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Effects of caffeine and isoprenaline on mammalian ventricular muscle

ANNE CLARK* AND CAMILLE B. OLSON

Department of Pharmacology, Harvard Medical School, Boston, Massachusetts, USA

Summary

1. Caffeine, 0·6–20·0 mM, altered the duration of the action potential recorded from kitten papillary muscles; low concentrations shortened and high concentrations prolonged the action potential.

2. Caffeine, 20 mM, prolonged the action potential by delaying the final phase of repolarization.

3. Caffeine, 2·0–20·0 mM increased the tension developed and the duration of the isometric contraction.

4. When large stimulating electrodes were used, all concentrations of caffeine increased the duration of the action potential; this effect was probably due to the interactions of caffeine and released endogenous catecholamines.

5. Concentrations of caffeine and isoprenaline, which separately caused little change in the duration of the action potential, greatly prolonged the action potential when used together.

6. The effects of caffeine may be due to an increase in membrane calcium current in addition to an action on intracellular calcium stores.

Introduction

Caffeine increases the force of contraction of both skeletal muscle (Sandow, 1965; Luttgau & Oetliker, 1968) and cardiac muscle (Rall & West, 1963; de Gubareff & Sleator, 1965; Blinks, Olson, Jewell & Braveny, 1972). In skeletal muscle, low concentrations of caffeine have no effect on the resting membrane potential (Luttgau & Oetliker, 1968) and little effect on the action potential (Taylor, Preiser & Sandow, 1969) and the potentiation of contractile force produced by the drug is attributed to an action on sarcoplasmic calcium stores (Weber & Herz, 1968). In atrial muscle, caffeine changes the shape of the action potential (de Gubareff & Sleator, 1965) but it is not clear that this is the primary cause of the increase in amplitude and duration of the muscle contraction; caffeine may act on sarcoplasmic calcium stores, as well as on the cell membrane in cardiac muscle.

In order to relate quantitatively the electrical and mechanical effects of caffeine, we have made simultaneous recordings of transmembrane potentials and tension developed during isometric contractions of kitten papillary muscles.

Methods

Experiments were done on papillary muscles taken from the right ventricle of kittens (body weight 0·3–1·5 kg). The muscles were 4–8 mm long and had a maximum diameter of 0·2–1·5 mm.

* Present address: The University Laboratory of Physiology, Oxford.

The muscles were supported horizontally in a small plastic bath through which there was a continuous flow (20 ml/min) of a physiological salt solution, maintained at 32·5° C and composed of Na^+ 140 mM, K^+ 5 mM, Ca^{++} 4·5 mM, Mg^{++} 2·0 mM, Cl^- 102 mM, HCO_3^- 24 mM, SO_4^{--} 1·0 mM, HPO_4^{--} 2·0 mM, acetate 20 mM, dextrose 10 mM. The solution was equilibrated with 95% oxygen, 5% carbon dioxide.

Initially, the muscles were positioned across two platinum wire electrodes (0·5 mm diameter) which were partially embedded in a potting resin (Sylgard 182). The ends of the muscles were pinned to the resin block with fine stainless steel pins. One to four muscles could be studied at the same time. Later, an arrangement which permitted simultaneous observation of the electrical and mechanical activity of the muscle was used. The papillary muscle was held, at the ventricular wall end, in a Perspex clamp, so that the muscle adjacent to the clamp was in close contact with a small punctate platinum cathode which, with a distant anode, was used to deliver electrical stimuli to the muscle. A short thread tied to the tendon connected the muscle to a variable inductance force transducer suitable for isometric recording. The resting tension of muscles set up in this way was standardized by stretching the muscle until a value was reached at which the subsequently developed tension was maximal. This length was maintained throughout the experiment. Muscles were stimulated with square wave pulses of 0·5 ms duration, just suprathreshold intensity and at pulse intervals of 3·0–30·0 s, but in any one experiment only one frequency was used. Conventional apparatus was used to record transmembrane potentials with glass microelectrodes suspended on chloride-coated silver wire (0·025 mm diameter). This very fine wire permitted movement of the electrode when the muscle contracted. Microelectrodes with tip potentials measuring less than 20 mV and of 5–50 MΩ resistance were used. The electrical and mechanical activity of the muscle were displayed on an oscilloscope and a permanent record was obtained on film.

The drugs used were caffeine (base) (this alkaloid is poorly soluble in water and it was necessary to warm the stock solution, 0·5 M, to dissolve the drug), (±)-propranolol and (−)-isoprenaline bitartrate. When isoprenaline was used, the disodium salt of ethylenediamine tetracetic acid (EDTA) was added to the solution to give a final concentration of 0·04 mM to prevent oxidation of the catecholamine.

In preliminary experiments, it was found that the duration of the action potential recorded from papillary muscles progressively increased during the first three hours after setting up a preparation ; at a pulse interval of 3·0 s there were increases of 30–90 ms in the time to 90% repolarization during the first three hours. However, the duration of the action potential remained relatively constant during the following 6–8 hours. Therefore a three-hour equilibration period was allowed before recording commenced.

To reduce the influence of any further changes in the action potential with time on the drug-induced changes, observations on the action potential in the presence of a drug were preceded and succeeded by periods of control observations. The effects of caffeine and isoprenaline were easily reversible, but at least 10 min was allowed for washout of the drugs from the tissue.

Recordings were made from arbitrarily selected sites on the muscle and records were obtained from as many cells as possible during the period of observation.

Results

Caffeine, in concentrations greater than 0·6 mM, produced the following effects:

(i) an increase in maximum tension developed by papillary muscles and a prolongation of the contraction;

(ii) an increase in the amplitude of the action potential, and

(iii) either an increase or decrease in the duration of the action potential dependent upon the concentration of the drug and the stimulus interval used. In the presence of caffeine and isoprenaline there was a marked increase in the duration and amplitude of the action potential.

Effects of caffeine on the action potential and muscle contraction recorded from muscles stimulated at 3·0, 10·0 or 30·0 s intervals

Caffeine, in concentrations of 0·6 and 2·0 mM, produced no significant change in the duration of the action potential recorded from muscles stimulated at 3·0 or 10·0 s intervals. When a pulse interval of 30·0 s was used there was a reduction of the duration measured at the time from onset of depolarization to both 50% and 90% repolarization. In the presence of higher concentrations of caffeine there was an increase in the duration of the action potential recorded from muscles stimulated at 3·0 and 10·0 s intervals, particularly during the final phase of repolarization. This prolongation was greatest in the presence of 20 mM caffeine (Figs. 1 and 2). In muscles stimulated at 30·0 s intervals there was a significant reduction in the duration of the action potential at the 50% repolarization level with both 6·0 mM and 20·0 mM caffeine. The final phase of repolarization, however, became very prolonged in the presence of caffeine 20 mM.

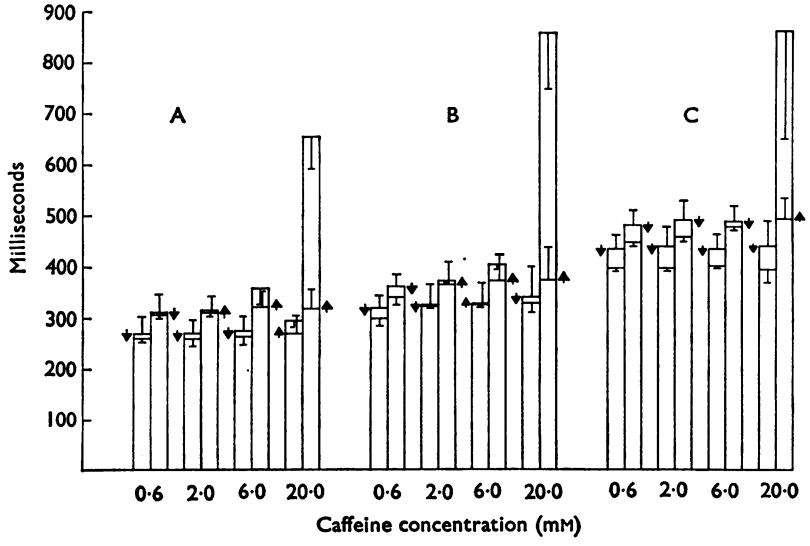

FIG. 1. Effect of caffeine on the duration of the action potential of kitten isolated papillary muscle. Stimulus interval; A. 3·0 s; B. 10·0 s; C. 30·0 s. Each pair of columns represents the duration measured at the time to 50% repolarization (left) and 90% repolarization (right). The heights of the columns show the pre-drug control level, mean with standard deviation (upwards), and the mean value in the presence of caffeine with the standard error of the change produced by the drug (downwards). The arrows show the direction of the change produced by caffeine. $n=3$ experiments in each group.

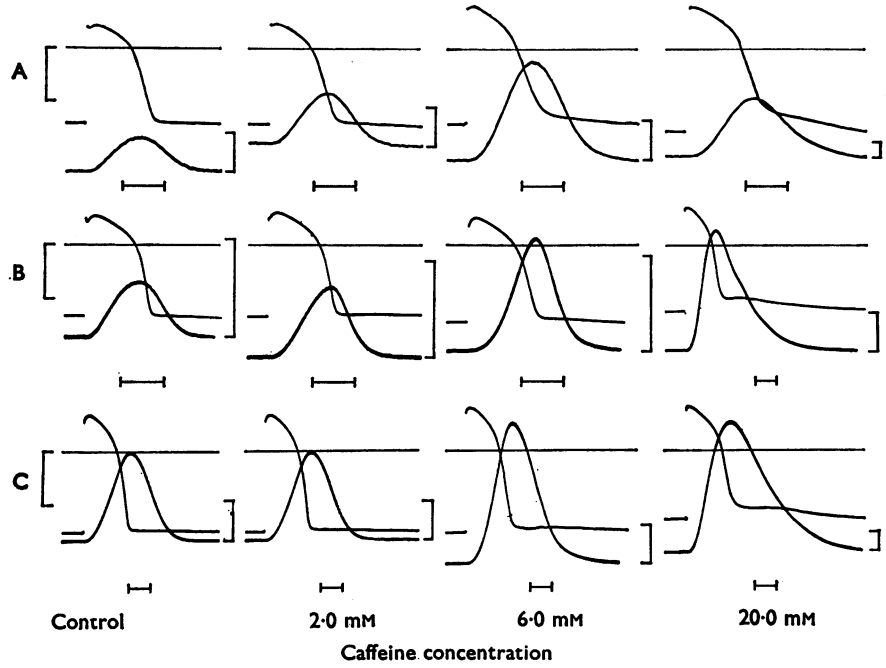

FIG. 2. Effect of caffeine on transmembrane potentials (upper trace) and isometric contractions (lower trace) of kitten isolated papillary muscle. Stimulus interval: A. 3·0 s; B. 10·0 s; C. 30·0 s. In each case the right-hand vertical scale represents 500 mg and the left-hand vertical scale represents 50 mV. Time marker 200 ms.

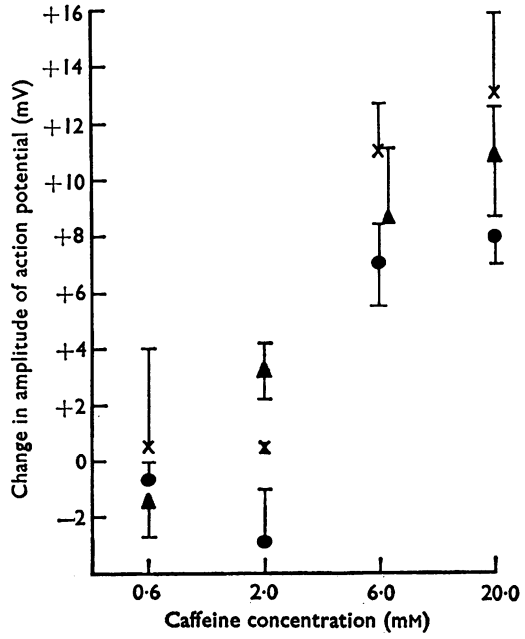

FIG. 3. Effect of caffeine on amplitude of action potential of kitten papillary muscles. Ordinates: mean (±S.E.) change produced by the drug. Abscissae: concentration of caffeine. Stimulus interval, ▲=3·0 s; ×=10·0 s; ●=30·0 s. $n=3$ in each case.

The resting membrane potential was unaffected by caffeine but there was an increase in the total amplitude of the action potential. This effect was dependent upon the concentration of the drug; in muscles stimulated at 3·0 s intervals there was a mean increase of 3·2 mV in the presence of 2·0 mM caffeine, and an increase of 10·7 mV in 20 mM caffeine (Fig. 3).

Caffeine 2·0–20·0 mM increased the peak tension developed and prolonged the duration of contraction recorded from muscles stimulated at all frequencies. The large increase in duration of the contraction in the presence of 6·0 and 20·0 mM caffeine was mainly due to delayed relaxation (Fig. 4).

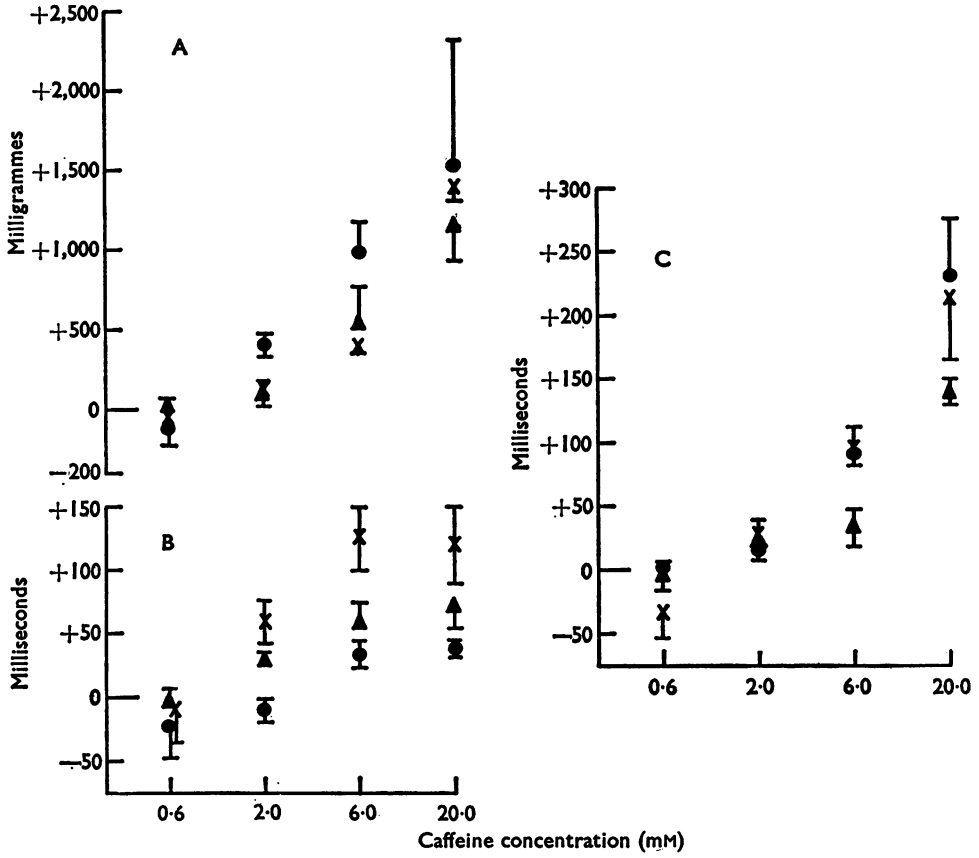

FIG. 4. Effect of caffeine on isometric contractions of kitten papillary muscle. A. Change in maximum tension. B. Change in time from onset of contraction to peak tension. C. Change in time from onset of contraction to half relaxation. Mean values ± S.E. Concentrations of caffeine shown on abscissae. Pulse intervals; ▲=3·0 s, ×=10·0 s, ●=30·0 s. $n=3$ in each case.

Effects of caffeine on action potentials recorded from papillary muscles pinned across platinum wire stimulating electrodes

The results from this series of experiments showed that caffeine in concentrations of 0·6–20·0 mM increased the duration of the action potential recorded from muscles stimulated at intervals of 3·0, 10·0 and 30·0 seconds. There was a mean increase in the time from onset of depolarization to both 50% and 90% repolarization with

all concentrations of the drug and at all frequencies of stimulation, e.g. for the action potentials shown in Fig. 5 there was an increase in duration of the action potential measured at 50% repolarization level of 69 ms and at 90% repolarization of 111 milliseconds. This contrasts with the results described in the previous section, where there was no effect on 50% repolarization time and a small increase in 90% repolarization time under the same conditions.

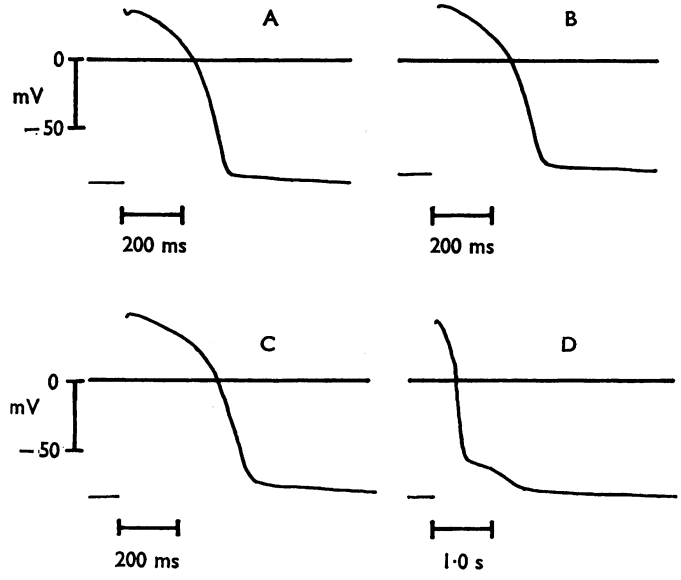

FIG. 5. Effect of caffeine on action potential when large electrodes were used for stimulating kitten papillary muscle. A. Pre-drug, control recording. B. 2·0 mM caffeine. C. 6·0 mM caffeine. D. 20·0 mM caffeine. Stimulus interval 10·0 s.

The magnitude of the increase in duration of the action potential increased with increasing concentration of caffeine (Fig. 6) but appeared to be dependent upon the frequency of stimulation: the effect of the drug was greater at 30·0 s than at 3·0 seconds. However, in 3 out of 6 muscles studied at 30·0 s stimulus interval, caffeine 20 mM decreased the time to 50% repolarization but increased the total duration of the action potential by delaying the final phase of repolarization. These were the only preparations in this series in which caffeine produced a decrease in the duration of the action potential at either 50% or 90% repolarization levels.

Caffeine increased the overshoot of the action potential without affecting the resting membrane potential. The magnitude of the effect increased with increased concentration of caffeine. In the presence of 20 mM caffeine there was an increase of 12–15 mV.

The effects of caffeine on the duration of the action potential obtained in this series of experiments contrast with the results obtained with the muscle clamp. The parallel platinum electrodes were relatively massive and when making observations on two or three muscles simultaneously it was sometimes necessary to increase the stimulus intensity to reach the threshold of activation of all muscles. It seemed likely that endogenous catecholamines were being released from muscles stimulated by the parallel electrodes, and that these amines were interacting with caffeine on the muscles. In order to examine this possibility experiments were done with the

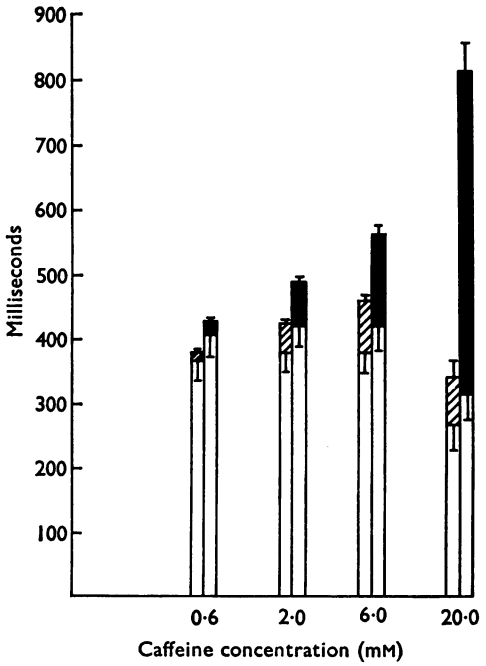

FIG. 6. Effect of caffeine on duration of action potential when large electrodes were used for stimulating kitten papillary muscle. Stimulus interval 3·0 s. Each pair of columns represents the duration measured at time to 50% repolarization (left) and 90% repolarization (right). Pre-drug, control values are shown, mean and S.D. (downwards), and the increases in duration produced by different concentrations of caffeine are represented by the cross-hatched and solid areas. $\bar{x}$ and S.E. of increase upwards (0·6 mM, $n=10$ experiments; 2·0 mM, $n=10$; 6·0 mM, $n=8$; 20·0 mM, $n=4$).

muscle clamp containing the small electrode to observe the effect of caffeine in the presence of (i) isoprenaline, 0·02 μM and (ii) a high stimulus intensity to release endogenous catecholamines.

Effects of isoprenaline and caffeine

In each experiment observations were made on the effects of isoprenaline and caffeine separately and then in combination. Isoprenaline (0·02 μM) produced no change in the resting membrane potential or the amplitude and duration of the action potential. There was an increase in the tension developed by the muscle, and a decrease in the duration of contraction. Caffeine, alone (6·0 mM) had little effect on the action potential but increased the developed tension and duration of contraction. However, when caffeine (6·0 mM) and isoprenaline (0·02 μM) were present at the same time there was an increase in the duration of the action potential at both 50% and 90% repolarization levels (Fig. 7). There was an increase in the amplitude of the action potential but no change in the membrane potential. The maximum developed tension was larger than when either drug was present separately and the duration of the contraction was increased.

The effects of caffeine and high stimulus intensity

Initially control observations were made when just suprathreshold stimuli were applied to the muscle. The stimulus intensity was then increased by 8–10 times

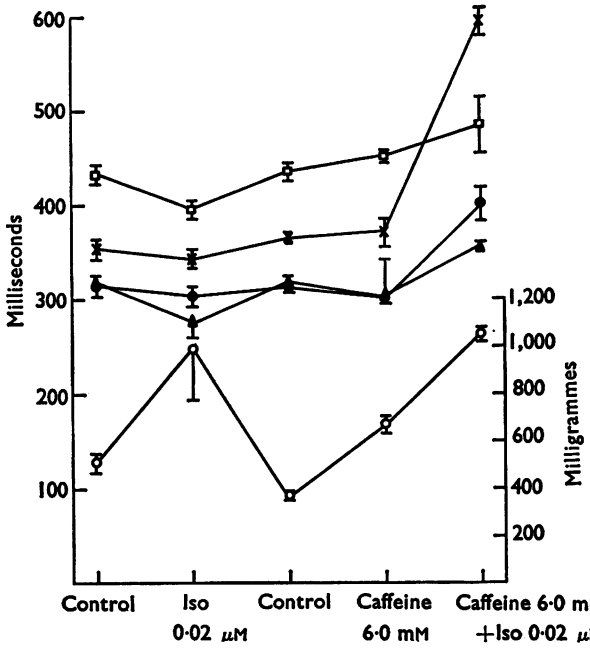

FIG. 7. Interaction of isoprenaline and caffeine on the action potential and contraction of kitten papillary muscle. Points show values obtained during a single experiment to demonstrate the effect of isoprenaline, caffeine and then the two drugs together. Pulse interval 3·0 s; ○=maximum tension; ▲=time from onset of contraction to peak tension; □=time from onset of contraction to half relaxation; ●=time to 50% repolarization; ×=time to 90% repolarization ($\bar{x} \pm$ S.D.).

in order to release some endogenous catecholamines. This produced a decrease in the duration of both the action potential and contraction but an increase in maximum developed tension. The stimulus voltage was then reduced and the effects of caffeine 6·0 mM were observed to be the same as described in the first section of the results. When the stimulus intensity was increased in the presence of caffeine the action potential became very prolonged and there was a further increase in the duration of the contraction and maximum developed tension.

Discussion

Comparison of results from the two experimental techniques

It now appears that the results presented in a preliminary report on the effect of caffeine on the cardiac action potential (Clark & Olson, 1968) reflect more than just the action of caffeine. These observations were made with the muscles pinned out across two large stimulating electrodes. The relatively massive electrodes used to excite the muscles, and the fact that the stimulating voltage was increased until all the muscles were excited, created a situation in which there was a very large electrical field in the region of the muscle: a small papillary muscle would have been situated almost entirely over the electrodes so that the whole muscle was subjected to a high current intensity. It has been shown (Blinks, 1966) that, when a large current field surrounds a cardiac muscle preparation, endogenous catecholamines are liberated in amounts large enough to produce positive inotropic or chronotropic effects. Therefore it is probable that these results represent the combined effects of catecholamines and caffeine on the cardiac action potential.

However, the muscle clamp was designed to contain a small electrode, against which the base of the papillary muscle was clamped. With this system, the threshold voltage for excitation was small and the area of the current field was reduced to a minimum, so that very small amounts, if any, of endogenous catecholamines were released.

We do not have any direct experimental proof that catecholamines were liberated by stimulation with the large electrodes but there is some evidence from experiments which supports this suggestion. (1) The effects of caffeine in the presence of a known concentration of a catecholamine (isoprenaline) or released endogenous amines (high stimulus intensity) are very similar to the results obtained with the large electrodes. (2) There were three experiments when the large electrodes were used in which caffeine decreased the duration of the action potential recorded from muscles stimulated at 30·0 s intervals. These experiments may demonstrate the effect of caffeine in the presence of very low concentrations of catecholamines released from the preparation. The concentration of catecholamines in the medium surrounding the muscle will depend upon the rate of stimulation: if the muscle is stimulated at an interval of 30·0 s, the amount of catecholamine released per minute will be less than if the muscle was stimulated at 3·0 s intervals. In two experiments with the muscle clamp, propranolol (0·01 mM) was used in an attempt to reduce the effects of the released catecholamines during excitation with high stimulus intensity. However, the presence of the β-adrenoceptor blocking agent produced no marked differences in the results.

De Gubareff & Sleator (1965) have suggested that caffeine may itself release endogenous catecholamines from atrial muscle. The increase in the duration of the action potential that we observed in the presence of 20·0 mM caffeine might then represent the combined effects of a catecholamine and caffeine. However, this seems unlikely, since the effect of caffeine (0·6–20·0 mM) on the isometric contraction of kitten papillary muscles is unaffected by pretreatment of the animals with reserpine or the presence of the β-blocking agent, propranolol (Blinks, Olson, Jewell & Braveny, 1972).

The action of caffeine

In cardiac muscle during excitation calcium is released from intracellular sites but there is also an inflow of calcium from extracellular spaces. This inward calcium current, which occurs during the early part of the plateau phase of the action potential, has been identified in both atrial (Rougier, Vassort, Garnier, Gargouil & Coraboeuf, 1969) and ventricular cells (Beeler & Reuter, 1970a). In the present experiments caffeine produced an increase in the amplitude of the action potential which probably represents an increase in the calcium current entering the cell. In atrial muscle (de Gubareff & Sleator, 1965) and in Purkinje fibres (Carmeliet & Vereecke, 1969), caffeine prolongs the plateau phase of the action potential; the effect of the drug in Purkinje fibres is reduced in the presence of manganese ions so that a large sustained calcium current is suggested. However, in our experiments caffeine increased the amplitude of the action potential but reduced the duration of the plateau phase. This is probably due to the influence of the calcium current on the time relations of the potassium currents responsible for repolarization in ventricular cells (Noble & Tsein, 1969).

The decrease in the duration of the action potential caused by caffeine was particularly marked when long stimulus intervals were used. This may be due to the influence of the frequency of stimulation of the muscle on calcium ion exchange across the cell membrane (Langer & Brady, 1963) and the effect of caffeine on these different rates of calcium flux.

It has been suggested that the inward current fills up partially depleted intracellular stores of calcium which can subsequently be released by an unknown mechanism to activate contraction (Beeler & Reuter, 1970b; Wood, Heppner & Weidmann, 1969). Any increase in the calcium current would therefore increase the concentration of bound intracellular calcium, resulting in an augmented release of calcium on excitation and an increase in the tension developed during contraction. Caffeine (2·0–20·0 mM) produced an increase in the developed tension in all of our experiments.

There was also an increase in the duration of the contraction in the presence of caffeine. In skeletal muscle caffeine decreases the ability of the sarcoplasmic reticulum to bind intracellular calcium (Weber & Hertz, 1968), and if it acts in a similar way on cardiac sarcoplasmic reticulum then the reuptake of intracellular calcium will be reduced. Calcium ions will therefore be retained in the region of the myofilaments for a longer time resulting in a prolongation of the contraction.

It appears, therefore, that caffeine may have two modes of action in mammalian ventricular fibres: (i) production of an increase of inward transmembrane calcium current during excitation, and (ii) a reduction in the accumulation of calcium in intracellular storage sites, although the two effects could be inter-related.

The effect of isoprenaline and caffeine

Isoprenaline produced the characteristic effects of a catecholamine on the isometric contraction; there was an increase in the maximum tension developed and a decrease in the duration of contraction. The amplitude and duration of the action potential were not significantly changed by the concentration of isoprenaline used, which is approximately 75% of the maximally effective concentration. It has been suggested that an increase in the duration and amplitude of the plateau phase of the action potential in the presence of adrenaline is due to an augmented inward calcium current (Reuter, 1967; Carmeliet & Vereecke, 1969; Vassort, Rougier, Garnier, Sauviat, Coraboeuf & Gargouil, 1969). If caffeine also increases the inward calcium current, when the two drugs are present there would be a very large influx of calcium in addition to a high intracellular calcium concentration due to the effect of caffeine on the sarcoplasmic reticulum. The increase in the action potential duration could then be due to the influence of the very large calcium current on currents responsible for repolarization.

It is known that theophylline potentiates the inotropic response to catecholamines (Rall & West, 1963). In the experiment shown in Fig. 7, the increase in maximum tension developed in the presence of isoprenaline and caffeine is less than would be expected by simple addition of the separate effects of the drugs. This represents a slight reduction in the positive inotropic response following repeated exposures of the muscle to drugs.

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