (to suit the diode) and then passed through a 'perfect diode' to the CR network. The voltage on the condenser was monitored via a high input resistance operational amplifier on to a pen recorder. With this system the charge on the condenser leaked away very slowly ($T_{\frac{1}{2}} > 1$ min), when a 100 μF condenser was used. When required, the condenser could be discharged during an 'action potential' by using another resistor and high speed relay, also driven from the Digitimer. The 'perfect diode' gave the most trouble. Normal diodes (e.g. OA 200) are non-linear at low voltages and were not suitable. Dr J. R. Greer devised a suitable circuit using a

SCN5 operational amplifier and a normal diode. The output of this circuit equals the input over the range of zero to 6–8 V depending on the conditions. The computations were done in real time, enabling a pen recorder to record the result. The operational amplifiers were part of a Heathkit Analogue Computer. In practice, the easiest way to curve fit was to bracket the experimental points with computed curves.

REFERENCES

ADRIAN, R. H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.* **133**, 631–658.

ADRIAN, R. H. & SLAYMAN, C. L. (1966). Membrane potential and conductance during transport of sodium, potassium and rubidium in frog muscle. *J. Physiol.* **184**, 970–1014.

AHLQUIST, R. P. (1948). A study of adrenotropic receptors. *Am. J. Physiol.* **153**, 586–600.

ANDERSON, ESTHER (1966). KCl distribution in frog ventricular muscle. Ph.D. Thesis, Glasgow University.

BAKER, P. F., BAUSTEIN, M. P. HODGKIN, A. L. & STEINHARDT, R. A. (1967). The effect of sodium concentration on calcium movements in giant axons of *Loligo forbesi*. *J. Physiol.* **192**, 43–44*P*.

BARCROFT, J. & DIXON, W. E. (1907). The gaseous metabolism of the mammalian heart. *J. Physiol.* **35**, 182–204.

BLACK, J. W. & STEVENSON, J. S. (1962). Pharmacology of a new adrenergic beta-receptor-blocking compound (nethalide). *Lancet*, **ii**, 311-314.

BROWN, G. L., GOFFART, M. & VIANNA DIAS, M. (1950). The effects of adrenaline and of sympathetic stimulation on the demarcation potential of mammalian skeletal muscle. *J. Physiol.* **111**, 184–194.

BÜLBRING, E. (1960). Biophysical changes produced by adrenaline and noradrenaline. In *Ciba Symposium on Adrenergic Mechanisms*, ed. VANE, J. R., pp. 275–287, London: Churchill.

BURRIDGE, W. (1911). Observations on the role of potassium salts in frog's muscle. *J. Physiol.* **42**, 359–382.

CARSTEN, M. E. (1967). Cardiac sarcotubular vesicles. *Circulation Res.* **20**, 599-605.

CARSTEN, MARY E. & MOMMAERTS, W. F. H. M. (1964). The accumulation of calcium ions by sarcotubular vesicles. *J. gen. Physiol.* **48**, 183–198.

CLARK, A. J. (1913). The action of ions and lipoids upon the frog's heart. *J. Physiol.* **47**, 66–107.

DUDEL, J. & TRAUTWEIN, W. (1957). Die Wirkung von Adrenalin auf das Ruhepotential von Myokardfasern des Vorhofs. *Experientia* **12**, 396–398.

DYDYŃSKA, M. & WILKIE, D. R. (1963). The osmotic properties of striated muscle fibres in hypertonic solutions. *J. Physiol.* **169**, 312–329.

ELLIOT, T. R. (1905). The action of adrenaline. *J. Physiol.* **32**, 401–467.

EVANS, C. L. & OGAWA, S. (1914). The effect of adrenaline on the gaseous metabolism of the isolated mammalian heart. *J. Physiol.* **47**, 446-459.

GASKELL, W. H. (1884). On the augmentor (accelerator) nerves of the heart of cold-blooded animals. *J. Physiol.* **5**, 46–48.

GERTZ, E. W., HESS, M. L., LAIN, R. F. & BRIGGS, F. N. (1967). Activity of the vesicular calcium pump in the spontaneously failing heart-lung preparation. *Circulation Res.* **30**, 477-484.

GOLD, H. & CATTELL, M. (1940). Mechanism of digitalis action in abolishing heart failure. *Archs intern. Med.* **65**, 263–278.

GRAHAM, J. A. (1966). The effect of adrenaline on mechanical activity and electrical events in frog ventricular muscle. M.Sc. Thesis, University of Glasgow.

GRAHAM, J. A. & LAMB, J. F. (1966). Effect of adrenaline on potassium contractures and membrane depolarization in frog ventricle. *J. Physiol.* **183**, 25–26*P*.

GRAHAM, J. A. & LAMB, J. F. (1967). The effect of adrenaline on action potential and twitch in frog ventricular muscle. *J. Physiol.* **188**, 25–26*P*.

HAAS, H. C. & TRAUTWEIN, W. (1963). Increase of sodium efflux induced by epinephrine in the heart of the frog. *Nature, Lond.* **197**, 80–81.

HANNAN, A. (1967). The effect of various inhibitors on the potassium and chloride fluxes in frog's ventricle. Ph.D. Thesis, Glasgow University.

HAUGAARD, N. & HESS, M. E. (1965). Actions of autonomic drugs on phosphorylase activity and function. *Pharmac. Rev.* **17**, 27-69.

HAUGAARD, N. & HESS, M. E. (1966). The influence of catecholamines on heart function and phosphorylase activity. *Pharmac. Rev.* **18**, 197-203.

HEPPNER, R. L., WEIDMAN, S. & WOOD, E. H. (1966). Posture and negative inotropic effects by constant electric currents or current pulses applied during the cardiac action potential. *Helv. physiol. pharmac. Acta*, **24**, C 94-96.

HESS, M. E., SHANFELD, J. & HAUGAARD, N. (1962). The role of the autonomic nervous system in the regulation of heart phosphorylase in the open chest rat. *J. Pharmac. exp. Ther.* **135**, 191–196.

HOLLAND, W. C. & SEKUL, A. A. (1959). Effect of ouabain on ^{45}Ca and ^{36}Cl exchange in isolated rabbit atria. *Am. J. Physiol.* **197**, 757–760.

KAVALER, F. (1959). Membrane depolarization as a cause of tension development in mammalian ventricular muscle. *Am. J. Physiol.* **197**, 968–970.

KAVALER, F. & MORAD, M. (1966). Paradoxical effects of epinephine on excitation-contraction coupling in cardiac muscle. *Circulation Res.* **18**, 492–501.

KEENAN, M. J. & NIEDERGERKE, R. (1967). Intracellular sodium concentration and resting sodium fluxes of the frog heart ventricle. *J. Physiol.* **188**, 235-260.

LAMB, J. F. & LINDSAY, R. (1968). The effect of adrenaline on the calcium fluxes in frog's ventricle. *J. Physiol.* **196**, 49–50*P*.

LAMB, J. F. & MCGUIGAN, J. A. S. (1966). Contractures in a superfused frog's ventricle. *J. Physiol.* **186**, 261–283.

LAMB, J. F. & MCGUIGAN, J. A. S. (1968). The efflux of potassium, sodium, chloride, calcium and sulphate ions and of sorbitol and glycerol during the cardiac cycle in frog's ventricle. *J. Physiol.* **195**, 283–315.

LEE, K. S., LADINSKY, H., CHOI, S. J. & KASUYA, Y. (1966). Studies on the in vitro interaction of electrical stimulation and Ca^{++} movement in sarcoplasmic reticulum. *J. gen. Physiol.* **49**, 689–715.

LÜTTGAU, H. C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.* **143**, 486–505.

MURAD, F., CHI, Y-M., RALL, T. W. & SUTHERLAND, E. W. (1962). Adenyl Cyclase III. The effect of catecholamines and choline esters on the formation of adenosine 3′,5′-phosphate by preparations from cardiac muscle and liver. *J. biol. Chem.* **235**, 1233–1238.

NIEDERGERKE, R. (1956*a*). The staircase phenomenon and the action of calcium on the heart. *J. Physiol.* **134**, 569–583.

NIEDERGERKE, R. (1956*b*). The potassium chloride contracture of the heart and its modification by calcium. *J. Physiol.* **134**, 584–599.

NIEDERGERKE, R. (1957). The rate of action of calcium ions on the contraction of the heart. *J. Physiol.* **138**, 506–515.

NIEDERGERKE, R. (1963*a*). Movements of Ca in frog heart ventricles at rest and during contractures. *J. Physiol.* **167**, 515–550.

NIEDERGERKE, R. (1963*b*). Movements of Ca in beating ventricles of the frog heart. *J. Physiol.* **167**, 551–580.

OLIVER, G. & SCHÄFER, E. A. (1895). The physiological effects of extracts of the suprarenal capsules. *J. Physiol.* **18**, 230–276.

ORKAND, R. K. & NIEDERGERKE, R. (1964). Heart action potential: dependence on external calcium and sodium ions. *Science, N.Y.* **146**, 1176–1177.

OTSUKA, M. & NONOMURA, Y. (1963). The influence of ouabain on the relation between membrane potential and tension in frog heart muscle. *J. Pharmac. exp. Ther.* **141**, 1-5.

ØYE, I. (1965). The action of adrenaline in cardiac muscle: dissociation between phosphorylase activation and inotropic response. *Acta physiol. scand.* **65**, 251–258.

PALMER, R. F. & POSEY, V. A. (1967). Ion effects on calcium accumulation by cardiac sarcoplasmic reticulum. *J. gen. Physiol.* **50**, 2085–2096.

SANDOW, A. (1965). Excitation-contraction coupling in skeletal muscle. *Pharmac. Rev.* **17**, 265–320.

SCHATZMAN, H. J. (1953). Herzglycoside als Hemstoffe für den aktiven Kalium und Natrium Transport durch die Erythrocytenmembran. *Helv. physiol. pharmac. acta* **11**, 346–354.

SHEPPARD, C. W. (1962). *Basic Principles of the Tracer Method.* London: John Wiley and Sons.

SONNENBLICK, E. H. (1962). Force velocity relations in mammalian heart muscle. *Am. J. Physiol.* **202**, 931–939.

SUTHERLAND, E. W. & ROBISON, G. A. (1966). The role of cyclic-3′,5′-AMP in responses to catecholamines and other hormones. *Pharmac. Rev.* **18**, 145–161.

THOMAS, L. J. (1960). Increase of labeled calcium uptake in heart muscle during potassium lack contracture. *J. gen. Physiol.* **43**, 1193–1206.

TRAUTWEIN, W. & SCHMIDT, R. F. (1960). Zur Membranwirkung des Adrenalins an der Herzmuskelfaser. *Pflügers Arch. ges. Physiol.* **271**, 715–726.

VINCENT, N. H. & ELLIS, S. (1959). Effects of epinephrine combined with acetylcholine or with epinephrine antagonists on cardiac rate amplitude and glycogen. *Pharmacologist, Lond.* **1**, 62.

WILBRANDT, W., BRAWAND, K. & WITT, P. N. (1953). Die quantitative Abhangigkeit der Strophanthosidwirkung auf des Froschherz von der Tatigkeit des Herzens und von der Glykosidkonzentration. *Naunyn-Schmiederbergs Arch. exp. Path. Pharmak.* **219**, 397–407.

WILBRANDT, W. & KOLLER, H. (1948). Die Calcium-Wirkung am Froschherzen als Funktion des Ionengleichgewichts zwischen Zellmembran und Umgebung. *Helv. physiol. pharmac. acta* **6**, 208–221.

WILLIAMSON, J. R. (1966). Kinetic studies of epinephrine effects in the perfused rat heart. *Pharmac. Rev.* **18**, 205–210.

WINEGRAD, S. & SHANES, A. M. (1962). Calcium flux and contractility in guinea pig atria. *J. gen. Physiol.* **45**, 371–394.

WOODBURY, J. W. (1962). Cellular electrophysiology of the heart. In *Handbook of Physiology*, Section 2: *Circulation*, vol. 1, ed. HAMILTON, W. F. Washington: American Physiological Soc.