Influence of Caffeine and Other Methylxanthines on Mechanical Properties of Isolated Mammalian Heart Muscle

EVIDENCE FOR A DUAL MECHANISM OF ACTION

By John R. Blinks, Camille B. Olson, Brian R. Jewell, and Pavel Braveny

ABSTRACT
Caffeine, theophylline, and theobromine have similar effects on the contractions of kitten atrial and papillary muscle preparations in vitro. In concentrations between 2 and 20 mM they both intensify and prolong the active state, as indicated by isometric and delayed-release isotonic contractions; contracture is not normally produced. Instantaneous force-velocity curves are shifted approximately symmetrically by caffeine; force-velocity curves derived from simple afterloaded contractions are misleading because of the great prolongation of activity. After the addition of caffeine the onset of the increased degree of activation is more rapid than that of the prolongation of activity; procaine antagonizes the prolongation of activity but not the intensification. In the presence of the methylxanthines, the duration of contraction is no longer abbreviated by isoproterenol, though it is still readily influenced by changes in frequency. The prolongation of activity by Sr²⁺ differs in significant respects from that induced by methylxanthines. The results suggest that the methylxanthines exert two effects on excitation-contraction coupling. One of these is presumed to be the inhibition of calcium sequestration by the sarcoplasmic reticulum; the other may be an effect on the cell membrane that leads to increased calcium entry. Most of the features of the altered mechanical response can be explained on this basis if it is assumed that intracellular calcium stores available for release are depleted as a result of the process that impairs calcium sequestration.

KEY WORDS theophylline theobromine procaine strontium isoproterenol active state excitation-contraction coupling force-velocity relation contracture cat papillary and atrial muscle

It has been known for more than a century that the methylated xanthine derivatives caffeine, theophylline, and theobromine...
increase the strength of contraction of heart muscle. This action of the methylxanthines has not been the subject of much detailed investigation, perhaps because the high concentrations required made it seem of doubtful therapeutic importance. Two recent developments have, however, rekindled interest in the inotropic effects of these compounds. One was the demonstration that caffeine causes the release of calcium from and decreases the net uptake of calcium in vitro by vesicles derived from the sarcoplasmic reticulum of muscle (1–7). This observation has been convincingly related to the effects of caffeine on the mechanical properties of skeletal muscle by Sandow and his co-workers (8–15, reviewed in 16–18). The other significant development was the discovery (19, 20) that the methylxanthines, especially theophylline, are inhibitors of phosphodiesterase, the enzyme responsible for the breakdown of cyclic 3', 5'-adenosine monophosphate (cyclic AMP) in the muscle cell. This observation, coupled with the demonstration that catecholamines stimulate the production of cyclic AMP in heart muscle (21–24), has led to widespread speculation that the catecholamines and the methylxanthines might exert their inotropic effects through a common pathway—an increased concentration of cyclic AMP in the muscle cell (e.g., 24–26).

An obvious corollary of the cyclic AMP hypothesis is that the inotropic effects of the two groups of substances ought to be similar, but this is a point that does not seem to have been examined, possibly because it has not been appreciated widely enough that there might be more than one sort of "positive inotropic effect." Unfortunately, little detailed information is available on this point because most investigations of the effects of methylxanthines on the heart have been limited to observation of the amplitude of isometric or isotonic responses recorded on slowly moving paper. Two exceptions are the papers by deGubareff and Sleator (27) and by Gibbs (28), which showed that caffeine differs from most other inotropic drugs (e.g., catecholamines) in one important respect, namely, that it prolongs the active state of cardiac muscle. Although neither paper was primarily concerned with the time course of activity, both showed that caffeine increased the time to peak tension and the overall duration of the contractions of heart muscle, an observation of great interest because of its obvious relevance to the recently discovered effects of caffeine on the sarcoplasmic reticulum.

The main purpose of the present study was to provide detailed information about the effects of the methylxanthines on the time course of activity in cardiac muscle, and the interactions of the effects with those of certain other agents known to influence excitation-contraction coupling in heart muscle. These effects have been assessed mainly from alterations in the isometric contractions, and preliminary accounts of some of the results obtained in this way have already been given (29–31). This paper also includes measurements of the time course of activity by the isotonic release technique (32, 33), which has been applied previously to cardiac muscle in investigations of the effects of other inotropic drugs (34–37). The results support some fairly straightforward inferences about the mechanisms by which the methylxanthines influence myocardial contractility.

**Methods**

**Preparations.**—Papillary muscles and strips of left atrium were removed from the hearts of kittens (0.5–1.2 kg) anesthetized with chloroform. Strips of left atrium from larger kittens were avoided because they tended to exhibit conduction blocks. Only the thinnest papillary muscles in the right ventricle were used: in most cases they were between 0.5 and 0.75 mm in greatest thickness; no muscle more than 1 mm thick was used.

**Muscle Holder.**—For most measurements of isometric contractions the preparations were mounted in an apparatus described previously (38). In this, the muscle was held vertically between a small acrylic plastic clamp below and a stainless steel wire hook hanging downward from a Statham GIA-350-1.0 tension (strain gauge) transducer. The muscle was tied to the hook with a short (about 2 cm) 5-0 cotton or braided

Circulation Research, Vol. XXX, April 1972
EFFECT OF CAFFEINE ON HEART MUSCLE

369

Tevdek thread. A punctate platinum stimulating cathode touched the tissue just above the point at which it was clamped, and field electrodes (platinum wire) ran parallel to the muscle on both sides. The isolated organ bath (volume 50 ml) provided a continuous stream of freshly oxygenated physiological salt solution circulating rapidly past the tissue. The organ bath was immersed in a large thermostatically controlled water tank which maintained temperature constant to within 0.5°C. Unless otherwise stated, experiments were carried out at 32.5°C.

Isotonic Lever.—Isotonic and quick-release studies were made with an apparatus that differed only in that the tension transducer was replaced by a combined length and tension transducer of the type described by Jewell et al. (39). This consisted of a lightweight magnesium lever on which both force and position sensing elements were mounted. The lever ratio was 9.35:1, and the lever had an equivalent mass of 80 mg at the point of muscle attachment (5 cm from pivot). Tension was measured as strain in the lever by means of semiconductor strain gauges (Baldwin-Lima-Hamilton type SP5-10-12, unbacked) which were cemented onto the upper and lower surfaces of the lever near its tip. Length changes were also measured near the tip of the lever, where an aluminum foil vane threw a shadow on the surface of a solar cell. The latter was treated as a current source, and a Philbrick P855AU operational amplifier was used to convert current into a voltage signal. The quick-release catch was a small, lightweight disc held by vacuum against a tubular stop. The vacuum was controlled with a solenoid valve. The muscle was connected to the lever by means of a carefully straightened stainless steel wire (mass 5 mg) 12 cm long and 20 μ in diameter. A minute load-extension curve of the equipment (i.e., lever, hard and soft connections, and muscle holder) was nonlinear, so the strain compliance cannot be specified by a simple value; in the 1-2-g range the slope compliance was 6μ/g, the value being greater at lower tensions and less at higher. In some experiments, the lever was damped by a 10-mm² vane mounted 2.5 cm from the pivot and immersed in mineral oil (viscosity 335-350 Saybolt Universal seconds).

Stimulation and Recording.—In most experiments, the left atrial strips and papillary muscles were driven through the punctate cathode and a large distant anode by square-wave pulses of 5-msec duration and just threshold voltage (usually 250-500 mv) at frequencies determined by the needs of the particular experiment. Field stimulation was used in some experiments; in these, papillary muscles were driven by 100-ma, 2-msec pulses passed between the two parallel field electrodes with a pulse amplifier of the type described by Waud (40). Tektronix 3C66 carrier amplifiers were used with both types of tension transducers; signals were displayed on a Tektronix 502 or 565 oscilloscope and recorded simultaneously on a Sanborn 7700 pen recorder. In some experiments the signal indicating lever displacement was differentiated with a Tektronix 3A8 operational amplifier (no added input resistance or feedback capacitance)² to provide a record of the velocity of shortening. Multiple exposures on Polaroid film were made to superimpose oscilloscope tracings. The stimulator, oscilloscope sweep, delayed release solenoid, camera shutter, and pen recorder paper drive were coordinated by signals derived from a digital programmer. In some cases (indicated in figure legends), experiments were recorded on magnetic tape and replayed to provide sequences of multiple exposures that otherwise would have been difficult or impossible to obtain. The Ampex SP-300 tape recorder had a frequency response flat to within 2 db from 0 to 625 Hz at the tape speed used, but for these studies the frequency response was limited by a simple R-C filter (break frequency = 160 Hz) on the output of the tape recorder. This virtually eliminated tape recorder noise without introducing detectable distortion of the contraction curves.

Solutions.—For most experiments the muscles were bathed in a solution of the following composition (mM): Na+ 140, K+ 5, Ca2+ 2.25, Mg2+ 1.0, Cl- 98.5, HCO3- 29, HPO42- 1.0, SO42- 1.0, fumarate 5.0, pyruvate 5.0, glutamate 5.0, glucose 10; and insulin 5 units/liter. For the delayed release and length-tension studies, a solution that contained a simpler mixture of metabolic substrates was used: 20 mM acetate replaced the other organic anions, the chloride concentration was 102 mM, and the bicarbonate concentration was 24 mM. Both solutions were equilibrated with 95% O2-5% CO2. No differences in the behavior of the muscles were noted in the two solutions.

¹ Fluorocarbon-treated polyester fiber manufactured by J. A. Deknatel and Son, Queens Village, N. Y.

² Designed and built in the department shop by Mr. John Dill.
Experimental Procedure.—Muscle preparations were driven at a constant frequency (usually 2- or 5-second intervals) to allow stabilization before the experimental procedures were begun (up to 3–4 hours). Papillary muscles were stretched to the length at which developed tension was maximal; the length of the left atrial strips was adjusted to give a resting tension approximately one-half that associated with maximal developed tension, because the resting tension at the optimal length was relatively high in atria and tended to be associated with troublesome stress-relaxation. Unless otherwise specified, recordings from which measurements were made were taken after at least 15 minutes of exposure to any given concentration of a methylxanthine. For some studies the muscles necessarily were exposed to a given concentration of a drug for an hour or more. The length of each papillary muscle was measured at the end of the experiment, while the muscle was still stretched to its optimal length. The muscle was removed from the holder and kept moist while its tendon and the clamped portion were severed under a dissecting microscope. The muscle was then rolled over gently on filter paper and weighed on a Cahn electrobalance within 10–15 seconds.

Drugs.—To avoid complications from undetermined effects of other substances, we used all of the methylxanthines (Nutritional Biochemicals Co.) in the poorly soluble free alkaloid form. It was necessary to heat the stock solutions to dissolve the alkaloids, and the more concentrated solutions (up to 1M caffeine) were kept in stoppered vessels in a hot water bath during the experiment. The volume of drug solution added never exceeded 4% of the bath volume. The upper limit for the concentration of theobromine used was dictated by the low solubility of the base, and for the higher concentrations it was necessary first to dissolve the drug in hot distilled water; after it had cooled, this solution was used instead of distilled water to make up the physiologic salt solution. Procaine hydrochloride, l-isoproterenol bitartrate, dl-propranolol hydrochloride, atropine sulfate, reserpine phosphate, and strontium nitrate were also used. All stock solutions were made up in distilled water, except that the isoproterenol stock (10 mM) was acidified to pH 3 with HCl during the initial dilution. Ethylenediamine tetraacetic acid, disodium salt (EDTA), 40 μM, was added to the physiologic salt solution whenever isoproterenol was to be used. The pH of the physiologic salt solution was changed no more than 0.05 pH units by any concentration of any drug used. Drug concentrations are expressed in molar terms; for this purpose the molecular weight of atropine sulfate was taken as 347.4 (atropine [SO₄]½).

Results

EFFECTS OF METHYLMETHANINES ON THE ISOMETRIC CONTRACTION

Experiments carried out under a wide variety of conditions (described below) indicated that the methylxanthines produce five characteristic alterations in the isometric contractions of heart muscle: (1) an increase in tension development, (2) a prolongation of the time to peak tension, (3) an increase in the total duration of contraction, (4) an increase in the maximal rate of tension

\[ 370 \]
EFFECT OF CAFFEINE ON HEART MUSCLE

development (dP/dt max), and (5) a more gradual onset of contraction. Representative records are reproduced in Figure 1, which shows superimposed oscilloscope tracings of isometric contractions of a left atrial strip and a papillary muscle in the presence of various concentrations of caffeine.

The relative prominence of the five effects varied considerably from one preparation to another, and from one xanthine concentration to another. All of the effects were usually clearly distinguishable in both atrial and ventricular muscle at concentrations from 2 to 6 mM, but some were more prominent in the latter tissue. Very low concentrations (0.2-0.6 mM) often increased peak tension slightly without appreciably prolonging the contractions. Very high concentrations of caffeine (>20 mM) usually depressed dP/dt max and peak tension while further increasing the other effects. The relative importance of the various changes described was greatly influenced by the frequency of contraction. This will be considered in detail below, as will the time course of the onset of these actions, the reproducibility of responses, the comparative effects of the three xanthine derivatives, and the fact that contracture was not normally encountered in these experiments.

The effects of the methylxanthines on isometric contractions of heart muscle differ strikingly from those of most other inotropic agents (e.g., isoproterenol; see Fig. 13A) in that caffeine markedly prolongs the contraction. Figure 2 shows a series of contractions recorded to contrast the effect of varying the extracellular calcium concentration (records 1-4) with that of adding caffeine (record 5). Increases in the extracellular calcium concentration were associated with increases in the maximal rate of tension development and in peak developed tension. Although there was a slight prolongation of the time to peak tension and the total duration of contraction when the extracellular calcium concentration was increased from 1.125 mM to the usual 2.25 mM, further increases in calcium concentration did not substantially alter the time course of contraction. It should be noted that increases in calcium concentration do not slow the onset of tension development in the way that the methylxanthines do.

ONSET AND DURATION OF ACTION

The effects of a methylxanthine on preparations of heart muscle begin to develop within seconds of its addition to the bathing solution, and the full effects are attained within 10-15 minutes. The increase in peak tension in the papillary muscle occurred in three distinct phases under the conditions of our experiments. There was always a small but very rapid increase in tension development during the first one to three contractions after the first addition of caffeine (Fig. 3) or after an increase in its concentration. This was followed by a brief phase in which for several beats there was a reduction in the rate at which twitch tension increased. Sometimes peak tension actually decreased during this phase (as in Fig. 3); more often it leveled off for a few beats or simply reduced its rate of rise. Finally, there was a slowly progressive increase in peak tension over a period of 10-15 minutes. This last phase was sometimes absent after the addition of minimally effective doses of caffeine. High speed tracings (Fig. 3) indicated that in the first phase there was an increase in dP/dt, with little or no change in the time to peak or in the total duration of contraction; during the second phase there

FIGURE 2

Comparison of influences of caffeine and calcium on isometric contractions of kitten papillary muscle driven at 3-second intervals. 1 = 1.125 mM Ca^2+; 2 = 2.25 mM Ca^2+; 3 = 4.5 mM Ca^2+; 4 = 6.75 mM Ca^2+; 5 = 2.25 mM Ca^2+ and 6 mM caffeine. Length of muscle, 4.7 mm; weight, 0.75 mg; 32.5°C.

Circulation Research, Vol. XXX, April 1972
was a slight reduction in $dP/dt$, and the onset of the other features of the xanthine effect became detectable; the third phase saw a gradual prolongation of the time to peak tension and total duration of contraction, usually accompanied by a further increase in $dP/dt_{\text{max}}$, and a further decrease in the abruptness of the onset of contraction. This triphasic change in peak tension is seen after each of several successive increases in caffeine concentration in studies of the cumulative dose-response relation, though the third phase may be absent at very low concentrations and the first may be absent at very high concentrations. This suggests that the various phases result from the interaction of at least two separate actions of caffeine, each action increasing with drug concentration but having a characteristic rate of onset (see Discussion).

The effects of the methylxanthines persist as long as the drug is present, but they are readily reversed when the preparation is washed. The time required for disappearance of the drug effect after washout is roughly similar to that for onset, with a halftime of 5 to 7 minutes. Dose-response curves can be repeated many times in the same preparation with virtually identical results.4

**COMPARISON OF EFFECTS OF CAFFEINE, THEOPHYLLINE, AND THEOBROMINE**

Because it is commonly accepted (e.g., 43) that the three methylxanthines—caffeine, theophylline, and theobromine—are beneficial in cardiac disease, it is our impression that muscles exposed to caffeine from time to time are more stable than those that are not. Interestingly, other workers (12, 41) have reported the opposite experience in frog skeletal muscle and related it to reports of histologic damage induced by caffeine (42).

**FIGURE 3**

*Time course of onset of inotropic effect of caffeine in kitten papillary muscle. Top: Low-speed oscillograph tracing of tension developed in isometric contractions. Bottom: Superimposed oscilloscope tracings of selected contractions from sequence shown in top tracing. Photographs made from tape recording with camera shutter open only during contractions indicated on photographs. Contraction 0 is before caffeine; contraction 1 is the first after the addition of 4 mM caffeine; contraction 180 is at the steady state. (Note that vertical gain in left photograph is twice that in right.) Muscle stimulated at 5-second intervals: time of stimulus recorded as mark above force tracing 100 msec from edge of grid. Length of muscle, 5.0 mm; weight, 3.20 mg; 32.5°C.*
EFFECT OF CAFFEINE ON HEART MUSCLE

theophylline, and theobromine—differ substantially in their effects on heart muscle, and theophylline is more potent than the others as a phosphodiesterase inhibitor (20). We compared the effects of these three compounds on the isometric contractions of kitten papillary muscles. Cumulative dose-response curves were determined in at least six papillary muscles for each of the three methylxanthines (one drug per muscle). Since other experiments had shown that caffeine concentrations higher than 20 mM depressed peak tension and frequently were associated with a considerable elevation in the threshold for stimulation, we did not use such concentrations routinely in dose-response curves. Theobromine concentrations higher than 6 mM were not used because of the low solubility of this substance. The results of these experiments are summarized in Figure 4. Dose-response curves for the three xanthine derivatives are indistinguishable; peak tension, rate of development of tension, time to peak tension, and total duration of contraction were increased concomitantly by each drug, and the potencies of the three drugs were similar. Substantial variability between preparations was encountered in the relative prominence of the various effects of each of the methylxanthines, with the result that the minimum significant changes between doses were rather large. Whereas Figure 4 indicates that none of the xanthine derivatives produced statistically significant effects at concentrations below 2 mM, individual tracings often showed a distinct effect associated with the addition of the 0.2 or 0.6 mM dose (Figs. 1, 11, 12, 14). As concentration was increased above these levels, all of the methylxanthines produced effects that increased progressively up to the 20 mM concentration. Although on average, peak tension increased through the 20 mM concentration level, a depression of peak tension began to appear at the 20 mM level in some experiments. (As was noted earlier, this occurs regularly at still higher concentrations.) This reflected a decrease in the rate of tension development, but occasionally time to peak tension became shorter as well; the total duration of contraction was always prolonged progressively by increasing doses.

In the presence of high concentrations (6 and 20 mM) of the methylxanthines, transient automaticity frequently occurred, but this usually subsided within 10 to 20 minutes. Although Figure 4 shows that no great differences exist between the effects of the various methylxanthines on cardiac contractility, the experiment is clearly incapable of...
revealing subtle differences. Because of the
great stability of the preparations and the
reproducibility of successive responses to the
methylxanthines, any such differences would
best be studied by comparisons on the same
muscle. We have determined dose-response
curves for both caffeine and theophylline on
each of three papillary muscles without
finding consistent differences between the
effects of the two methylxanthines.

INFLUENCE OF METHYLXANTHINES
ON LENGTH-TENSION RELATION

In contrast to the situation in amphibian
skeletal muscle (16, 17), contracture is not a
prominent feature of the response of kitten
heart muscle to the methylxanthines. In the
dose-response curves for which quantitative
data are reported in this paper, no concentra-
tion of any of the methylxanthines produced
a significant change in tension in the resting
(unstimulated) muscle. Slight increases in
resting tension (no more than 10% of maximal
twitch tension) were sometimes noted in
relatively fresh muscles (up to 12 hours old)
exposed to caffeine concentrations between 20
and 60 mM. However, in three muscles that
had been isolated at 32.5°C for 36–48 hours,
obvious contractures did appear over the same
range of caffeine concentrations that had
earlier produced only twitch potentiation.
Increases in extracellular calcium concentra-
tion (to 10 mM) also favored the development
of caffeine contractures, which appeared only
during periods of moderate to high frequency
stimulation and gradually subsided after
stimulation was stopped.

The influence of 6 and 20 mM caffeine on
the passive and active length-tension curves
was examined in detail on six papillary
muscles. No changes in the passive length-
tension relation attributable to caffeine were
observed. Although the active tension was
greater at all muscle lengths after caffeine,
there was no change in the muscle length
optimal for tension development. The prolong-
ation of the time to peak tension associated
with increases in muscle length (45) was
reduced or eliminated in the presence of
caffeine, though the total duration of contrac-
tion was still prolonged by stretch.

INFLUENCE OF TEMPERATURE

In three papillary muscles, cooling from
32.5°C to 22.5°C was found to decrease both
the minimum effective concentration of
caffeine and the concentration associated with
maximum tension development by a factor of
about 3. Warming from 32.5°C to 38°C did not
materially alter the response to caffeine.
Contracture was not induced in fresh prepara-
tions by concentrations of caffeine up to 20
mM at any of the temperatures studied.

INFLUENCE OF FREQUENCY OF CONTRACTION

The influence of the methylxanthines on the
isometric contraction curve is highly depen-
dent on the frequency of contraction. This is
illustrated by the representative superimposed
oscilloscope tracings in Figures 5 and 6, and
summarized in somewhat different perspective
in Figure 7.

Control contractions from a papillary mus-
cle driven at intervals ranging from 300 to 0.55
seconds are illustrated in the upper panel of
Figure 5. Contractions of the same muscle at
the same intervals in the presence of 6 mM
caffeine are shown in the lower panel. With
and without caffeine, progressive shortening of
the interval between contractions caused an
increase in dP/dt and an abbreviation of the
time to peak tension. In the presence of 6 mM
caffeine, however, the interval-force relation
was altered in certain respects. The rate of
tension development was relatively high even
at low frequencies of contraction, and as the
interval between contractions was decreased
from 300 to 3 seconds (curves 1 through 5 in
lower panel of Fig. 5), dP/dt increased to an
apparent maximum. As the interval was
shortened further, dP/dt stayed constant, but
the time to peak tension continued to decrease
(curves 5 through 8) and peak tension de-
creased. The main effects of caffeine on papil-
lary muscles contracting at various steady-
state frequencies can be summarized as
follows: (1) The total duration of contraction
is increased at all intervals. (2) Developed
tension is increased at all but very short
intervals, and there it is actually reduced. (3)
The time to peak tension is prolonged at all
intervals except the longest and sometimes the
EFFECT OF CAFFEINE ON HEART MUSCLE

Figure 5
Top: Control. Bottom: Influence of 6 mM caffeine on isometric contractions of kitten papillary muscle at various frequencies. Intervals (seconds) between contractions (at steady state): 1 = 300; 2 = 100; 3 = 30; 4 = 10; 5 = 3; 6 = 1.7; 7 = 1.0; 8 = 0.55. Muscle length, 5.5 mm; weight, 1.52 mg; 32.5°C.

Figure 6
Top: Control. Bottom: Influence of 6 mM caffeine on isometric contractions of kitten left atrium at various frequencies. Intervals (seconds) between contractions (at steady state): Top (in order of increasing tension), 3, 10, 30, 1.7, 100, 1.0, 300, 0.55; bottom (virtually superimposed curves), 30, 100, 300; in order of increasing tension, 10, 3, 1.7, 1.0; abbreviated curve, 0.55 sec; 32.5°C.
peak tension and the total duration of contraction are prolonged at all intervals; dP/dt is increased by caffeine at all intervals except the shortest (0.55 seconds); the gradual onset of contraction is present, but less prominent than in papillary muscle; the influence of the interval between contractions on the tension developed by atrial muscle is much diminished by caffeine; and dP/dt is made high at all frequencies.

Figure 7 summarizes the findings on 4-8 preparations in which high-speed tracings were made when steady states had been achieved at various frequencies of contraction in the absence and in the presence of 6 mM caffeine. In this graph each measurement made in the presence of caffeine is plotted as percent of the value obtained from steady-state control contractions at the frequency in question, and a given change appears especially large under those conditions in which control measurements have small values. This method of plotting puts stress on the apparent differences in caffeine effect that one might encounter by working at different frequencies and facilitates choice of frequency for maximum relative drug effect.

**STUDIES BY THE DELAYED ISOTONIC RELEASE METHOD**

Isometric contractions clearly indicate that the time course of activity is prolonged by methylxanthines. This observation has been extended by the use of the isotonic release technique, which allows the degree of activity of the muscle to be assessed in terms of its ability to shorten against a load when suddenly released at various times after stimulation. The time course of activity can be judged from various measurable parameters when this technique is used (33), and we have followed the recent practice in the cardiac muscle field of using the postrelease velocity of shortening against a light load (34, 36, 37). Figure 8 shows the influence of 20

---

**FIGURE 7**

Relative changes in tension development and time course of contraction induced by 6 mM caffeine in kitten atrium and papillary muscle at various frequencies of contraction. All measurements indicate value after caffeine as percent of value before caffeine at the same frequency of contraction. Means and standard errors of 4-8 preparations at 32.5°C. Note change in scale above middle of vertical axis.
Influence of caffeine on time course of activity in kitten papillary muscle. Both before and after the application of 20 mM caffeine, the muscle was released at various times from optimal fiber length against an afterload of 0.4 g wt (lever undamped). The prerelease tension was the tension reached by the muscle at the end of the isometric phase of contraction before release; the curve of prerelease tensions differs from the isometric contraction curve only in that successive points were measured from different contractions. The postrelease velocity was determined graphically from oscilloscope photographs as the mean velocity of shortening during the period from 10 to 30 msec after release and plotted at the time corresponding to 20 msec after release. Length of muscle, 7.0 mm; weight, 4.0 mg; stimulation at 5-second intervals; 32.5°C. Solid symbols = control; open symbols = 20 mM caffeine.

The delayed isotonic release technique was also used to estimate instantaneous force-velocity curves for the papillary muscle. As one might surmise from the similarity of the caffeine-induced shifts in the two indexes of
Influence of 6 min caffeine on force-velocity curves of kitten papillary muscle determined in two ways. A: Instantaneous curves plotted from delayed-release contractions in which release occurred 200 msec after stimulus and velocity was measured 50 msec after release. B: Curves plotted from simple afterloaded contractions of the same muscle. Velocities are maximum velocities of shortening against various afterloads; small figures beside points indicate times after stimulation at which maximum velocity was attained. Each isotonic or delayed-release contraction was preceded by at least ten isometric contractions to assure comparable steady-state conditions. Lever oil-damped, velocities measured by electronic differentiation. Length of muscle, 9.0 mm (95% of optimal); weight, 4.08 mg; resting tension, 75 mg; stimulation at 5-second intervals; 32.5°C.

activity (which are the two ends of the force-velocity curve) the instantaneous force-velocity curve was shifted approximately symmetrically by caffeine (Fig. 10A). As has been noted before (46, 47), force-velocity curves plotted from simple afterloaded contractions can be misleading in situations like this because apparent changes in the shape of the force-velocity curve may arise from changes in the time course of activity. Figure 10B shows such curves from the same muscle for comparison.

Experiments with field stimulation were performed to test the possibility that the slowed onset of tension development might reflect a retardation of the spread of the action potential from the site of stimulation. These experiments also served to show that β-receptor blockade and reserpine pretreatment had no significant effect on the inotropic response to caffeine. In each of six papillary muscles, a dose-response curve to caffeine was determined, and high-speed tracings of isometric contractions were photographed at each dose level. After the caffeine had been washed out, 0.6 μM propranolol was added to prevent the rather substantial effects of norepinephrine that would be released by subsequent field stimulation (48). Thirty minutes later, a second dose-response curve to caffeine was determined. When the steady-state effect had been achieved at each caffeine concentration level, field stimulation was instituted for several minutes. After the second dose-response curve had been completed, photographs of superimposed high-speed tracings were made by replaying magnetic tape recordings of the results. This permitted superimposing the contraction curves from all concentration levels for threshold stimulation on one photograph and for field stimulation on another, though both photographs were obtained from the same dose-response curve. All experiments showed the same three things, which are illustrated by the tracings of Figure 11: (1) dose-response curves for caffeine are highly reproducible in a given preparation; (2) β-receptor blockade has virtually no effect on the inotropic effect of caffeine; (3) the slowed onset of tension development is not appreciably altered by field stimulation.

Dose-response relations for caffeine were also examined in four papillary muscles from kittens given reserpine (5 mg/kg sc 18–24 hours before death). The results were not significantly different from those in untreated muscles. The results of field stimulation (two muscles) were as described above though propranolol was not used.
EFFECT OF CAFFEINE ON HEART MUSCLE

1950 msec

FIGURE 11
Lack of influence of β-receptor blockade or field stimulation on caffeine effect. Cumulative dose-response relations for caffeine in kitten papillary muscle (three photographs from same muscle). Isometric contraction curves; 5-second intervals; 32.5°C. A: First dose-response curve for caffeine. B and C: Second dose-response curve determined in presence of 6 x 10^{-7} M propranolol and 10^{-6} M atropine. Numbers beside contraction curves indicate caffeine concentration (mM). In A and B the muscle was driven with threshold stimuli applied through a punctate cathode; in C it was driven with intense field pulses. Photographs were made from magnetic tape recordings of experiment; see text for details. Muscle length, 6 mm; weight 2.03 mg.

Circulation Research, Vol. XXX, April 1972

EFFECTS OF PROCAINE

Procaine and certain other local anesthetics have long been known to prevent the contracture caused by caffeine in skeletal muscle (15, 41, 49–51). Furthermore, local anesthetics have been shown to antagonize the effect of caffeine on calcium retention by preparations of isolated sarcoplasmic reticulum (2, 3, 5, 7). For these reasons, we examined the effect of procaine on the response of the kitten papillary muscle to caffeine. Preliminary experiments on seven muscles showed that procaine alone, in concentrations above 0.6 mM, produced a marked increase in peak tension accompanied by a substantial decrease in the time to peak tension. Because of the resemblance of this effect to that of the catecholamines, the influence of propranolol on the response to procaine was tested. It was found that in the presence of 0.6 μM propranolol, no concentration of procaine shortened the time to peak tension, and concentrations of procaine that formerly increased tension development now decreased it markedly. (The procaine used did not contain epinephrine; furthermore, the sympathomimetic effect was observed with procaine from three different sources.) Dose-response relations for procaine were then examined in four papillary muscles from reserpine-treated kittens (5 mg/kg sc 18 hours before death); no sympathomimetic effects were observed. Thus it appears that the positive inotropic effect of procaine results from the release of endogenous norepinephrine, and not from an action of procaine itself on the β-receptors of the heart.

Not surprisingly, concentrations of procaine that (after reserpine or in the presence of propranolol) decreased tension development also increased the threshold voltage for stimulation. Even field-stimulated muscles usually ceased to respond in procaine concentrations above about 5 mM. Procaine also frequently induced alternation of the strength of successive contractions, and bursts or continuous periods of rapid spontaneous activity in previously nonspontaneous muscles.
PROCaine-CAFFEINE ANTAGONISM

To be able to drive preparations in the presence of the greatest possible concentrations of procaine (i.e., 2–4 mM) as well as to obviate problems that might be introduced by procaine-induced conduction blocks, we performed experiments on procaine-caffeine antagonism with field-stimulated muscles. Nine muscles were used; all were driven at 5-second intervals. Propranolol, 0.6 μM, was present in seven experiments and served a dual purpose by antagonizing the effects of norepinephrine released by the field stimuli and by preventing the sympathomimetic effect of procaine; in experiments on two muscles taken from reserpine-treated kittens (5 mg/kg sc 18 hours before death) propranolol was omitted. Atropine, 1 μM, was added in three of the nine experiments to antagonize the effects of acetylcholine released by the field stimuli, but this had no apparent effect on the results. Two of the nine preparations (both with propranolol alone) become spontaneously active after the addition of procaine and did not yield meaningful results.

The experiments on procaine-caffeine antagonism were conducted as follows: A dose-response curve to caffeine was determined in a field-stimulated muscle (Fig. 12A) (the final dose-response curve of the experiments on the effects of field stimulation served for this in most of the experiments on propranolol-treated muscles). The preparation was washed, the propranolol replaced if it had been present, and 4 mM caffeine added. When the preparation had stabilized, a contraction curve was photographed, and this was compared to contractions recorded after the addition of successively higher concentrations of procaine. Procaine reversed the caffeine-induced prolongation of the contraction in a dose-related manner (Fig. 12B). Under these conditions, 1–2 mM procaine was usually required to produce a marked abbreviation of the active state. Though lower concentrations tended to increase peak tension, these concentrations usually reduced it, but never to an extent approaching that produced by procaine in the absence of caffeine. The experiment illustrated in Figure 12 was unusual in that the active state was markedly abbreviated by a concentration of procaine that did not depress peak tension.

_Circulation Research, Vol. XXX, April 1972_
In the next stage of the experiment, the caffeine was washed out of the preparation, and the propranolol and atropine (if used) and 2 mM procaine replaced. Peak tension invariably decreased far below the original control level, though the time to peak tension and total duration of contraction remained similar to control. The dose-response curve to caffeine was now repeated with results in all experiments essentially like those shown in Figure 12C. Caffeine now increased the rate of rise of tension and peak tension with little or no prolongation of contraction until concentrations of 6 mM were exceeded. It is noteworthy that the caffeine-induced slowing of the onset of contraction is also prevented by procaine. The effect of low concentrations of caffeine had a rapid onset; higher concentrations readily overcame the procaine effect, and the prolongation of activity had a slow onset. The effects of procaine were rapidly reversed on washing.

INTERACTIONS OF CAFFEINE AND ISOPROTERENOL

Catecholamines cause a substantial abbreviation of the active state of heart muscle. Gibbs has reported (28) that this effect is prevented by caffeine. The following experiments were done to examine this point in more detail. Dose-response curves for L-isoproterenol were determined both before and after the addition of various concentrations of caffeine to the organ bath, and superimposed photographs of high-speed tracings of isometric contractions were taken at each dose level. Full dose-response relations for isoproterenol were determined in eight preparations, and then repeated in the presence of 2, 4, 6, 10, or 20 mM caffeine (one or two concentrations of caffeine per preparation). Representative results are shown in Figure 13. Even the lowest concentration of caffeine consistently prevented the abbreviation of the active state by isoproterenol (Fig. 13B). Isoproterenol still increased dP/dt (except sometimes at the higher caffeine concentrations) but, in contrast to the normal situation, relaxation was progressively delayed. The result of these changes is that instead of being sharpened by isoproterenol, as it is normally, the peak of the contraction becomes broadened, sometimes into a virtual plateau. Indeed, instantaneous force-velocity curves determined by the isotonic release technique in a preparation in which a virtual plateau had been produced were practically superimposable at 200, 300, 400, and 500 msec after stimulation (A. Clark and C. B. Olson, unpublished experiments).

In any given preparation, the effect of isoproterenol on the contraction curve was regularly diminished as the caffeine concentration increased, and in two preparations isoproterenol had no discernible effect in the presence of 20 mM caffeine (Fig. 13D). The ability of isoproterenol to abbreviate the contraction returned rapidly when the preparation was washed free of caffeine.

COMPARISON OF THE ACTIONS OF CAFFEINE AND STRONTIUM

Strontium ions have long been known to prolong the contractions of cardiac muscle (52-60). To test the specificity of caffeine’s antagonism of the abbreviation of the active state by catecholamines, we compared the actions of strontium and caffeine on three papillary muscles.

Dose-response curves to Sr$^{2+}$ (as Sr(NO$_3$)$_2$) were determined in phosphate-free physiological salt solution containing the usual concentration of Ca$^{2+}$ (4.5 mEq/liter). The results in all experiments were like those shown in Figure 14B. The effects of strontium ions resembled those of the methylxanthines in all respects except that strontium did not slow the onset of contraction. Since such a slowing was strikingly apparent in the tracings of Thomas (54), Reiter (57), and de Hemptinne et al. (59), we did several additional experiments in calcium-free solutions like theirs. The slowed onset was then clearly apparent, though a great prolongation of the refractory period of the muscle prevented studies at the usual frequency of contraction unless small amounts of calcium remained in the bath.

Isoproterenol was still able to shorten the time course of contraction in muscles exposed to 20 mM Sr$^{2+}$ (Fig. 14C). This concentration...
Influence of caffeine on inotropic effect of isoproterenol. Isometric contractions of kitten papillary muscles driven at 5-second intervals. Cumulative dose-response relation for l-isoproterenol in each photograph: 0, 10^-11, 10^-10, 10^-9, 10^-8, 10^-7, 10^-6 M. A, B, and C from same muscle; length, 4.8 mm; weight, 1.41 mg. D from different muscle; length, 4.2 mm, weight, 2.35 mg. The two muscles had almost identical control responses to isoproterenol; 32.5°C.

Discussion

COMPLEXITIES IN THE MEASUREMENT OF MYOCARDIAL CONTRACTILITY

The rather complicated actions of the methylxanthines on the mechanical properties of cardiac muscle illustrate well the difficulty of describing a change in myocardial contractility in simple terms. Results such as these have led us to believe that no simple, universally applicable index of contractility is ever likely to be devised. In particular, the inadequacy of describing the action of a drug only as a positive or negative inotropic effect is evident, and the strikingly different effects of the catecholamines and the methylxanthines on the contractions of heart muscle make it clear that the mechanism of action of the methylxanthines is far more complicated than the proponents of the cyclic AMP hypothesis (see Introduction) must have originally supposed.

In recent years more sophisticated approaches to the study of cardiac muscle mechanics have led to the widespread use of the force-velocity curve as an index of myocardial contractility. Such curves have most commonly been constructed from measurements of shortening velocity in simple afterloaded contractions in which the muscle develops tension isometrically up to a level determined by the afterload, and then shortens isotonically. In these contractions muscle shortening begins progressively later the greater the afterload, with the result that the velocities corresponding to different afterloads are necessarily measured at different times.
Comparison of the actions of caffeine and strontium ion. Isometric contractions of kitten papillary muscles driven at 5-second intervals. A: Cumulative dose-response relation for caffeine at concentrations (mM) shown on curves. B: Cumulative dose-response relation for Sr\(^{2+}\) at mM concentrations shown on curves. C: Cumulative dose-response relation for isoproterenol in the presence of 20 mM Sr\(^{2+}\): 0, 10\(^{-7}\), 10\(^{-6}\), 10\(^{-5}\), 10\(^{-4}\), 10\(^{-3}\), 10\(^{-2}\), 10\(^{-1}\) M isoproterenol. A and B from same muscle (length, 6.0 mm; weight, 2.03 mg) which had been exposed to propanolol and atropine in earlier experiments; C from different muscle (length, 4.2 mm; weight, 0.86 mg); 32.5°C.

during the active state. Since in heart muscle the intensity of the active state appears to change continually throughout the contrac-
tion, the various points on the force-velocity curve measured in this way correspond to different levels of activity. One result of this is that the shape of the curve has little fundamental significance (and estimates of V\(_{max}\) determined by extrapolation of the curve to the velocity axis have correspondingly less).

Another is that interventions which change the time course of activity may appear to alter the shape of the curve. The action of caffeine illustrates this point strikingly, as is shown in Figure 10B. Instantaneous force-velocity curves, as determined by the delayed isotonic release method (Fig. 10A), are not without their problems (47) but serve to show that the basic shape of the force-velocity curve is probably not altered by caffeine.

**PROBABLE BASIS FOR THE ACTION OF CAFFEINE IN SKELETAL MUSCLE**

Important clues to the mechanism of action of caffeine can be gained from detailed studies of high-speed tracings of contraction curves, as has been shown in painstaking studies on skeletal muscle by Sandow and his colleagues (review, 16-18). They have developed a most attractive framework of hypothesis within which to analyze these results, and we have found it instructive to try to fit our observations on heart muscle into that framework. Briefly, the analysis is as follows: Caffeine produces two major effects on skeletal muscle: twitch potentiation and contracture. In the frog sartorius muscle, twitch potentiation occurs at caffeine concentrations above about 0.1 mM and results primarily from a prolongation of the active state; tetanus tension is not affected (at least not at muscle lengths above the optimal [61]). Close scrutiny of high-speed tracings of isometric (12) and isotonic (9) contractions has revealed, however, that although the time to onset of mechanical activity is not changed, the early rate of tension development (dP/dt) or of isotonic shortening is increased by caffeine. Contracture occurs in frog muscle at concentrations above 2 to 5 mM, and increases with concentration. Twitch potentiation still occurs, but decreases as the contracture increases (12).
The mechanical threshold (the membrane potential beyond which depolarization produces a detectable mechanical response) is reduced (made more negative) by caffeine (8, 15, 41, 62, 63). Sandow (16–18) interprets these various observations in terms of the demonstration (1–7) that caffeine impairs the ability of the sarcoplasmic reticulum to sequester calcium and causes the release of calcium from the reticulum. This is believed to increase the concentration of free Ca²⁺ in the sarcoplasm of the unstimulated muscle. If the concentration is above the threshold level for the development of tension, contracture results. If it is below the threshold, only twitch potentiation occurs.

Twitch potentiation might result in part from the fact that the calcium released by the action potential is superimposed upon an increased initial level in the sarcoplasm. Sandow has pointed out to us that even subthreshold changes in the calcium concentration at rest could have substantial effects on the dynamics of the twitch because in the resting muscle there is far more calcium bound to the contractile proteins than free in the sarcoplasm, and about half the amount required for mechanical saturation is bound at the mechanical threshold (18, 64). In addition, an important factor contributing to twitch potentiation could be the decreased ability of the sarcoplasmic reticulum to compete with the contractile proteins for released calcium from the very beginning of the active state (15). Either or both of these factors could account for the early increase in dP/dt and shortening velocity induced in frog skeletal muscle by caffeine. According to Sandow’s hypothesis, the duration of the plateau of the active state in skeletal muscle is increased for two reasons: (1) because peak calcium levels are higher, more calcium must be sequestered by the sarcoplasmic reticulum before the intensity of the active state falls below its plateau level, and (2) the process of sequestration of calcium is slowed by the action of caffeine on the sarcoplasmic reticulum.

An observation that may force modification of this simple and attractive hypothesis is that although procaine antagonizes the ability of caffeine to produce contracture and also the effect of caffeine on calcium binding by isolated vesicles of sarcoplasmic reticulum (2, 3, 5, 7), it has been reported not to prevent the alteration of the mechanical threshold by caffeine (15). The implication of this observation is that the lowering of mechanical threshold by caffeine is not related to the postulated effect of caffeine on the sarcoplasmic reticulum. Thus it may be necessary to assume that caffeine exerts two separate actions on excitation-contraction coupling in skeletal muscle, one of which is procaine-sensitive and the other procaine-insensitive. This matter will be considered later in more detail.

**DIFFERENCES BETWEEN THE CONTRACTIONS OF CARDIAC AND SKELETAL MUSCLE**

We will attempt to compare the actions of caffeine on the contractions of skeletal and cardiac muscle within the framework of Sandow’s hypothesis. To do this, it will be helpful first to consider the probable basis for some important differences between the skeletal muscle twitch and the contraction of cardiac muscle. (1) In cardiac muscle the rise of the active state appears to be considerably less abrupt than in skeletal muscle (65, 66). (2) The tension developed per unit of cross-sectional area is usually somewhat greater in skeletal than in cardiac muscle, even when correction is made for actomyosin content (66). (3) The ability to bear tension rises to a plateau in the skeletal muscle twitch (65), whereas it normally does not in cardiac muscle (36, 66). (4) The level of the plateau in skeletal muscle cannot readily be altered (16), whereas the peak intensity of the active state in cardiac muscle is influenced profoundly by many factors. (5) Factors that increase the peak intensity of the active state in cardiac muscle tend as a rule slightly to prolong the plateau of the active state in skeletal muscle (16). All of these differences fall into place behind the assumption that during activity the myoplasmic calcium concentration rises far above the level required to saturate the contractile apparatus in skeletal muscle, but
normally does not reach that level in cardiac muscle. This assumption, plus the generally shorter time course of contraction in skeletal muscle, requires the existence of a far more active mechanism for calcium release and sequestration in skeletal muscle, and this is consistent with the presence of a more highly developed sarcoplasmic reticulum in skeletal than in cardiac muscle (67, 68).

**EFFECTS OF THE INHIBITION OF CALCIUM SEQUESTRATION: A MODEL**

Let us now consider the five effects of the methylxanthines within the framework of the hypothesis just discussed. On a qualitative level, and with certain assumptions, it is possible to interpret all five effects as deriving from the inhibition of calcium sequestration. The assumptions are that (1) calcium released by the action potential must diffuse through a zone in which it is subject to sequestration before it reaches the myofilaments; (2) the calcium "sinks" are active at all times, and are not "turned on" to initiate relaxation; and (3) the inhibition of sequestration leads to a decrease in the amount of calcium released by the action potential. The last might be expected if the inhibition of sequestration led to a depletion of releasable intracellular calcium stores.

Figure 15 shows an analog model that may be helpful in the visualization of what might be expected if the amount of calcium released and the rate of its subsequent sequestration were both reduced, but to varying relative degrees. The model does not take into consideration the time over which calcium release occurs (release is treated as occurring instantaneously), nor does it have any provision for changes in the amount of calcium required to reach the mechanical threshold.

**Circulation Research, Vol. XXX, April 1972**
Despite these omissions, it does provide a qualitative illustration of a possible basis for most of the effects of the methylxanthines on the cardiac muscle twitch. It is intuitively obvious that the inhibition of calcium sequestration will lead to an increase in time to peak tension and in the total duration of contraction. It is also fairly obvious that it will lead to an increase in peak tension unless the release of calcium is also substantially reduced. The maximum rate of rise of tension will also be increased if release is affected relatively little (Fig. 15A). The model is probably most useful in illustrating a possible basis for the slowed onset of contraction after caffeine (Fig. 15B and C).

It is clear from the action of Sr\(^{2+}\) in the presence of Ca\(^{2+}\) that marked prolongation of activity can be achieved without any slowing of the onset of contraction. According to the scheme just discussed, the effect of Sr\(^{2+}\) in the presence of a normal Ca\(^{2+}\) concentration is of the type that would result from a simple decrease in the rate of Ca\(^{2+}\) sequestration. (If Ca\(^{2+}\) sequestration takes place throughout the active state, an increase in dP/dt and peak tension would be expected on the basis of slowed sequestration alone. This could result if Sr\(^{2+}\) inhibited the uptake of Ca\(^{2+}\) into the sarcoplasmic reticulum but did not displace calcium to an appreciable degree from the sites of release.) A quite different effect is produced by Sr\(^{2+}\) when calcium is removed from the bathing medium. Heart muscle ceases to develop tension in the absence of Ca\(^{2+}\), but contraction is restored by the addition of Sr\(^{2+}\) (53, 54, 57, 59). It seems reasonable to suppose that in this circumstance Sr\(^{2+}\) substitutes for Ca\(^{2+}\) in the activation process in cardiac muscle as it does in skeletal muscle (69), and therefore it is not surprising that all the kinetics of activation are greatly altered.

**THE PROCAINE-INSENSITIVE EFFECT**

One major observation is not readily accounted for within the foregoing framework of hypothesis; it is that procaine antagonizes the prolongation of the active state by caffeine but not the intensification. This would imply that 2 mM procaine prevents caffeine's inhibition of Ca\(^{2+}\) sequestration in the living cardiac muscle cell, but leaves intact some other effect of caffeine that leads to an intensification of the active state. The rapid onset of the effect and the fact that contracture is not normally produced by even very high caffeine concentrations make us reluctant to attribute the effect to subthreshold changes in sarcoplasmic calcium concentration. Such a mechanism for the procaine-insensitive effect in heart muscle seems even less likely in the light of the fact that it is the presumptive basis of the procaine-sensitive effect of caffeine on skeletal muscle. It is tempting to relate the procaine-insensitive effect in cardiac muscle to the report by Taylor et al. (15, 70) that procaine does not prevent the lowering of the mechanical threshold by caffeine in skeletal muscle, though evidence is lacking on this point in the case of cardiac muscle.

Since caffeine has been found not to sensitize the contractile apparatus to calcium (71), an effect on calcium release would seem the most likely mechanism for the procaine-sensitive effect. Perhaps caffeine, in addition to its action on calcium sequestration, has an effect that tends to increase the amount of calcium released into the sarcoplasm by the action potential. Whether this tendency actually results in increased calcium release might depend on the extent to which releasable stores of calcium are depleted by the concurrent impairment of calcium sequestration. In other words, the two postulated effects of caffeine normally might tend to counteract one another as far as the release of calcium is concerned. If this occurs, one would expect the effect of caffeine on calcium release to become unmasked when sequestration is no longer impaired, as in the presence of procaine. This could explain the increase in dP/dt and tension development induced by procaine in experiments like that shown in Figure 12B.

The antagonistic effects on calcium release of the two postulated actions of caffeine could also explain the biphasic nature of the onset of action of caffeine on tension development.
EFFECT OF CAFFEINE ON HEART MUSCLE

The initial rapid increase in dP/dt and peak tension may be presumed to represent the onset of the procaine-insensitive effect (see Results). If this effect is in fact a manifestation of increased calcium release, the subsequent decrease in tension development might reflect a decrease in releasable stores stemming from the inhibition of sequestration. The final gradual increase in tension development could then result from a progressive dominance of the effects of impaired calcium sequestration over those of decreased release. The slow onset of the procaine-sensitive effect of caffeine is consistent with an action on the sarcoplasmic reticulum; the more rapid onset of the procaine-insensitive effect suggests a more superficial site of action such as the cell membrane (see below).

SOURCE OF CALCIUM FOR EXCITATION-CONTRACTION COUPLING

We have suggested that the procaine-insensitive effect of caffeine may represent an increase in the amount of calcium released into the sarcoplasm by the action potential. In principle, this calcium could be derived from intracellular stores, it could enter the cell during the action potential, or both. There is evidently a good deal of species variation in the relative importance of these two sources (72). Beeler and Reuter (73) have shown that in dog ventricular muscle, the calcium entering during a given action potential has relatively little effect on the contraction triggered by that action potential. Its chief effect is on the strength of subsequent beats, presumably through an influence on the size of intracellular stores of calcium available for release by subsequent action potentials. Similar observations (G. W. Beeler, Jr., unpublished) have been made on the cat papillary muscle. In that tissue, though over a certain range the duration of the action potential may influence the time to peak tension of the corresponding contraction somewhat (72, 74, 75), the chief effect of an imposed change in action potential duration is on the strength of subsequent beats. Thus the calcium released from intracellular stores by an action potential appears to play a much greater role in excitation-contraction coupling than does the calcium entering the cell during that action potential (72, 75, 76). The amount of calcium released from intracellular stores appears highly dependent on the extent to which these stores have been precharged with calcium, but it is probably not important whether the calcium involved enters during the action potential or at rest.

One might speculate then that the procaine-insensitive effect of caffeine stems ultimately from an effect on the cell membrane that leads to increased calcium influx at rest, during activity, or both. Caffeine in concentrations as low as 1 mM is known to stimulate calcium influx in the resting frog sartorius muscle (14, 77). Nayler (78) has shown a similar effect in the quiescent ventricle of the toad. Caffeine prolongs the plateau phase of the action potential in mammalian atrial (27) and ventricular (79) muscle, and it seems quite probable that the prolongation is associated with an increased inward calcium current. Thus there is ample evidence to suggest that caffeine has one or more actions on the cell membrane that facilitate the entry of calcium into the cell. There is, unfortunately, no information about the procaine sensitivity of the action or actions. The rapid onset of the procaine-insensitive effect of caffeine is consistent with the notion that it reflects an action on the surface membrane. The fact that the first contraction after the addition of caffeine is substantially augmented suggests that if increased calcium entry is involved, it is not limited to the period of the action potential, but occurs during rest as well.

INTERACTIONS WITH CATECHOLAMINES

Similarities.—We have already noted certain similarities between the procaine-insensitive effect of caffeine and the effect of the catecholamines on myocardial contraction, and have suggested that the former might reflect an increase in the amount of calcium that is released into the sarcoplasm in response to the action potential. It is reasonable to suppose that the catecholamines also increase the release of calcium that is triggered by the action potential, and it is in...
the context of this effect that a role for the involvement of cyclic AMP in the action of caffeine (see Introduction) might best be considered. Rasmussen (80) has recently speculated on the relation between cyclic AMP and calcium ion in the regulation of various cellular responses. Mayer (81) has reviewed the evidence for the idea that changes in intracellular levels of cyclic AMP may be responsible for inotropic effects. Though there is no evidence so far that cyclic AMP has anything directly to do with muscular contraction, the possibility must be considered that the catecholamines and caffeine might exert a similar effect on the release of calcium by the action potential through this or some other common pathway. Certainly caffeine does not exert its procaine-insensitive effect through the release of norepinephrine or through a direct effect on the β-receptor, since the effect is undiminished in the presence of 0.6 μM propranolol. If the procaine-insensitive effect results from the inhibition of phosphodiesterase, then theophylline ought to be substantially more potent than caffeine in eliciting it, since theophylline is the more potent phosphodiesterase inhibitor (20). Our results to date do not suggest that this is so, though experiments with theophylline have not been carried out in the presence of procaine.

Differences.— Though caffeine and the catecholamines seem to have one effect (an increased degree of activation) on cardiac contraction in common, and low concentrations of theophylline do potentiate the inotropic effect of norepinephrine (25), there is no solid evidence at present to indicate that these interactions are more than fortuitous. We are more impressed by the opposite effects of the methylxanthines and the catecholamines on the relaxation phase of cardiac contraction. Catecholamines are known to decrease the time to peak tension and accelerate relaxation in cardiac muscle. These actions can plausibly be attributed to a stimulation of calcium sequestration (82–84) and Entman et al. (85) have reported that 1 μM epinephrine stimulates the uptake of calcium by a microsomal preparation from cardiac muscle. Results with other catecholamines have been less in accord with this interpretation, however. In another cardiac microsomal preparation, Shinebourne et al. (86) found norepinephrine to be effective only in very high concentrations (1mM), but White and Shinebourne (87) found no consistent effect of isoproterenol on calcium uptake.

Antagonism.— We were surprised to find that the abbreviation of the active state by isoproterenol is totally prevented by even low concentrations of caffeine; this antagonism cannot be surmounted by high concentrations of isoproterenol, and thus stands in sharp contrast to the limited antagonism of caffeine toward the effects of high frequency, low fiber length, and increased temperature on the time course of contraction. The fact that Sr2+ does not prevent the abbreviation of the active state by isoproterenol indicates that the effect of caffeine cannot be explained simply on the basis of its prolonging the active state. Nor can it be argued that caffeine, in contrast to Sr2+, prolongs the active state in some insurmountable way, because increasing frequency is still able to abbreviate contractions in the presence of high concentrations of caffeine. These observations suggest the existence of a relatively specific antagonism by caffeine toward at least certain of the effects of the catecholamines. As has been reported elsewhere (88), this antagonism extends to the positive chronotropic effect of isoproterenol. However, caffeine antagonizes the positive chronotropic effects of other substances as well (88), so the antagonism presumably does not occur at the level of the adrenergic receptor.

Contracture and Tetanus

The prominent contracture produced by caffeine in frog skeletal muscle has been studied so widely that contracture has come to be commonly regarded as the expected outcome of the exposure of a muscle to caffeine. It is less widely appreciated that there are considerable differences in the susceptibility of various types of skeletal muscle to caffeine contracture (89, 90) and
EFFECT OF CAFFEINE ON HEART MUSCLE

that the sensitivity of a given muscle is readily modified by experimental conditions (90-93). Specifically, gradually cooling rat skeletal muscle from body temperature to 20°C reduces the susceptibility to contracture (90, 92), and cooling from 37° to 25°C markedly decreases the effectiveness of caffeine in the inhibition of calcium sequestration by preparations of isolated sarcoplasmic reticulum from rabbit skeletal muscle (6). It was for this reason that we studied the effect of caffeine at 38°C as well as at lower temperatures. The fact that we did not observe contracture except under certain extreme conditions may be presumed to indicate that the calcium-sequestering mechanism of cat myocardium is able to keep the sarcoplasmic calcium concentration below the threshold for tension development even in the face of considerable inhibition by caffeine. In this respect cat heart muscle may not be qualitatively different from other muscles; rather, it probably lies toward one end of a spectrum of sensitivities.

High contraction frequency appears to be a most potent stimulus to the calcium-sequestering mechanism, and at normal calcium concentrations it is possible to practically overcome the effect of 6 mM caffeine by driving the muscle at high frequencies (Fig. 6). Nevertheless, if the caffeine concentration is increased further, or if the extracellular calcium concentration is increased in the presence of 6 mM caffeine, even this stimulus to rapid relaxation is no longer sufficient to prevent extensive fusion of contractions when the muscle is driven at high frequencies. By pushing this phenomenon to the extreme, one can produce complete or almost complete fusion of contractions, and this is the basis of a method for tetanizing heart muscle recently employed in Sonnenblick’s laboratory (94).

References


EFFECT OF CAFFEINE ON HEART MUSCLE


Influence of Caffeine and other Methylxanthines on Mechanical Properties of Isolated Mammalian Heart Muscle: EVIDENCE FOR A DUAL MECHANISM OF ACTION
JOHN R. BLINKS, CAMILLE B. OLSON, BRIAN R. JEWELL and PAVEL BRAVENÝ

doi: 10.1161/01.RES.30.4.367

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/30/4/367

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/