BRIEF COMMUNICATION

CAFFEINE CONTRACTURE AND IODOACETATE RIGOR IN FROG SKELETAL MUSCLE

A COMPARISON

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ABSTRACT Frog sartorius muscle treated with 5.0 mM or greater caffeine exhibits stiffness similar to that obtained from muscle in iodoacetate rigor. The data provide quantitative evidence that suggests that caffeine at irreversible contracture-producing concentrations somehow induces a rigor or rigorlike state in skeletal muscle.

INTRODUCTION

The effects of the alkaloid caffeine on skeletal muscle have been studied by numerous investigators from both physiological and biochemical perspectives. The drug has often been used to produce the active state in muscle by its observed action of causing calcium release from the sarcoplasmic reticulum (Weber and Herz, 1968; Endo, 1975). Two effects on mechanical behavior are observed, depending upon the concentration of the drug. At low levels (1.0–3.0 mM) it causes potentiation of the individual twitch, characterized by increases in the magnitude and the rate of development of tension during an isometric twitch, as well as a decreased rate of relaxation (Sandow and Brust, 1966). At higher concentrations (3.0 mM or greater) it brings about a contracture, as evidenced by an increase in muscle rest tension (Axelsson and Thesleff, 1958). Some workers have remarked that contracture-producing concentrations of caffeine appear to cause irreversible changes in muscle, similar to the appearance of rigor; indeed, the term "caffeine rigor" has occasionally been used. However, the stiffness of caffeine-treated muscle has not been quantitatively measured.

The most salient feature of rigor is a large increase in stiffness, as reflected by an in-

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creased resistance to mechanical stretch (Murphy, 1956; Mulvaney, 1975). An examination of the elastic properties of skeletal muscle in caffeine contracture and in rigor might therefore determine whether caffeine at higher concentrations produces a state mechanically similar to rigor.

METHODS

Whole sartorius muscle from the frog *Rana pipens* was used for these experiments, all of which were performed at 20°C. Normal Ringer's medium consisted of 116.8 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 2.0 mM Tris-HCl buffer (pH 7.2 and 2.0×10^{-2} mg ml^{-1} curare. Caffeine Ringer's medium contained, in addition to the above, caffeine (Sigma Chemical Co., St. Louis, Mo.) in the desired concentration. Rigor was produced by treatment with 0.50 mM iodoacetic acid (IAA, Sigma), according to the method of Mulvaney (1975). A modified Levin-Wyman ergometer was used to produce a linear stretch of 0.05 L_0 in 100 ms, corresponding to a stretch speed of 0.50 L_0 s^{-1}. Tension was recorded with an RCA 5734 transducer (RCA Solid State Div., Somersville, N.J.) while length changes were determined by standard light beam interruption methods. The quantitative measure of muscle stiffness used was Young's elastic modulus, aquivalent to the ratio of stress to strain, where stress is the resistive force exerted by the muscle per unit cross-sectional area and strain is the imposed length change expressed as a fraction of L_0. Rest length was recorded as the length at which peak active tension (P_0) was elicited with a supramaximal stimulus of duration 0.3 s, pulse duration 0.3 ms and pulse frequency 200 s^{-1}

RESULTS AND DISCUSSION

Fig. 1 shows the results from a typical experiment performed at a caffeine concentration of 3.0 mM. Fig. 1 A is the stress-strain record for the muscle in normal Ringer's medium. The average value for the elastic modulus obtained under such conditions was $5.23 \pm 0.26 \times 10^5$ N m^{-2} (57 muscles). 1 h after the addition of 3.0 mM caffeine, the elastic modulus has increased by a factor of 4.5, as shown in Fig. 1 B. In addition, the muscle has developed a contracture tension, as seen by an increase in the rest tension. When returned to the rest length, this contracture tension drops to zero but then redevelops to its initial value in less than 1 min (1 C). This redevelopment of the tension upon return to the rest length suggests that caffeine contracture at 3.0 mM is due to the presence of the active state, according to the criterion of tension redevelopment upon quick release (Ritchie, 1954). Stretching the same muscle 2 h after returning it to normal Ringer's medium (1 D) shows that the elastic modulus has dropped to 1.4 times the normal value; in addition, the contracture tension is still present. This demonstrates that treatment with 3.0 mM caffeine causes an increase in muscle stiffness, which is only partially reversible.

Fig. 2 shows the results from representative experiments performed with 5.0 mM caffeine (2 A, B, and C) and with muscle in IAA rigor (2 D, E, and F). Several interesting features are in evidence. First, the response to stretch 1 h after the addition of 5.0 mM caffeine (2 B) is similar to that obtained for a muscle in IAA rigor (2 E). In each case, the muscle exhibits an elevated resting tension, a constant elastic modulus over the first 2% of L_0, and a pronounced plasticity thereafter. Second, when released back

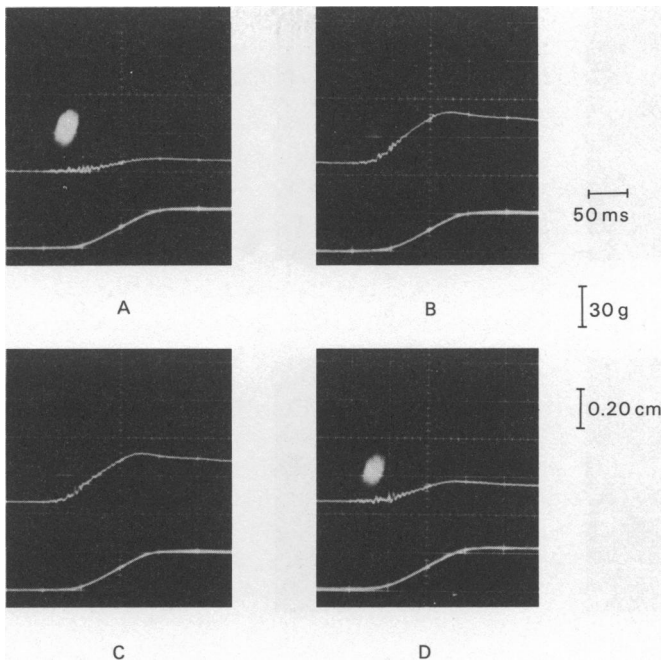

FIGURE 1 Effects of 3.0 mM caffeine on elastic modulus of frog sartorius muscle at 20°C. Upper trace in each record is tension (stress) output and lower trace is length (strain) record. Scale factors are indicated. (A) shows stress-strain record for a muscle in normal Ringer's (no caffeine). (B) is record obtained 1 h after addition of 3.0 mM caffeine, depicting elevated rest tension and increased elastic modulus. Upon returning to rest length, contracture tension quickly redevelops and elastic modulus is the same during subsequent stretch (C). Returning muscle to normal Ringer's for 2 h (D) does not completely eliminate elevated rest tension and increased elastic modulus. The muscle rest length (L_0) is 4.0 cm and the muscle cross-sectional area (determined by weighing) is 4.5×10^{-2} cm^2.

to the rest length, the elevated resting tension does not redevelop in either case, and subsequent restretching yields a constant elastic modulus of 6.7 times normal for the muscle in 5.0 mM caffeine (2 C) and 7.0 times normal for the rigor muscle (2 F). Although not shown in Fig. 2, rebathing either the caffeine-treated or IAA rigor muscle in normal Ringer's medium for 2 h did not reduce this stiffness at all, implying that in both cases the increase in stiffness is irreversible.

Experiments performed at 1.5 mM caffeine (a potentiation-producing concentration) did not reveal any detectable increase in the elastic modulus compared to the untreated muscle, even though the typical effects of caffeine's action as a type A potentiator (Sandow and Brust, 1966) were observed. The elastic moduli obtained at caffeine concentrations of 7.5 mM and 10.0 mM were essentially the same as those observed in the 5.0 mM caffeine and IAA rigor muscles.

These results are summarized in Table I. The magnitude of contracture tension as a function of caffeine concentration is in agreement with values reported by several other workers (Axelsson and Thesleff, 1958; Caputo, 1966; Gebert, 1968; Sakai et al.,

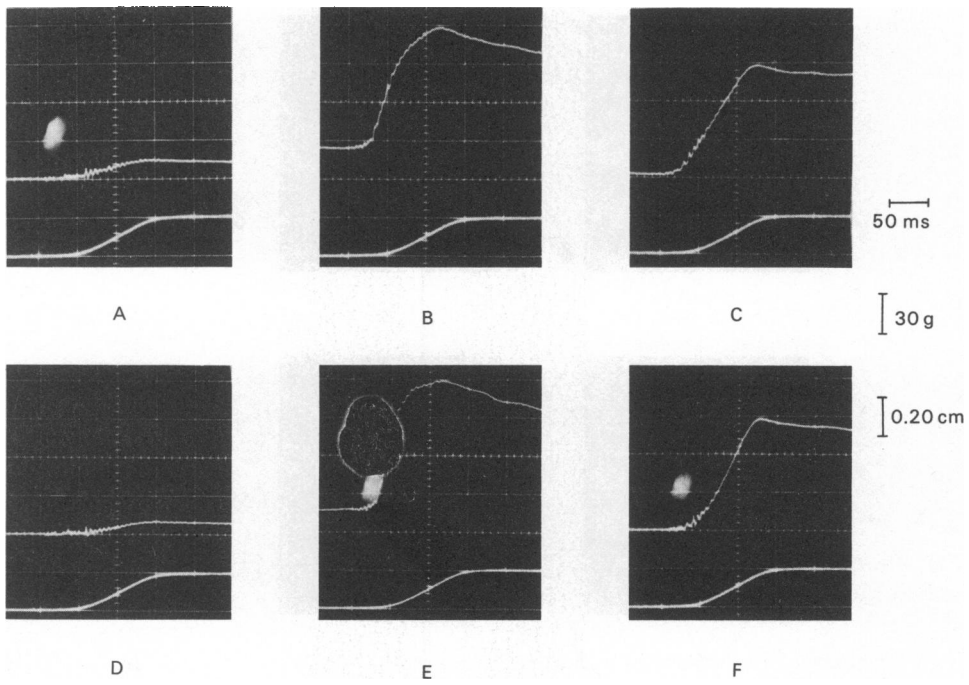

FIGURE 2 Similar effects of 5.0 mM caffeine (A, B, C) and 0.50 mM iodoacetate (D, E, F) on elastic modulus of frog sartorius muscle at 20°C. (A) and (D) are stress-strain records for muscles in normal Ringer's. (B) and (E) are for muscles in 5.0 mM caffeine contracture and iodoacetate rigor, respectively. Release to rest length does not result in redevelopment of elevated rest tension in either case, and subsequent stretch yields similar stress-strain output (C, F).

1970). Stress-strain results for muscle in IAA rigor similar to those reported here have also been obtained (Mulvaney, 1975). Elastic moduli for skeletal muscle at rest that agree with the values obtained in these experiments have been reported by Halpern and Moss (1976). The elastic moduli given in Table I, when adjusted for the level of tension present, are consistent with the findings of Halpern and Moss for the stiffness of actively contracting muscle at peak isometric tension.

These results suggest that caffeine contracture (5.0 mM or greater) and IAA rigor are similar in: (*a*) the level of elevated resting tension, (*b*) the increase in elastic modulus, and (*c*) the extent of reversibility.

Although the results seem to indicate that higher concentrations of caffeine produce a state mechanically similar to rigor, they do not directly suggest how. Rigor is associated with a decrease in intracellular ATP; therefore, the most plausible explanation is ATP depletion resulting from prolonged contracture. However, the irreversible nature of the stiffness increase implies that some form of metabolic inhibition might be involved, as in the case of IAA inhibition of 3-phosphoglyceraldehyde dehydrogenase. One possible agent for producing the rigorlike state in caffeine-treated muscle is the

TABLE I

SUMMARY OF MECHANICAL PROPERTIES OF MUSCLE IN CAFFEINE CONTRACTURE AND IODOACETATE RIGOR

Conditions	Number of muscles	Tension developed $\times P_0$ (1 h)	Y_C/Y_N	Reversibility
Caffeine				
1.5 mM	6	0	0.99 ± 0.02	Complete
3.0 mM	7	0.11 ± 0.01	4.45 ± 0.30	Partial
4.0 mM	6	0.15 ± 0.02	4.93 ± 0.17	Partial
5.0 mM	12	0.21 ± 0.02	6.65 ± 0.38	Irreversible
7.5 mM	11	0.23 ± 0.01	6.76 ± 0.33	Irreversible
10.0 mM	9	0.25 ± 0.02	6.04 ± 0.27	Irreversible
Iodoacetate				
0.5 mM	6	0.17 ± 0.02	7.03 ± 0.25	Irreversible

P_0 = maximum isometric tension (rest length). Y_N and Y_C are the elastic moduli in normal Ringer's and caffeine Ringer's, respectively. Values are stated as mean ± SD.

elevated sarcoplasmic calcium concentration. Yabu (1963) has reported that 5.0 mM caffeine caused an increase in O_2 consumption in normal muscle (but not in calcium-deficient muscle) like that resulting from treatment with 2,4-dinitrophenol, a respiratory uncoupler. Evidence has been presented (Borys and Karler, 1971) that 10.0 mM caffeine causes a decrease in microsomal calcium content and a concomitant increase in mitochondrial calcium content, the amount lost by the former being approximately equal to the amount accumulated by the latter. The similarity between this effect and respiration-dependent mitochondrial uptake (Lehninger, 1970) is further extended by the electron micrographs of Borys and Karler, which demonstrate that treatment with 10.0 mM caffeine results in pronounced swelling of the mitochondria with their christae in various states of dissolution. More recently, Burt et al. (1977) have determined by means of phosphorus-31 nuclear magnetic resonance spectroscopy that treatment of frog muscle with 20.0 mM caffeine results in total depletion of intracellular ATP and phosphocreatine, along with large increases in sugar phosphates and inorganic orthophosphates. These alterations in metabolism are very similar to those believed to result from glycolytic inhibition during IAA rigor.

No one of these corroborative results, however, establishes the irreversibility of 5.0 mM caffeine. If metabolic inhibition of some kind is a result of caffeine treatment, it must be irreversible to be consistent with the irreversible increase in stiffness for muscle in caffeine contracture. The results presented here strongly suggest that irreversible rigor results from higher caffeine concentrations, a conclusion for which there has been no prior quantitative evidence.

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