LETTER TO THE EDITOR

Comments on "Critical Dependence of Calcium-activated Force on Width in Highly Compressed Skinned Fibers of the Frog"

Dear Sir:

Gulati and Babu (1985) recently reported that low level compressions of the myofilament lattice by dextran T-500 do not increase the Ca^{2+} activated isometric force of skinned fibers, results that contradict the data of others (Godt and Maughan, 1977, 1981; Krasner and Maughan, 1981; April and Maughan, 1982, 1986; Kawai and Schulman, 1985). The discrepancy arises because Gulati and Babu introduce a force correction factor on the assumption that dextran addition reduces the ionic strength (I) of a solution. However, this assumption is in error; the addition of an anhydrous nonelectrolyte cannot change ionic strength because its definition is based on molality, i.e., the number of moles of solute per kilogram of solvent (Eq. 1). While the total molality changes, dextran addition does not alter the partial molality of each electrolyte because neither water nor salt is added. When ionic strength is calculated in terms of molarity, i.e., the number of moles of solute per liter of solution, a density term compensates for the relative decrease in molarity (Eq. 2). The dextran addition reduces the activity of the water and dissolved electrolytes because the relative mole fractions of each decrease. The myofilament lattice surface acts as a semipermeable barrier that prevents the entrance of dextran but allows the efflux of water and electrolytes. The lattice compresses until the hydrostatic pressure within the fiber counterbalances the osmotic pressure created by dextran addition.

$$I = 0.5 \sum_i m_i z_i^2 \qquad (1)$$

$$I = (0.5 \sum_i c_i z_i^2)/(\phi - \sum_i c_i M_i/1{,}000), \qquad (2)$$

where m_i is the molality, z_i is the number of charges, c_i is the concentration (molarity), M_i is the molecular weight, ϕ is the density of solution, and the subscript i represents the ith species.

While the argument against an ionic strength change after dextran addition is theoretically straightforward, physiological tests were conducted to confirm that the dextran effect on the pCa/tension curve is not related to ionic strength. Previously, we reported (Kawai et al., 1981; Brandt et al., 1982) that an ionic strength change causes a shift of the midpoint of the pCa/tension curve. More recently, other investigators (Ogawa, 1985) have observed that Ca^{2+} binding to both the high and low affinity sites on troponin C depends on ionic strength. If dextran addition decreases ionic strength we would expect the midpoint of the pCa/tension curve to shift to the left (higher pCa). Gulati and Babu (1985) themselves report (their Fig. 4), however, that the normalized pCa/tension relations for fibers activated in control ($I = 0.190$) and 20% dextran (which they assume as $I = 0.162$) solutions are identical in their calcium sensitivity despite the assumed difference in ionic strength. When Godt and Maughan (1981) investigated the pCa/tension relation for rabbit soleus muscle they found that fibers activated in 0 and 10% dextran solutions exhibited similar responses to Ca^{2+}; fibers activated in 5% dextran solution (approximately the lattice spacing found in vivo) had a slightly lower Ca^{2+} threshold and a left shifted curve. We determined the pCa/tension curves at different concentrations of dextran (0, 2, 4, 7, 10%; see Table I for protocol) on chemically skinned rabbit psoas fibers.

As the summarized pCa/tension data of Table I indicate, dextran addition does not continuously alter Ca^{2+} sensitivity. Our results qualitatively agree with those of Godt and Maughan (1981); the 0 and 10% dextran solutions have similar midpoints and thresholds, while the intermediate dextran solution (4–7%) have slightly higher calcium sensitivities. The shift of the pCa/tension curve, while experimentally significant, is less than that previously reported on rabbit soleus (Godt and Maughan, 1981). The shift at 4 to 7% cannot be the result of an ionic strength change, because at 10% dextran, when the effect should be the greatest, Ca^{2+} sensitivity is not significantly different from the control. Because Ca^{2+} sensitivity does not change systematically, dextran addition does not alter the ionic environment within the myofilament lattice as Gulati and Babu expect. Furthermore, Donnan calculations indicate that while the concentration of individual species of co-ions and counter-ions varies within the lattice during compression by uncharged polymers, the net ionic strength does not change (April and Maughan, 1986).

TABLE I
EFFECT OF DEXTRAN ON THE PARAMETERS OF THE pCa/TENSION RELATION OF SKINNED RABBIT PSOAS FIBERS

Experiment	n	log K (midpoint)	Tension
			kg/cm^2
0% Dextran	6.1 (0.5)	6.08 (0.01)	2.1 (0.1)
2% Dextran	5.5 (0.2)	6.06 (0.02)	2.3 (0.1)
4% Dextran	3.4 (0.1)	6.11 (0.01)	2.2 (0.1)
7% Dextran	3.9 (0.3)	6.16 (0.02)	2.2 (0.2)
10% Dextran	4.2 (0.1)	6.07 (0.02)	2.3 (0.1)

At each dextran concentration 10 single rabbit psoas fibers were activated through a pCa series by the method of serial dilution (Brandt et al., 1980, 1982, 1984). The activating solution without dextran contains (in millimolars): 6 $K_2CaEGTA$, 5.1 Na_2MgATP, 5.17 Na_2K_2ATP, 15 Na_2creatinine phosphate (CP), 8 $K_{1.4}PO_4$, 29.1 K propionate, 25.5 Na propionate, 10 MOPS, and 80 U/ml CP kinase. Dextran solutions are prepared so that ionic concentrations were unaffected: a 4% solution is made by adding 4 g of dextran per 100 ml of polymer-free solution (Godt and Maughan, 1981). Tension is normalized to fiber cross-sectional area and represents the maximum force generated in response to pCa 4.97. The standard error of the mean values are indicated in parenthesis for 10 experiments. The log K is the midpoint of the curve and n is the Hill coefficient calculated by fitting the data to the equation: $P/P_0 = ([Ca^{2+}]K)^n/\{1 + ([Ca^{2+}]K)^n\}$.

Gulati and Babu (1985) provide an interesting explanation for the effects of skinned fibers at higher level compressions (>13% dextran). They observe, as have others (Godt and Maughan, 1981; April and Maughan, 1982, 1986; Kawai and Schulman, 1985) that force declines sharply after a skinned fiber is compressed beyond 58% of its original width. From stiffness measurements, Gulati and Babu found that crossbridges could attach and cycle; they suggest that compression probably blocks the power stroke step in the crossbridge cycle, or inhibits some other molecular movements that are essential for tension generation, ideas that agree with interpretations of others (Kawai and Schulman, 1985; April and Maughan, 1986).

Since fibers swell upon skinning (Rome, 1967; Reuben et al., 1971; April et al., 1972; Ford and Podolsky, 1972; Matsubara and Elliot, 1972; Godt and Maughan, 1977), low level compressions have been introduced to generate in situ lattice spacings. The investigations have helped define the limitations of skinned fiber experiments and the extent of their applicability for physiological study. Because isometric tension changes as a function of lattice spacing, the compression studies suggest to us that the length–tension relationship (Gordon et al., 1966) may not be characterized exclusively by actin–myosin overlap (Brandt et al., 1967; April et al., 1971; Godt and Maughan, 1977; April and Maughan, 1986).

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REFERENCES

April, E. W., P. W. Brandt, and G. F. Elliott. 1971. The myofilament lattice: studies on isolated fibers. I. The constancy of the unit-cell volume with variation in sarcomere length in a lattice in which the thin-to-thick myofilament ratio is 6:1. *J. Cell Biol.*51:72–82.

April, E. W., P. W. Brandt, and G. F. Elliott. 1972. The myofilament lattice: studies on isolated fibers. II. The effects of osmotic strength, ionic concentration and pH on the unit-cell volume. *J. Cell Biol.*53:53–65.

April, E. W., and D. W. Maughan. 1982. Correlation between interfilament spacing and force generation in striated muscle. *Biophys. J.* 37:129a. (Abstr.).

April, E. W., and D. W. Maughan. 1986. Active force as a function of filament spacing in crayfish skinned muscle fibers. *Pfluegers Arch. Eur. J. Physiol.* In press.

Brandt, P. W., R. N. Cox, and M. Kawai. 1980. Can the binding of Ca^{2+} to two regulatory sites on troponin-C determine the steep pCa/tension relationship of skeletal muscle? *Proc. Natl. Acad. Sci. USA.* 77:4717–4720.

Brandt, P. W., R. N. Cox, M. Kawai, and T. Robinson. 1982. Regulation of tension in skinned muscle fibers: effect of cross-bridge kinetics on apparent Ca^{2+} sensitivity. *J. Gen. Physiol.* 79:977–1016.

Brandt, P. W., M. S. Diamond, B. Gluck, M. Kawai, and F. H. Schachat. 1984. Molecular basis of cooperativity in vertebrate muscle thin filaments. *Carlsberg Res. Commun.* 49:155–167.

Brandt, P. W., E. Lopez, J. P. Reuben, and H. Grundfest. 1967. The relationship between myofilament packing density and sarcomere length in frog striated muscle. *J. Cell Biol.* 33:255–264.

Ford, L. E., and R. Podolsky. 1972. Intracellular calcium movements in skinned muscle fibers. *J. Physiol. (Lond.).* 223:21–33.

Godt, R. E., and D. W. Maughan. 1977. Swelling of skinned muscle fibers of the frog. Experimental observations. *Biophys. J.* 19:103–116.

Godt, R. E., and D. W. Maughan. 1981. Influence of osmotic compression on calcium activation and tension in skinned muscle fibers of the rabbit. *Pfleugers Arch. Eur. J. Physiol.* 391:334–337.

Gordon, A. M., A. F. Huxley, and F. J. Julian. 1966. The variation of isometric tension with sarcomere length in vertebrate muscle fibers. *J. Physiol. (Lond.).* 184:170–192.

Gulati, J., and A. Babu. 1985. Critical dependence of calcium-activated force on width in highly compressed skinned fibers of the frog. *Biophys. J.* 48:781–787.

Kawai, M., R. N. Cox, and P. W. Brandt. 1981. Effect of Ca ion concentration on exponential processes in rabbit psoas fibers: evidence for the presence of two Ca-activated states of actin. *Biophys. J.* 35:375–384.

Kawai, M., and M. I. Schulman. 1985. Crossbridge kinetics in chemically skinned rabbit psoas fibers when the actin-myosin lattice spacing is altered by dextran T-500. *J. Muscle Res. Cell Motil.* 6:313–332.

Krasner, B., and D. W. Maughan. 1981. Dextran T-500 decreases skinned fiber width, tension and ATPase. *Biophys. J.* 33:27a. (Abstr.).

Matsubara, I., and G. F. Elliott. 1972. X-ray diffraction studies on skinned single fibers of frog skeletal muscle. *J. Mol. Biol.*72:657–659.

Ogawa, Y. 1985. Calcium binding to troponin C and troponin: effects of Mg^{2+}, ionic strength and pH. *J. Biochem (Tokyo).* 97:1011–1023.

Reuben, J. P., P. W. Brandt, M. Berman, and H. Grundfest. 1971. Regulation of tension in the skinned crayfish muscle fiber. I. Contraction and relaxation in the absence of Ca (pCa > 9). *J. Gen. Physiol.* 57:385–407.

Rome, E. 1967. Light and x-ray diffraction studies of the filament lattice of glycerol extracted rabbit psoas muscle. *J. Mol. Biol.* 27:591–602.

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