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THE EFFECTS OF CAFFEINE ON THE CONTRACTION OF THE FROG HEART

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SUMMARY

1. The force of contraction and the membrane potentials have been measured from preparations of frog heart, with methods that also allow the rapid exchange of the extracellular fluid.

2. In regularly beating preparations, caffeine induces only weak contractures at temperatures above 15° C, but it does cause a marked potentiation of the twitch responses; longer exposure results in a depression of contraction. The build up and decline of the twitch strength, on addition and on removal of caffeine approximates to a single exponential, time constant 26–45 sec, and this time constant is not altered by variation of the $[Ca]_o$, the stimulus rate or the caffeine concentration. This time course of the change of twitch strength is less complex than the changes seen when either $[Ca]_o$ or the stimulus rate is altered, suggesting a more direct action of caffeine on excitation-contraction coupling.

3. Caffeine increases the strength of the contractures initiated by potassium-rich or sodium-depleted solutions in isolated atrial trabeculae.

4. After the spontaneous relaxation of the contracture, evoked by either sodium-free or potassium-rich fluids, the application of caffeine initiates a redevelopment of tension. This caffeine contracture is transient and its strength is dependent on the caffeine concentration. The response in sodium-free solution can be elicited in the virtual absence of extracellular calcium.

5. Local anaesthetics antagonize the caffeine contracture.

6. The results suggest that the sarcoplasmic reticulum of frog heart muscle plays an important role in the initiation and control of contraction and relaxation.

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INTRODUCTION

Caffeine potentiates the twitches and initiates contractures in a variety of skeletal muscles. Its effect on contraction is likely to be exclusively due to the action of caffeine on the sarcoplasmic reticulum to raise the sarcoplasmic calcium concentration (Weber & Herz, 1968), because caffeine contractures can be evoked in the absence of extracellular calcium (Frank, 1962), and the alkaloid does not influence the calcium binding of isolated mitochondria or sarcolemmal vesicles (Weber, 1968; Thorpe & Seeman, 1971) or affect the contractile proteins (Korey, 1950; Hasselbach & Weber, 1955).

In smooth muscle caffeine has a potentiating action upon the spontaneous contractions and sometimes it induces contractures; however, longer exposures result in the depression of contraction. Caffeine will also antagonize the development of the potassium contracture (Ito & Kuriyama, 1971; Sunano & Miyazaki, 1973; Osa, 1973; Magaribuchi, Ito & Kuriyama, 1973). Although caffeine does influence the electrical properties of the cell membrane, the dual effect of caffeine on contraction is explained by assuming that the potentiation is a result of calcium release from the sarcoplasmic reticulum and that the rapid depletion of the calcium reserves, due to the paucity of the sarcoplasmic reticulum in these muscles, brings about the later depression and the antagonism of the potassium contracture (Magaribuchi *et al.* 1973).

In cardiac muscle the situation is not as clear as in skeletal muscle, for although caffeine does affect the contraction of frog and mammalian heart, reasons other than an effect on the sarcoplasmic reticulum have been proposed to account for this action (Suzuki, 1962; de Gubarref & Sleator, 1965; Blinks, Olson, Jewell & Braveny, 1972; Kimoto, 1972). Other structures, apart from the sarcoplasmic reticulum, have been proposed as the relaxing system in heart muscle (Nayler & Hasker, 1966; Carafoli, Patriarca & Rossi, 1969). In mammalian heart, the increase in the duration of the action potential has been considered to underlie the potentiation of the heart beat that occurs on exposure to caffeine. Moreover, Morad & Orkand (1971) propose that the contraction of frog heart is activated solely by the calcium entering from the bathing medium during the action potential, and they refer to the sparsity of the sarcoplasmic reticulum, in this tissue, to support their idea (Staley & Benson, 1968).

The dependence of the contraction of the frog heart on the bathing calcium concentration has been investigated using methods that reduce the clearance time of the extracellular space to a minimum. This work suggests that the calcium which activates contraction originates from two, if not three sites, one of which at least is intracellular (Chapman &

Niedergerke, 1970*a*,*b*; Chapman & Tunstall, 1971; Chapman, 1973*a*,*b*; 1974). In this and the following paper, these methods have provided a means of assessing the action of caffeine on frog ventricle and auricle preparations in an attempt to estimate the physiological significance of the sarcoplasmic reticulum in this muscle without the ambiguity of long diffusion delays.

Preliminary reports of part of this work have already been published (Chapman & Miller, 1971, 1972).

METHODS

The preparations were taken from the hearts of healthy frogs of the species *Rana pipiens* kept in running water at 4° C. The frogs were killed by pithing the central nervous system, either directly or after a few days at the experimental temperature; no difference was noted in the response of frogs treated in either way.

Two preparations of frog ventricle have been used. The first, the superfused half-ventricle preparation of Lamb & McGuigan (1966) as adapted by Chapman & Niedergerke (1970*a*), provided the means of studying the action of caffeine on the twitch responses of the heart. Further modifications were made, so that the experimental solutions were driven by a constant gas pressure (oxygen, air or an oxygen-carbon dioxide mixture), from closed reservoirs. The gas also ventilates the solution. A two-way tap allowed unused solutions to run to waste before and after they were switched to flow over the preparation. The force generated by the half-ventricle was measured by a semiconductor force transducer (Devices ST 102), and the maximum rates of tension changes were obtained by electronic differentiation. The criteria proposed by Chapman & Niedergerke (1970*a*) for the acceptability of the results have been rigorously applied. The second type of preparation was a strip cut from the upper ring of the ventricle less than 300 μm in diameter and up to 3 mm long. This strip was mounted and perfused in the way described for isolated atrial trabeculae (Chapman & Tunstall, 1971). This preparation was used for the measurement, with conventional 3 M-KCl-filled glass micro-electrodes, of the intracellular resting and action potentials. The force generated by the strip was simultaneously measured with a semiconductor force transducer.

The method for trabeculae isolated from the atrium of the frog's heart was essentially the same as that described by Chapman & Tunstall (1971). The method was modified as described in Chapman (1973*a*). The criteria for accepting results for quantitative assessment are those suggested by Chapman & Tunstall.

In each preparation pacemaker tissue was removed and the heart stimulated electrically via platinum electrodes adjacent to the preparation. Hypodynamia was allowed to develop before quantitative measurements were made (Chapman & Tunstall, 1971). At the end of each experiment, the piece of tissue was drained and then weighed on a torsion balance, so that the force exerted by the muscle could be expressed as a function of its wet weight.

The composition of the perfusing solutions is shown in Table 1. Calcium was added as 1 M-$CaCl_2$ solution (B.D.H. volumetric standard) while the caffeine was added as a solid, without adjustment for the small change in tonicity that results. Some caffeine was purified by precipitation with ether from saturated chloroform solution. No differences between the responses of the heart caused by exposure to either type of caffeine were noted.

All measurements were made from traces of a U.V. recorder, a pen recorder or from oscilloscope film. All were subjected, where necessary, to regression analysis on a Wang programmable desk calculator, to give an equation for the regressed line,

TABLE 1. Composition of the physiological solutions

(*a*) Ventricle experiments

Solution	NaCl (mM)	KCl (mM)	Na_2HPO_4 (mM)	NaH_2PO_4 (mM)	$NaHCO_3$ (mM)	pH	Ventilating gas
Phosphate Ringer	117·0	3·0	0·8	0·2	—	7·3	O_2
Low bicarbonate Ringer	115·0	3·0	—	2·97	2·38	7·3	Air
High bicarbonate Ringer	94·0	3·0	—	2·97	23·80	7·3	5% CO_2 95% O_2

(*b*) Auricle experiments

Solution	NaCl (mM)	KCl (mM)	TrisHCl (mM)	NaH_2PO_4 (mM)	Na_2HPO_4 (mM)	Glucose (mM)	pH	Gas
Phosphate Ringer	117·0	3·0	—	0·2	0·8	5	7·3	O_2
Tris Ringer	117·0	3·0	2·0	—	—	5	7·3	O_2
Sodium-free Ringer	—	3·0	126·8	—	—	5	7·3	O_2
High potassium Ringer	117·0	100·0	2·0	—	—	5	7·3	O_2

the coefficient of correlation and the standard error of the points about the regressed line.

All experiments were carried out in a room cooled to between 15 and 22° C with a fluctuation of less than one degree during an experiment.

RESULTS

EXPERIMENTS ON VENTRICLE MUSCLE

The effect of caffeine on the resting and action potentials of frog ventricle

The simultaneous recording of the membrane potentials and tensions generated by ventricle strips shows that although the twitch tension increases there is no significant change in the action potential when caffeine is added to the bathing medium (Table 2). The duration of the action potential shortens slightly during the first 15–30 sec of perfusion with caffeine (Fig. 1*a*). Shortening of the action potential duration will tend to reduce the twitch tension, when all other factors are unchanged (Morad & Trautwein, 1968), therefore the potentiation of the contraction caused by caffeine would seem in this respect to be different to that described for mammalian heart (Blinks *et al.* 1972). This view is supported by the observation that perfusion with an inactive analogue of caffeine, 1,9-dimethylxanthine also causes a slight shortening of the action potential, but does not affect the contraction of the heart (Fig. 1*b*).

The effects of caffeine on the beating ventricle

The addition of caffeine to a solution bathing a regularly stimulated preparation causes a progressive increase in the strength of the twitch response. To avoid complications in the analysis of this response due to the saturation of the contractile response or other non-linearities on the addition of caffeine, a bathing calcium concentration was chosen so that the response of the heart was close to the bottom of the linear part of the

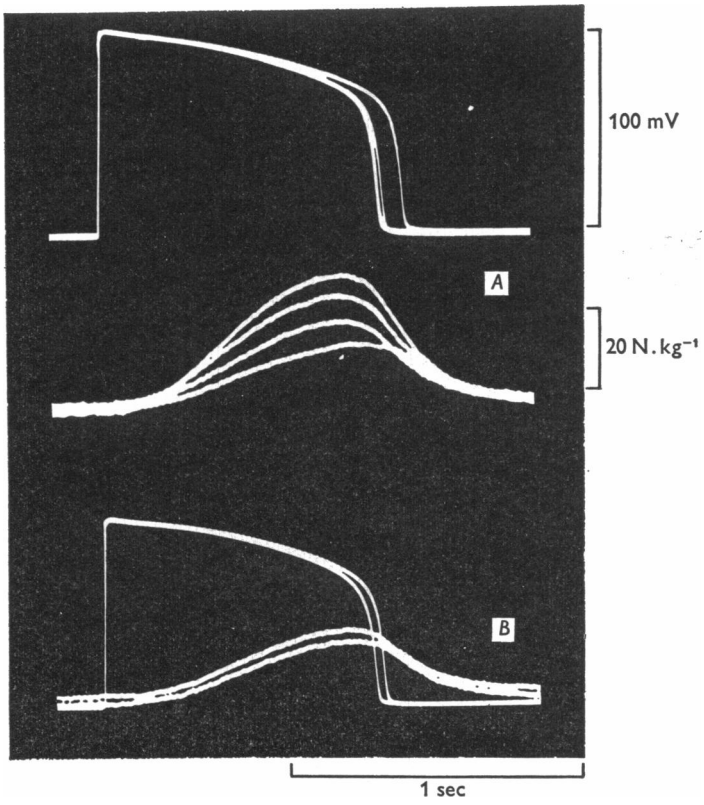

Fig. 1. The influence of methylxanthines on the twitch and action potential recorded from a strip of ventricle.

A, four superimposed action potentials (upper trace) and the accompanying twitches (lower trace) recorded immediately before and then the first, second and fourth after the inclusion of 5 mM caffeine in the bathing solution.

B, an action potential and the twitch response before and after the application of 1,9-dimethylxanthine are superimposed. The tension trace was purposely displaced to show that this methylxanthine, unlike caffeine, did not potentiate the strength of contraction. Phosphate Ringer, 0·25 mM calcium; stimulus rate 4 min^{-1}; 22·0° C.

calcium dose–tension curve (cf. Chapman & Niedergerke, 1970*a*). Under these conditions, the increase of the twitch response reaches a maximum after about 3 min, and subsequently the twitch strength falls, stabilizing after about 20 min, above, at or even below the pre-caffeine level. Removal of the alkaloid always causes a rapid decline in the twitch strength to

TABLE 2. The effects of caffeine on the resting and action potentials of frog ventricle muscle cells

	0·25 mM-Ca Ringer	0·25 mM-Ca Ringer + 5 mM caffeine
Resting potential	77·1 ± 5·8 mV (48)	78·6 ± 5·2 mV (14)
Action potential	104·7 ± 4·1 mV (48)	105·9 ± 4·6 mV (14)
Mean overshoot	27·6 mV	27·3 mV
Duration	1·00 ± 0·11 sec (48)	0·96 ± 0·09 sec (14)

The figures are the means with S.D., the numbers in parentheses show the total number of cells recorded from four preparations. The low $[Ca]_o$ in these experiments was chosen for two reasons, because it reduced the size of the twitch response, thereby making it easier to maintain the electrode within a cell while the heart was beating, and because at low $[Ca]_o$ the twitch response in caffeine is relatively larger (see Fig. 3*a*). Phosphate Ringer, 19·0° C.

below the pre-caffeine level, followed by a slower recovery back to the original tension. The time courses of the changes in the response of the muscle are always approximately symmetrical irrespective of the concentration of caffeine or the period of exposure: after a short application and hence a curtailed second phase of twitch tension decline, the recovery to the pre-caffeine level is also short. The effects of caffeine are therefore fully reversible.

When Ringer saturated with caffeine was applied to the ventricle ($\simeq$ 50 mM), only small contractures (5% of the maximum tension) developed at 20 °C. The depressive phase of caffeine's action varied from one preparation to another, but with the individual ventricle this depression did not depend on the heart rate or the $[Ca]_o$.

The time course of the response to brief exposures to caffeine

To avoid or reduce the influence of the depressive action of caffeine, in some experiments the alkaloid was removed as soon as the twitch responses had reached a steady level. Fig. 2*a* shows part of such an experiment. The rise and fall of the twitch tension (P_{max}) and the maximum rate of tension development ($[dP/dt]_{max}$), in response to the addition and removal of three different caffeine concentrations are illustrated. The changes in these features of the contraction, on addition and removal of the drug, are parallel and symmetrical.

The time course of the changes of P_{max} and $[dP/dt]_{max}$ caused by caffeine

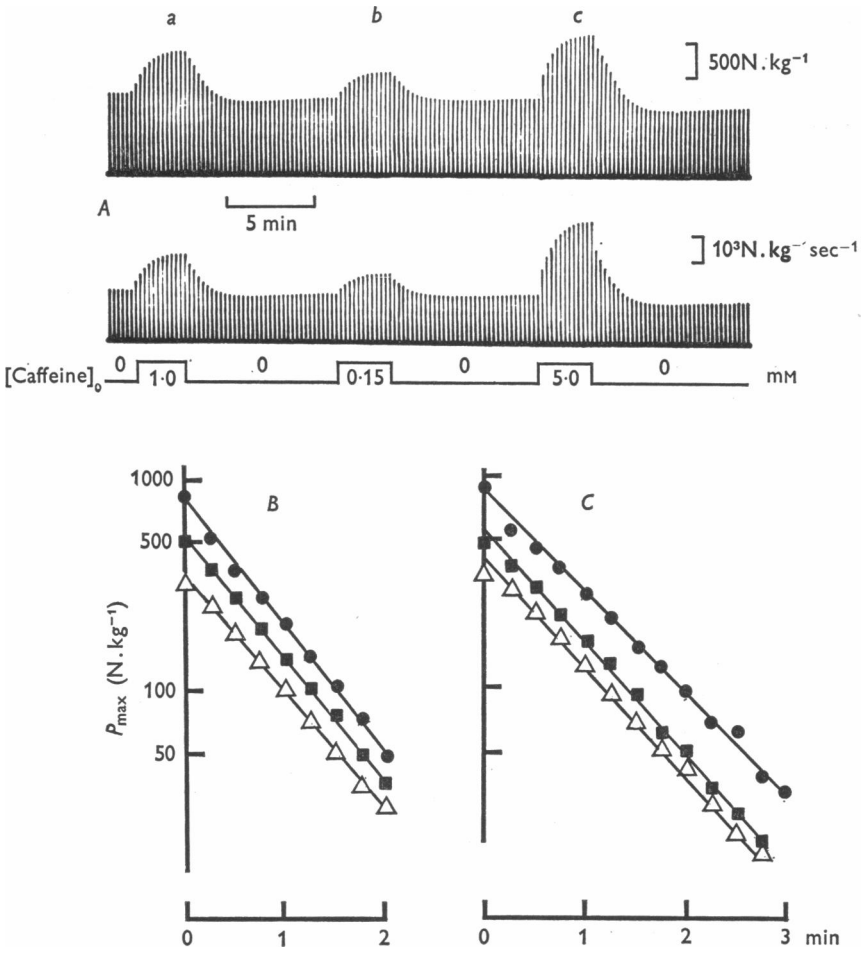

Fig. 2. The effects of different caffeine concentrations on a regularly beating ventricle preparation.

A, the upper trace shows the changes in the twitch tension (P_{max}), the middle trace the change in the maximum rate of tension development ($[dP/dt]_{max}$) when the heart was perfused by Ringer containing 1·0 mM caffeine (*a*), 0·15 mM caffeine (*b*) and 5·0 mM caffeine (*c*), as indicated by the lower trace.

B, semilogarithmic plots of the build up in the strength of the twitch response (P_{max}) caused by the application of caffeine as shown in part *A*, with 1·0 mM caffeine (■–■), 0·15 mM caffeine (△–△) and 5·0 mM caffeine (●–●).

C, the decline of P_{max} on semilogarithmic co-ordinates, on the removal of the caffeine from the bathing solution, (■–■) the decline after 1·0 mM caffeine, (△–△) the decline after 0·15 mM caffeine and (●–●) the decline after 5mM caffeine.

High-bicarbonate Ringer; 1 mM calcium; stimulus rate 4 min^{-1}; 20·0° C.

application and removal are generally well approximated by single exponential functions (Fig. 2*b*, *c*). The time constant of the change of the twitch tension is independent of the caffeine concentration up to about 10 mM. This time constant does not vary with the bathing calcium concentration (Table 3*a*) or with the rate of beating of the heart in the same or in different bathing calcium concentrations (Table 3*b*). A small difference in the time constants of the build up and decline of the twitch tension (Fig. 2) is always observed and is due to the small change in the time to peak of the twitch that invariably occurs in the presence of caffeine, as well as to the development and reversal of the depressive action of caffeine.

TABLE 3. Effect of $[Ca]_o$, stimulus rate and caffeine concentration on the time course of the response of frog ventricle to caffeine application

(*a*)

	[Caffeine]$_o$ mM		
	5	1	0·5
$[Ca]_o$ mM 0·5	37·8 (2)	41·2 (2)	37·2 (2)
$[Ca]_o$ mM 1·0	37·8 (2)	38·7 (4)	46·1 (4)
$[Ca]_o$ mM 2·0	40·0 (1)	32·5 (2)	45·6 (3)

The values shown in the Table are the mean time constants of the change or P_{max} in sec obtained from the semilogarithmic plots of the rise and fall of the twitch responses associated with the exposure of a half ventricle preparation to various doses of caffeine, at three outside calcium concentrations. The numbers in parentheses correspond to the total number of times the heart was exposed to that particular experimental condition. 20° C, stimulation rate 4 min^{-1}. High-bicarbonate Ringer.

(*b*)

Stimulus rate (min^{-1})	2	4	8	15
$[Ca]_o$	1·0	0·5	0·5	0·3
Time constant ± S.D. sec	33·8 ± 1·3	32·2 ± 1·3	32·4 ± 0·4	32·0 ± 3·9

The mean time constants for both the changes in P_{max} and $[dP/dt]_{max}$ shown in the Table, are for the build up and decline of the twitch response on the application and removal of 2 mM caffeine at four different stimulus rates (thirty-eight experimental changes in all). 20° C, high-bicarbonate Ringer.

The depressive action of caffeine is still apparent as evidenced by the biphasic nature of the time course of the twitch tension change when caffeine is removed. At caffeine concentrations above 10 mM the time to peak of the twitch shortens considerably so that the time course of the rise and fall of the twitch tension are no longer exponential, even at 5 mM some deviation from exponential is sometimes seen (Fig. 2*c*). By measuring the amplitude of the twitches at the peak of the potentiating phase, caused by the application of various caffeine concentrations, the relationship between

the caffeine concentration and the degree of twitch potentiation can be determined. Caffeine increased the twitch strength at all calcium concentrations, so long as the response of the muscle is submaximal, and therefore caffeine steepens the dose–response curve for calcium (Fig. 3*a*). The results can be expressed in another way which shows that the incremental twitch tension, due to the addition of caffeine, is related to the concentration of this agent by a rectangular hyperbola (Fig. 3*b*).

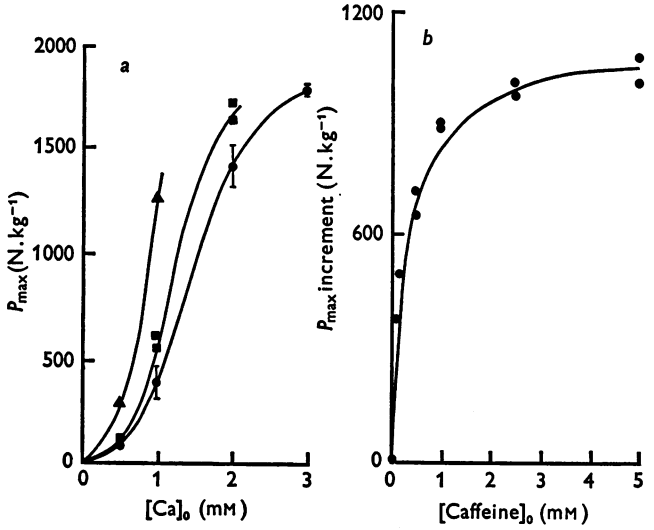

Fig. 3. *a*, dose–action curve for the twitch heights (P_{max}) against $[Ca]_o$, in the absence of caffeine (●–●), in the presence of 0·5 mM caffeine (■–■) and in the presence of 5 mM caffeine (▲–▲). The results in the absence of caffeine were obtained before and after each exposure to caffeine and the points represent the means and the vertical bars the S.D. of several determinations at each $[Ca]_o$. 20·0° C; stimulus rate 4 min^{-1}; ventricle preparation; phosphate Ringer.

b, the increment in the twitch response caused by exposure to caffeine is plotted against the caffeine concentration. 1 mM calcium; 20·0° C; stimulus rate 4 min^{-1}; ventricle preparation; phosphate Ringer.

Comparison of the changes of twitch responses induced by caffeine with those caused by changing the $[Ca]_o$ and the stimulus rate

The experiments described above show some similarities in the action of calcium and caffeine on the strength of the twitch response.

The time course of changes in the strength of twitch responses associated with both the alteration of the bathing calcium concentration and the frequency of beating of the heart, has been studied in some detail in both frog ventricle and auricle (Chapman & Niedergerke, 1970*a*, *b*; Chapman, 1971). In the present experiments, performed at a lower temperature than

those of Chapman & Niedergerke, three sequential exponential phases of twitch tension change are recognized when the $[Ca]_o$ is reduced and two phases when the heart rate is lowered (Fig. 4). These results, like those reported for atrial preparations (Chapman, 1971), mean that the change of the twitch response when the calcium concentration is reduced can be fitted by eqn. (1).

$$\psi_u - \psi_1 = (\psi_u - \psi_i) \exp -\frac{t}{\tau_I} + (\psi_i - \psi_{ii}) \exp -\frac{t}{\tau_{II}} + (\psi_{ii} - \psi_1) \exp -\frac{t}{\tau_2}, \quad (1)$$

where ψ_u is the initial upper twitch tension developed in the high calcium concentration: ψ_1 the final steady level of the twitches in the subsequent lower concentration; ψ_i the intercept of the intermediate fraction at zero time and ψ_{ii} the intercept of the slowest fraction. τ_I, τ_{II} and τ_2 are the time constants of these tension changes and t is the time since the $[Ca]_o$ was reduced.

The initial rapid exponential phase, seen when the calcium concentration is lowered, is absent from the staircase response, so that eqn. (1) simplifies to eqn. (2) in this case:

$$\psi_u - \psi_1 = (\psi_u - \psi_{ii}) \exp -\frac{t}{\tau_{II}} + (\psi_{ii} - \psi_1) \exp -\frac{t}{\tau_2}, \quad (2)$$

where t is now the time since the stimulus rate was reduced.

The mean values of the time constants τ_I, τ_{II} and τ_2, obtained by regression analysis of the results of the two types of experiment (as illustrated in Fig. 4) are compared in Table 4. The time constants of the two slower exponential phases of tension change that occur in both types of response are not significantly different, as found by Chapman (1971) in atrial trabeculae. The time constant of the change in twitch tension caused by addition and removal of caffeine from the bathing fluid is found to be close to the value obtained for τ_{II} and not significantly different from it (Table 4).

The hypothesis put forward to account for the changes of the twitch strength that occur as the consequence of alteration of the $[Ca]_o$ or the heart rate, assumes that the strength of contraction is controlled by calcium ions liberated from three sources (Chapman, 1971). The three sources are visualized as three calcium compounds (designated Ca_I, Ca_{II} and Ca_2) which act co-operatively to determine the eventual strength of the contraction.

$[Ca_I]$ changes the fastest and its time constant is the same as that of the exchange time of the extracellular space, in these preparations, suggesting that the physical counterpart of a change in $[Ca_I]$ is a change in the influx of calcium at each action potential. The calcium that has entered the cell is then supposed to accumulate within the muscle cells as two other calcium compounds, Ca_{II} and Ca_2 at the rates of τ_{II} and τ_2 respectively. During

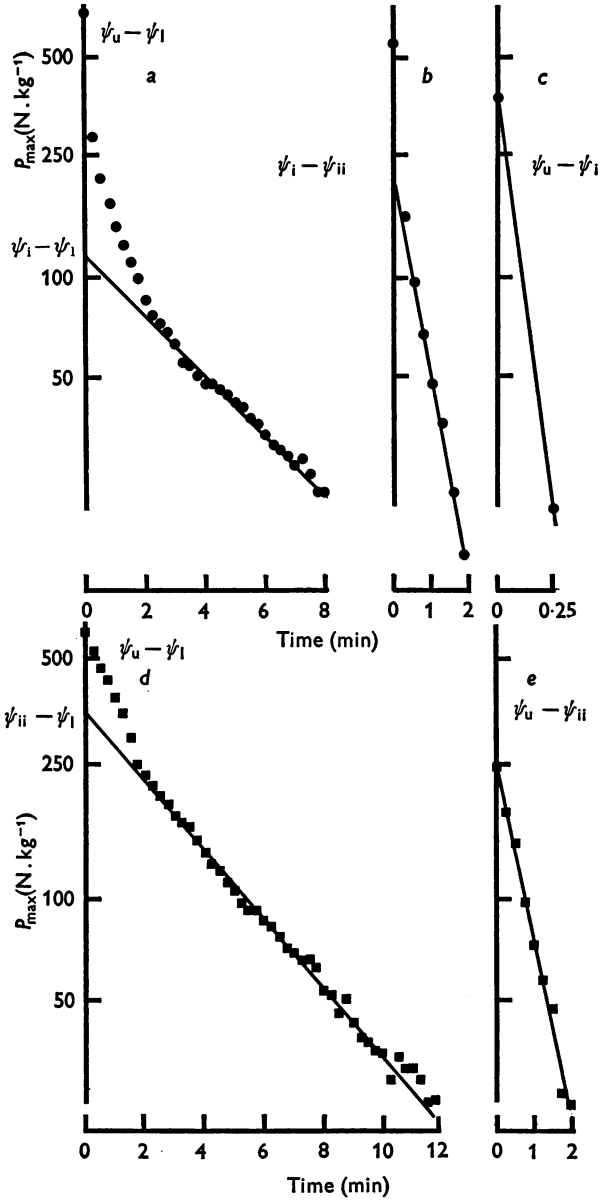

Fig. 4. All graphs are semilogarithmic plots of the decline of P_{max} with time when the $[Ca]_o$ is reduced from 3 to 1 mM at a constant heart rate of 4 min^{-1} (*a*, *b* and *c*) or when the heart rate is reduced from 12 to 4 min^{-1} at a constant $[Ca]_o$ of 1 mM (*d* and *e*). The change in P_{max} on reduction of $[Ca]_o$ is composed of three exponential phases which are revealed by multi-compartmental regression analysis and are shown by continuous lines. The composite form of the staircase response is revealed in the same way. The tension levels and the intercepts as expressed in eqns. (1) and (2) are shown. All the regression lines have correlation coefficients better than 0·990. 20·0° C; ventricle preparation; phosphate Ringer.

the staircase response it would seem that the influx of calcium per action potential is constant, i.e. there is no change in $[Ca_I]$, but there is an increase in the influx of calcium per unit time so that the staircase response occurs as the consequence of the build up of intracellular calcium, i.e. increases of $[Ca_{II}]$ and $[Ca_2]$. In the terms of this hypothesis caffeine could potentiate contraction by one of three ways; either increasing the influx of calcium at each action potential, i.e. the response should be like raising

TABLE 4. Time constants derived by multicompartmental regression analysis of the time courses of the changes of P_{max} and $[dP/dt]_{max}$ when either the $[Ca]_o$ or the stimulus rate was reduced, are compared with the time constant of the caffeine effect on the twitch response in ten ventricle preparations

Experimental change	τ_I (sec)	τ_{II} (sec)	τ_2 (sec)
Reduction of $[Ca]_o$ (3 to 1 mM) (2 to 1 mM)	4·6 ± 2·4	30·1 ± 10·6	191·2 ± 45·1
Reduction of stimulus rate from 12 to 4 min^{-1} (1 mM-Ca)	—	34·1 ± 9·5	168·0 ± 80·1
Addition or removal of caffeine	—	34·9 ± 10·1	—

The numbers shown were calculated by averaging the data for each experiment and then obtaining an over-all mean and standard deviation for the ten experiments. Application of a Student t test to the data reveals that the values of τ_{II}, estimated from the staircase response and from the response on reduction of $[Ca]_o$, are not significantly different ($P > 20\%$), and furthermore that these time constants are not different from that obtained from the caffeine response ($P > 20\%$). The values of the time constants, τ_2, obtained from the staircase response or from the responses following a reduction of $[Ca]_o$ are also not significantly different ($P > 10\%$). All experiments were done at 20·0° C. Phosphate Ringer.

the bathing calcium concentration; or by increasing the calcium influx per unit time, i.e. like a staircase response; or by changing one or more of the time constants τ_I, τ_{II}, or τ_2. The changes of the twitch response caused by caffeine are unlike the staircase response or that caused by variation of the bathing calcium levels (compare Figs. 2 and 4) suggesting that caffeine does not directly increase the influx of calcium into the muscle cells.

One must be cautious when looking for changes in the time constants τ_I, τ_{II}, τ_2, because if the tension developed by the muscle is the product of $[Ca_I]$, $[Ca_{II}]$ and $[Ca_2]$ (Chapman, 1971), then the decline of the twitch strength on reducing the $[Ca]_o$ is the sum of seven exponential and not three. In the present experiments where the ratio of $\tau_I:\tau_{II}:\tau_2$ is 1:6:42, this effect can be shown to be small because the seven exponentials fall into three groups of similar numerical value so that the decline of twitch tension caused by reduction of $[Ca]_o$ does not vary significantly from the sum of three exponentials as expressed in eqn. (1).

The exponential phases that comprise the tension decay on reduction of the $[Ca]_o$, derived from experiments made after equilibrations in the presence and in the absence of caffeine, are compared in Table 5. Both the time constants and the intercepts of the various phases are not significantly altered by exposure of the muscle to caffeine. Similarly, no change in the time constants or intercepts of the staircase response is produced by caffeine,

TABLE 5. The effects of caffeine on the response of frog ventricle to changes in $[Ca]_o$

a, time constants of the various exponential components of the decline in tension on reduction of the bathing calcium concentration with and without caffeine, four experiments. Phosphate Ringer, 20·0° C

	Time constants ± S.D. (sec)			
Change in solution	τ_I	τ_{II}	τ_2	No. of changes
3 to 1 mM-$[Ca]_o$	4·6 ± 0·7	24·5 ± 8·5	203·3 ± 40·4	19
1·5 to 0·5 mM-$[Ca]_o$ in the presence of 5 mM caffeine	4·9 ± 1·3	23·7 ± 6·5	207·6 ± 59·0	12
P	> 10%	> 20%	> 20%	—

b, the percentage of the total tension change of each of the exponential components (∓ S.D.) that compose the change in tension associated with a reduction of the bathing calcium concentration, are compared in the presence and in the absence of caffeine

	$\psi_u - \psi_i$	$\psi_i - \psi_{ii}$	$\psi_{ii} - \psi_1$	No. of changes
3 to 1 mM-$[Ca]_o$	61·0 ± 13·5	15·5 ± 7·7	23·5 ± 9·4	19
1·5 to 0·5 mM-$[Ca]_o$ in the presence of 5 mM caffeine	62·0 ± 6·2	12·0 ± 2·1	26·1 ± 10·9	12
P	> 20%	> 10%	> 5%	—

The calcium changes, in the presence of caffeine, were made after the depressive phase of caffeine's action had stabilized. The calcium concentrations were selected so that the consequent changes in twitch tension in the presence of caffeine were over the same tension range as those in the absence of the alkaloid.

confirming that τ_{II} and τ_2 are not influenced by caffeine. The estimate of τ_I is least accurate because it is the resultant of 4 exponentials. The time constant τ_I can best be estimated from the fall of the twitch response on reducing the bathing calcium concentration to zero when the heart is stimulated at a high rate, estimated in this way τ_I is between 2 and 5 sec and is not changed if caffeine is present in the bathing fluid. These experiments show that caffeine does not alter the form of the response of the heart to changes of the $[Ca]_o$ or the stimulus rate. In a few preparations the onset of the depressive phase of caffeine's action was delayed or the depression

was small and slow to develop. In these ventricles it is possible to compare the effect of reducing the stimulus frequency in Ringer without caffeine, to the response produced by the simultaneous reduction of the stimulus rate and the removal of caffeine at the height of its potentiating action. These experiments show no change in the time constant τ_{II} and τ_2 but

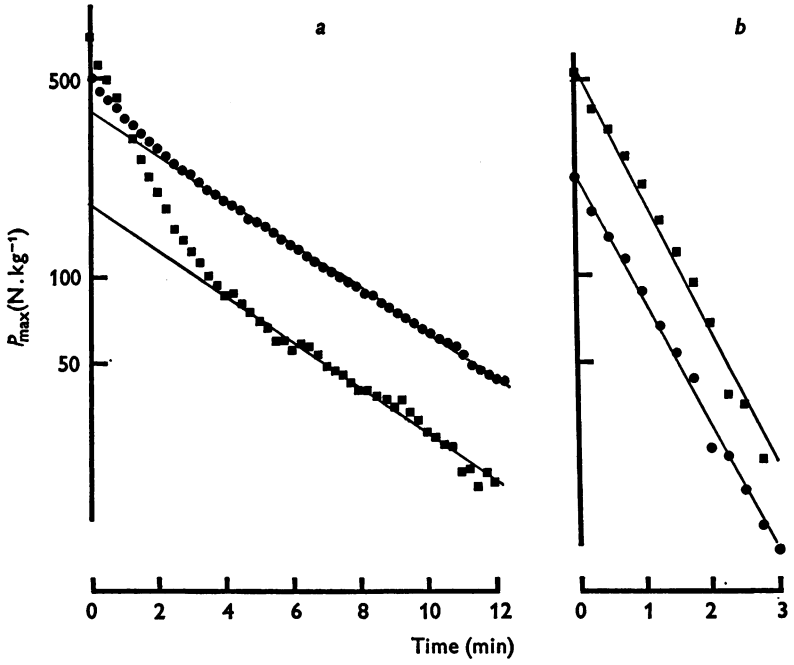

Fig. 5. The effects of caffeine pre-treatment on the staircase response. Semi-logarithmic plots obtained in the same way as described in Fig. 4.

a, the change in the twitch tension on reduction of the stimulus frequency from 12 to 4 min^{-1} in normal Ringer (●–●), is compared to the staircase response to the same change in heart rate made at the peak of potentiation caused by 5 mM caffeine. The caffeine is removed at the same time as the stimulus frequency is reduced (■–■). The continuous lines are regression lines of time constant 242 sec for the normal staircase and 254 sec for the caffeine-treated staircase response.

b, the fast phase of the two staircase responses are compared. The time constant is 45·5 sec for the normal staircase and 48·0 sec for the caffeine-treated staircase. Low-bicarbonate Ringer; 1 mM calcium; 20·0° C.

there is a large increase in the size of the initial phase of the staircase $\psi_u - \psi_{ii}$, i.e. in the [Ca_{II}] and large reduction in the intercept of the slow phase of tension decline $\psi_{ii} - \psi_1$, i.e. of the [Ca_2] (Fig. 5).

EXPERIMENTS ON ISOLATED ATRIAL TRABECULAE

The effects of caffeine on the beating atrial muscle

The changes in the twitch responses on exposure to caffeine are much the same as those described for the ventricle except that applications of caffeine concentrations above 10 mM often evoke a rapidly transient contracture that could be up to 25% of the maximum tension the trabecula could develop. The development of these contractures shows a strong temperature dependence (Chapman & Ellis, 1973).

The effects of caffeine on the potassium contracture of atrial trabeculae

The effects of caffeine on the tension developed during a potassium contracture depend upon the timing of its application. If caffeine is added before the potassium concentration is raised and included in the contracture fluid, the amplitude of the subsequent contracture is enhanced. The over-all relationship between the strength of the potassium contracture and the bathing calcium concentration is still proportional to $[Ca]_o^3$ when this concentration is less than 1 mM, but in the presence of caffeine the tensions generated are increased by a constant factor depending upon the caffeine concentration.

These results mean that if the potassium contracture tension can be described by eqn. (3);

$$C_{\max [Ca]_o < 1 \text{ mM}} = P_1 [Ca]_o^3, \tag{3}$$

where P_1 is constant and C_{max} is the isometric contracture tension; and then in the presence of caffeine, the tension will be described by eqn. (4).

$$C_{\max \text{ caffeine}} = P_1 [Ca]_o^3 . [\text{Caffeine}]_o P_2, \tag{4}$$

where P_2 is a constant. The fact that the results can be expressed in this way suggests a first order relationship between the caffeine concentration and the degree of potentiation of the potassium contracture.

If caffeine is applied after the spontaneous relaxation of a potassium contracture, its addition to the contracture fluid initiates a second contracture that develops very rapidly (Chapman, 1973*a*). The strength of this caffeine contracture increases with the concentration of the added alkaloid (Fig. 6). Between 1 and 20 mM the contracture tension is related to the caffeine concentration by a rectangular hyperbola when plotted on linear co-ordinates; on logarithmic co-ordinates these results have a mean maximum slope of + 0·67 S.D. 0·17, when the $[Ca]_o$ was 1 mM. This result supports a first order relationship between the strength of the contracture and the concentration of caffeine applied. An estimate of the half-saturation constant, made by means of a double reciprocal plot, has a mean

value of 3·40 S.D. 0·20 mM, but the correlation coefficients of the regression lines are rather low ($r = 0{\cdot}85$, $P > 0{\cdot}05$).

The caffeine contracture, evoked after the spontaneous relaxation of a potassium contracture, in turn relaxes spontaneously. When the concentration of the alkaloid is below 5 mM, the spontaneous relaxation of the caffeine contracture is fitted by a single exponential which has a

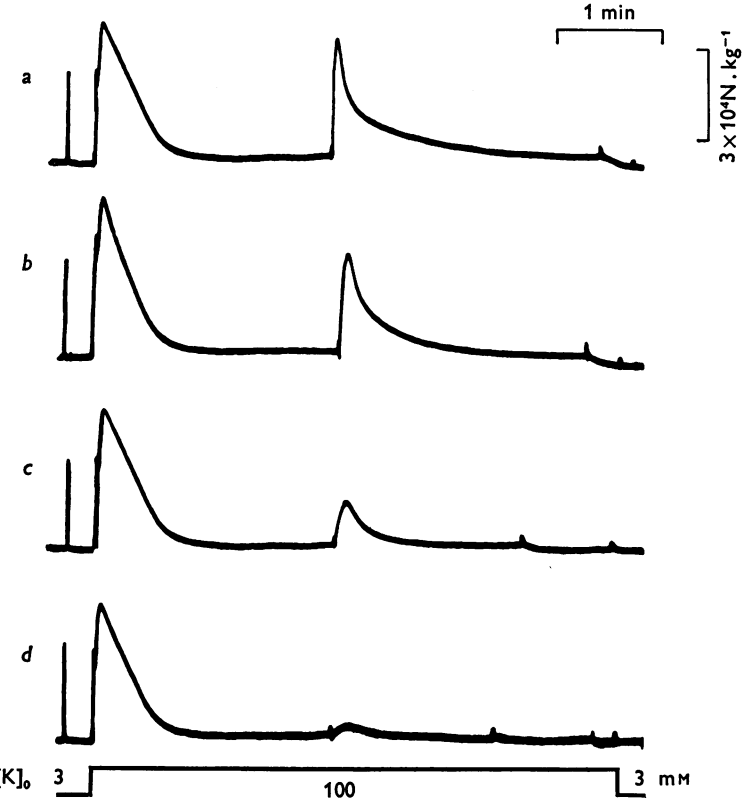

Fig. 6. Caffeine contractures can be elicited in depolarized atrial trabeculae. Records of contractures evoked by a range of caffeine concentrations applied after the spontaneous relaxation of a preceding contracture initiated by raising the bathing potassium concentration from 3 to 100 mM (as indicated by the lower trace). The caffeine, 20 mM in *a*, 5 mM in *b*, 2 mM in *c* and 0·8 mM in *d* is applied in high-potassium Ringer, 135 sec after the increase in the $[K]_o$. 2 mM calcium; 20·0° C. Phosphate Ringer.

time constant similar to that of the exponential phase of the spontaneous relaxation of the previous potassium contracture (Chapman, 1973*a*). Above this concentration the spontaneous relaxation of the caffeine contracture is composed of two phases and the second slow phase becomes slower the higher the caffeine concentration. A very rapid phase of relaxation at high

caffeine concentrations means that the peak of these contractures is not a reliable measure of the strength of contraction, i.e. these contractures do not conform to the criteria suggested by Chapman & Tunstall (1971) for the use of the peak contracture tension as an index of the contractile strength.

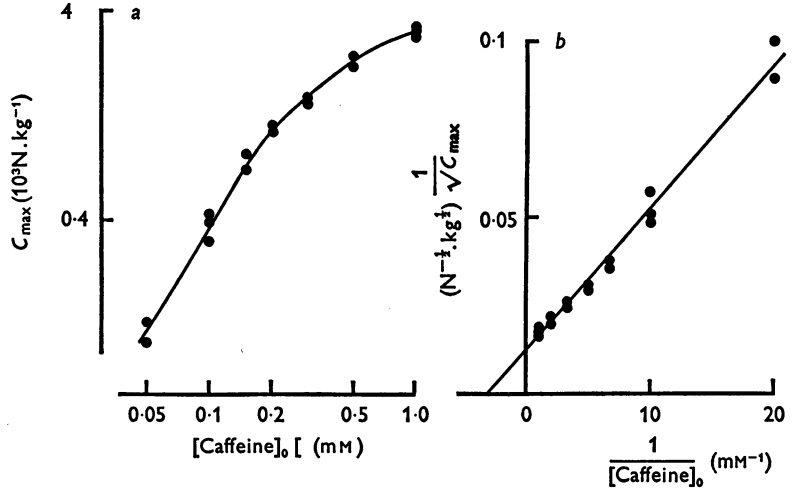

Fig. 7. *a*, a logarithmic plot of the dependence of the caffeine contracture elicited following the spontaneous relaxation of a contracture induced by sodium-free solution upon the caffeine concentration. The log of the force generated by an atrial trabecula is plotted against the log of the caffeine concentration. The slope of the continuous line, between 0·2 and 0·05 mM caffeine is +1·64.

b, the same results as those in part *a*, are plotted as the reciprocal of the square root of the caffeine contracture tension against the reciprocal of the caffeine concentration. The continuous line is a regression line (r = 0·99; $P < 0{\cdot}001$) and the intercept with the abscissa yields an affinity constant for caffeine of 0·33 mM. 1 mM calcium; 19·5° C. Phosphate Ringer.

The effects of caffeine on the sodium-free contracture of atrial trabeculae

The amplitude of the contracture evoked by sodium-free solutions is not altered by caffeine, but caffeine does potentiate the contractures evoked by solutions of a reduced sodium content. Following the spontaneous relaxation of a contracture evoked by perfusion with a sodium-free solution, application of caffeine at relatively low concentrations i.e. around 2 mM, induces a maximal but transient contracture. This caffeine contracture develops rapidly and subsides more slowly, so that the peak tension may be taken as a reliable estimate of the contractile strength. The caffeine contracture is steeply related to the caffeine concentration with a mean maximum slope, on logarithmic co-ordinates of +1·59 S.D. 0·11 (Fig. 7).

These results suggest a second order relationship between the caffeine concentration and the tension induced in sodium-free conditions. If $1/\sqrt{}$contracture tension is plotted against $1/[\text{caffeine}]_o$, the resulting straight lines have high coefficients of correlation and low P values; and give a half-saturation constant for caffeine of 0·26 S.D. 0·08 mM (when the $[Ca]_o$ is 1 mM).

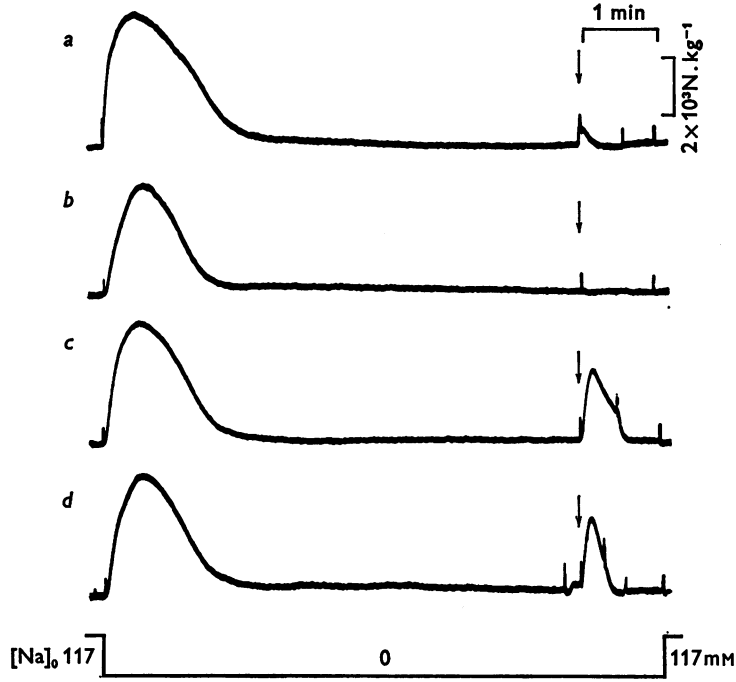

Fig. 8. After the spontaneous relaxation of a contracture initiated by removing the sodium in the presence of 0·1 mM calcium (as indicated by the lower trace), depolarizing the muscle by raising the $[K]_o$ from 3 to 100 mM (at the arrow in trace *a*) induces a small contracture. Raising the calcium concentration to 10 mM (at the arrow in trace *b*) has no effect on the tension generated by the trabecula. Adding 5 mM caffeine (at arrow in trace *c*) induced a large contracture. 5 mM caffeine still induced a contracture (at arrow in trace *d*) even when the $[Ca]_o$ is reduced to less than 10^{-8} M by the omission of calcium chloride and the addition of 1 mM-EGTA to the perfusing fluid for 15 sec before the caffeine is added (still without calcium) 19·0° C. Tris Ringer.

Reduction of the bathing calcium concentration to less than 10^{-8} M by the addition of EGTA prevents the development of the sodium-free contracture (Chapman, 1974). If a similar reduction is made after the spontaneous relaxation of the sodium-free response, caffeine will still evoke a significant contracture, showing that the caffeine response does

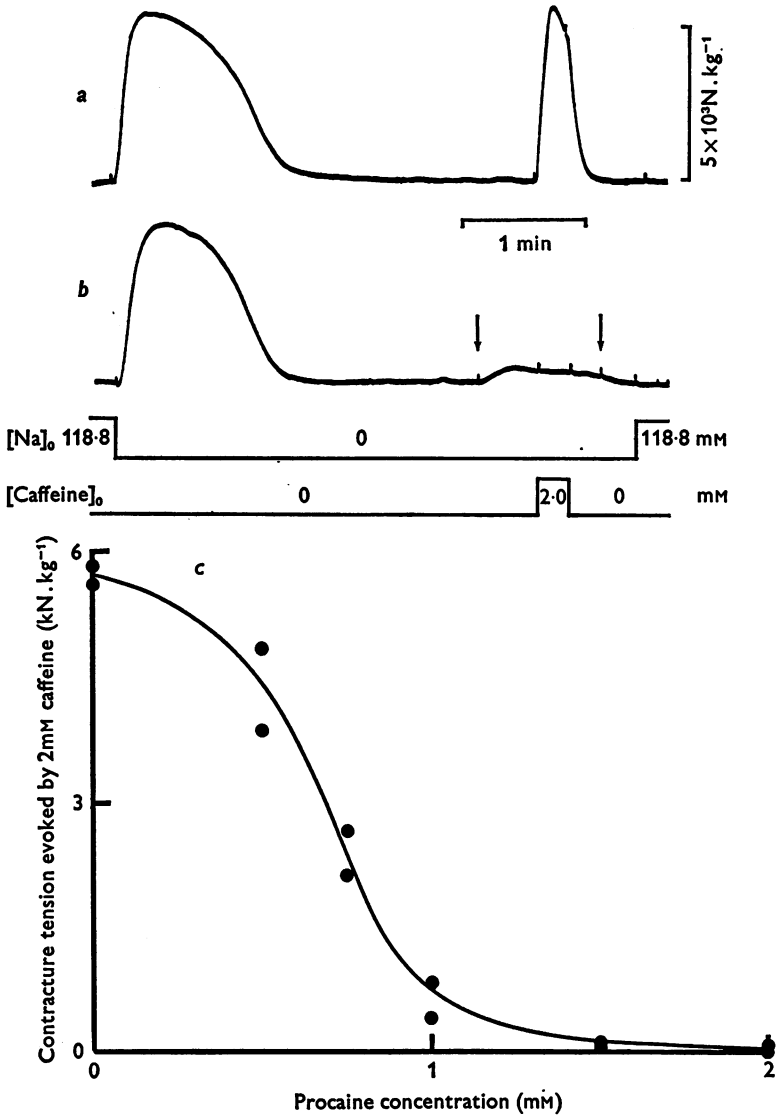

Fig. 9. Effects of procaine on the caffeine contracture evoked in sodium-free solution. *a*, the trace of the contractures elicited initially by the withdrawal of sodium and that initiated by the application of 2 mM caffeine 3 min later, after the sodium-free contracture had spontaneously relaxed. *b*, a trace showing that a similar application of caffeine fails to induce a contracture if the muscle is first exposed to 2 mM procaine for 30 sec (as indicated by the arrows). In this trabecula procaine induced a small contracture at this concentration. The traces below *b* show the timing and extent of the changes in the bathing sodium and caffeine concentrations. *c*, the strength of the caffeine contracture, from the same experiment as *a* and *b* is plotted against the concentration of procaine in the bathing medium before and during the application of 2 mM caffeine. 1 mM calcium; 20·5° C. Phosphate Ringer.

not require the presence of calcium ions in the bathing medium (Fig. 8). This result clearly favours an intracellular site for caffeine's action, especially as raising the bathing calcium or potassium concentrations fails to elicit a comparable contractile response (Fig. 8).

The size of the caffeine contracture does show a dependence on the calcium concentration present while the sodium-free contracture is relaxing. The contracture is larger when this preceding calcium level is high.

The effects of local anaesthetics on the caffeine contracture

Local anaesthetics are known to antagonize the effects of caffeine on skeletal muscle and isolated sarcoplasmic reticulum (Feinstein, 1963; Weber & Herz, 1968). In frog atrial preparations, a 15–30 sec exposure to procaine, tetracaine or chloroform, after the spontaneous relaxation of a sodium-free contracture, antagonizes the development of a subsequent caffeine contracture (Fig. 9*a*, *b*). This inhibition of the response to caffeine is sigmoidally related to the concentration of the anaesthetic (Fig. 9*c*), with tetracaine being some 5 times more potent than procaine. The concentration of local anaesthetic that blocks the caffeine contracture after the spontaneous relaxation of the sodium-free contracture is much less effective in blocking the response to the same caffeine concentration after the spontaneous relaxation of a potassium contracture. The typical effect is that the application of the local anaesthetic abolishes the rapid phasic part of the caffeine contracture to leave the more slowly relaxing part of the contracture unaffected. Procaine-insensitive effects of caffeine also occur in mammalian heart (Blinks *et al.* 1972).

DISCUSSION

To increase the strength of the twitches and to induce contractures, caffeine must presumably increase the sarcoplasmic calcium concentration. This calcium must come directly from the bathing medium or from an intracellular store. A number of observations suggest that caffeine releases calcium from an intracellular site. First, the potentiation of the twitch, in frog heart occurs without change in the action potential, except for a small shortening of about 4 %. Secondly, the time course of the change in twitch tension on exposure to caffeine is simple in comparison to the changes produced when the bathing calcium concentration or the stimulus rate is altered. Thirdly, caffeine fails to alter the rate of the disappearance of the twitch response when all the calcium ions are removed from the bathing fluid. Fourthly, in frog atrial preparations caffeine can be made to induce contractures in the virtual absence of extracellular calcium. The idea that caffeine acts on an intracellular site, to release calcium, is in agreement with the supposed mode of action of this alkaloid on other muscles

and isolated cell organelles (Caldwell & Walster, 1963; Weber & Herz, 1968; Chiarandini, Reuben, Brandt & Grundfest, 1970; Sakai, Geffner & Sandow 1970; Thorpe & Seeman, 1971).

The responses of frog heart on exposure to caffeine are intermediate to those induced in frog skeletal muscle (Sandow & Brust, 1966; Lüttgau & Oetliker, 1968) and those found in mammalian smooth muscle (Ito & Kuriyama, 1971; Sunano & Miyazaki, 1973; Osa, 1973; Magaribuchi, Ito & Kuriyama, 1973). The sarcoplasmic reticulum is highly organized in frog skeletal muscle (Page, 1964), less so in frog heart (Page & Niedergerke, 1972), and least organized in smooth muscle (Somlyo & Somlyo, 1968), however the responses of frog heart most closely resemble those found in crustacean skeletal muscle (Chiarandini *et al.* 1970) where the sarcoplasmic reticulum is extensively developed.

The results of the experiments on frog ventricle show that caffeine has little or no effect on the time constant τ_I, τ_{II}, and τ_2, as determined from the twitch changes associated either with a reduction of $[Ca]_o$ or of the heart rate (Table 5). The experiment illustrated in Fig. 5 shows that, despite the fact that the twitch response at the higher stimulus rate in the presence of caffeine is very large, the intercept $(\psi_{ii} - \psi_1)$ of the staircase response is reduced. This change suggests that caffeine reduces the effective $[Ca_2]$ and increases $[Ca_{II}]$. This change could be achieved if caffeine rapidly liberates ionic calcium from the Ca_2-complex which is then converted to Ca_{II}. As the rate of penetration of caffeine into the muscle cells is likely to be fast (Bianchi, 1962), this scheme would account for a variety of observations: the similarity between the time constant for the caffeine effect on the twitches and the value of τ_{II} (Table 4); the single exponential form of this caffeine effect (Fig. 2); the rapid development of the caffeine contractures in atrial trabeculae; and the long term depressive action of caffeine, for with prolonged exposure the increased liberation of calcium from the Ca_2-complex will result in the eventual depletion of this compound, resulting in turn in the depression of the twitch strength.

It would seem that caffeine acts at a site corresponding to the hypothetical calcium compound Ca_2 and that if caffeine is specific in its action on the sarcoplasmic reticulum, then this structure is the physical counterpart of the Ca_2 compound.

The ability of caffeine to evoke contractures after the spontaneous relaxation of a contracture initiated by potassium-rich or sodium-free solutions shows that calcium accumulates at a site sensitive to caffeine during this relaxation. This idea is confirmed by the failure of caffeine to induce a contracture when the spontaneous relaxation has been blocked by metabolic inhibitors (Chapman, 1973*b*). To determine the relationship between the caffeine concentration and the tension it elicits, caffeine

contractures evoked after the spontaneous relaxation of both the potassium and the sodium-free contractures have been used. The results of the two types of experiment are not in agreement. A first order relationship is suggested by the caffeine responses elicited after a potassium contracture and a second order one is suggested by the contractures evoked under sodium-free conditions. The results obtained after the spontaneous relaxation of the sodium-free contracture are, however, likely to be a more reliable estimate of the effect of caffeine on contraction. There are several reasons for considering this to be the case: the caffeine contracture in sodium-free media does not show the two phases of relaxation seen when the potassium concentration is high; local anaesthetics completely block the caffeine response in sodium-free solutions but are less effective after a potassium contracture; the rapid initial phase of the spontaneous relaxation of the caffeine contracture, evoked following a potassium contracture, reduces the reliability of the peak tension as a measure of the contractile strength; the solutions used to evoke potassium contractures are hypertonic to the normal Ringer, and hypertonicity can influence the contractility of skeletal and cardiac muscle (Länngergren & Noth, 1973; Chapman & Léoty, 1974; R. A. Chapman, unpublished). The state of the muscle after the spontaneous relaxation of the two types of contracture may also not be the same, for the contractures show a different sensitivity to manganese (Chapman & Ochi, 1972), and to an increase in the outside calcium concentration (Chapman, 1973*a*, 1974).

Any interpretation of the relationship between the tension generated during the application of caffeine and the concentration of the drug depends on two other relationships not fully understood. The first is the relationship between the intracellular calcium concentration and the degree of activation of the contractile apparatus. Fuchs & Briggs (1968) and Ebashi, Endo & Ohtsuki (1969) suggest a first order relationship, while Ashley (1971) supports a second order one. Secondly, the relationship between the caffeine concentration and the amount of calcium released by the sarcoplasmic reticulum is also unknown. Recent experiments, in our laboratory, have shown that caffeine has two actions on the calcium transport by isolated cardiac and skeletal sarcoplasmic reticulum (Patricia A. Barford & R. A. Chapman, unpublished). Below 3 mM, caffeine competes with ATP for the calcium transport system, but with higher concentrations the inhibition of calcium transport is non-competitive. These results could explain the difficulties encountered in the present work, for low doses of caffeine induce large contractures after the spontaneous relaxation of the sodium-free contracture, and under these conditions the first action of caffeine would be expected to predominate. Larger doses of caffeine are necessary to induce similar tensions after the relaxation of the potassium contracture,

so that the second non-competitive action of caffeine could complicate the results and may account for the complex form of the spontaneous relaxation of the caffeine contracture in these solutions.

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