8. C. S. Chandler and F. J. Ballard, *Biochem. J.* **176**, 151 (1978); M. Rabinowitz and J. M. Fisher, *Biochim. Biophys. Acta* **91**, 313 (1969); Y. Klemes, J. D. Etlinger, A. L. Goldberg, *J. Biol. Chem.* **256**, 8436 (1981).
9. R. Uy and F. Wold, *Science* **198**, 890 (1977).
10. H. M. Ranney and H. Lehmann, in *The Red Blood Cell*, D. M. Surgenor, Ed. (Academic Press, New York, ed. 2, 1975), pp. 873–908.
11. E. Beutler, *Pharmacol. Rev.* **21**, 73 (1969).
12. C. C. Winterbaum and R. W. Carrel, *Nature (London)* **240**, 150 (1972).
13. F. S. Boches and A. L. Goldberg, *Science* **215**, 978 (1982).
14. H. Hsieh and E. R. Jaffe, in *The Red Blood Cell*, D. M. Surgenor, Ed. (Academic Press, New York, ed. 2, 1975), pp. 799–824.
15. B. Poole, S. Ohkuma, M. Warburton, in *Protein Turnover and Lysosome Function*, H. L. Segal and D. J. Doyle, Eds. (Academic Press, New York, 1978), pp. 43–58; P. Libby, S. Burstajn, A. L. Goldberg, *Cell* **19**, 481 (1980).
16. T. Aoyagi and H. Umezawa, in *Proteases and Biological Control*, E. Reich, D. B. Rifkin, E. Shor, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1975), p. 429; P. Libby and A. L. Goldberg, *Science* **199**, 534 (1978).
17. L. Waxman, in *Protein Turnover and Lysosome Function*, H. L. Segal and D. J. Doyle, Eds. (Academic Press, New York, 1978), pp. 363–377.
18. S. Speiser, M. J. Glucksman, J. D. Etlinger, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1682 (Abstr.) (1980).
19. F. S. Boches, Y. Klemes, A. L. Goldberg, *ibid.*, p. 1682.
20. F. S. Boches, L. Waxman, A. L. Goldberg, in preparation.
21. M. Muller, W. Dubiel, J. Rathmann, S. Rapoport, *Eur. J. Biochem.* **109**, 405 (1980).
22. R. Kirschner and A. L. Goldberg, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, in press.
23. A. L. Goldberg and F. S. Boches, unpublished observations.
24. G. McLendon and E. Radany, *J. Biol. Chem.* **253**, 6335 (1978).
25. T. P. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.* **50**, 733 (1957).
26. R. H. Rice and G. E. Means, *J. Biol. Chem.* **246**, 831 (1971).
27. Supported by grants from the National Institute of Neurological and Communicative Disorders and Stroke and from the Muscular Dystrophy Association to A.L.G. and by fellowships from the Massachusetts Heart Association and the Muscular Dystrophy Association to F.S.B. We thank J. Goldberg for technical assistance and R. Levine and M. Rush for help in preparing the manuscript.
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22 June 1981; revised 21 September 1981

Tonicity Effects on Intact Single Muscle Fibers: Relation Between Force and Cell Volume

Abstract. *Contraction of isolated, intact frog muscle fibers under increasing tonicity of the external solution was studied by adding (i) effectively impermeant sodium chloride and sucrose and (ii) permeant potassium chloride. Force of isometric contraction decreased as a function of tonicity, independent of the permeability of the solute. In contrast, cell volume changed with tonicity in impermeant solutes and was constant with potassium chloride. The results are evidence that ionic strength in the sarcoplasm directly influences the contraction mechanism. Also, the findings show that force development is unaffected by changes in fiber volume, suggesting that the force per cross-bridge is constant at different distances between the thin and thick myofilaments. Finally, in light of the length-force relation, the results support the idea that cross-bridges are independent force generators.*

Muscle force is generated in the cross-bridges that form between the thin and thick myofilaments during contraction. According to the current sliding filament models of muscular contraction, the cross-bridges act independently of each other. The length-force relation found for frog muscle fibers was important in establishing this conclusion because force levels closely corresponded to the filament overlap in the cross-bridge region at sarcomere lengths between 2.0 and 3.5 μm (*1*). However, the lattice volume of the intact muscle is constant with change in length; therefore the lateral (radial) distance between thin and thick filaments decreases with increasing sarcomere length (*2*, *3*). These observations indicate that the contraction mechanism is sensitive to radial separation and that this is a factor in the length-force relation (*1*). The explanation of this relation assumes that the decrease in radial spacing has no effect on force. Several attempts have been made to test this assumption indirectly (*4*–*6*). In more direct tests on demembranated frog muscle fibers, fiber width and the radial separation between the filaments were found to decrease in the presence of polyvinylpyrrolidone (*7*), and these effects were accompanied by changes in force and fiber stiffness (*8*). These results undermine the assumption that the decrease in radial spacing has no effect on force.

In an effort to clarify this issue, we investigated the influence of raising the tonicity of the external bathing medium on force development in isolated frog muscle fibers with the following strategy (*9*). We applied the principles of Boyle and Conway (*10*) to manipulate cell volume and ionic strength by adding (i) effectively impermeant NaCl and sucrose, which change the volume and the concentrations of intracellular solutes, and (ii) permeant KCl, which changes only the ionic concentrations in the cell. The fiber was maximally contracted under both conditions. The results provide direct evidence that force development in frog muscle is independent of changes in fiber volume and presumably, therefore, of changes in radial separation between the myofilaments.

Single intact fibers were isolated from the semitendinosus muscle in the frogs *Rana pipiens pipiens* and *Rana temporaria* (*11*). Resting sarcomere length was 2.2 to 2.3 μm (*12*, *13*). Each fiber was activated by a rapid temperature step in the presence of caffeine (*14*), achieved by transfer of the fiber from a chamber at 25°C to a chamber at 0°C. The transfer was usually made within 2 seconds (*15*), and the temperature step was practically instantaneous. This technique produced fiber activation in all solutions, including KCl, in which muscle fibers are depolarized and therefore electrically inexcitable.

Figure 1A shows two activation-relaxation cycles of one fiber in two solutions. The fiber, which was relaxed at 25°C, contracted quickly at 0°C, the force reaching a stable plateau. The force response in a solution with a tonicity (T, the total molarity of all solutes) of 1.0 (left-hand trace) is compared with a typical record obtained in a hypertonic solution (1.8 T with KCl) (right-hand trace). The response is nearly the same in each case except that the force level is lower in the hypertonic solution. Similar responses were also seen with NaCl and sucrose. It generally was possible to record 15 to 20 isometric contraction cycles in a fiber with a change from the original force level of less than 10 percent and no significant alteration in the laser diffraction pattern. In hypotonic solutions the force plateau was less stable. In the presence of high calcium, the force plateau for fibers from *R. temporaria* was somewhat more stable than that for fibers from *R. pipiens pipiens*.

The force response with NaCl and sucrose is shown in Fig. 1B. Tension measurements were made at the plateau region of the force response in normal and hypertonic solutions and at the response peak in hypotonic solutions. There is a linear decrease in force with increasing tonicity in the range 0.6 to 1.8 T. The average force at 0.6 T is about 1.3 times greater than that at 1.0 T, and at 1.4 T the relative force is 0.75. These results agree with the corresponding mean values for tetanic force levels obtained with electrical stimulation in single fibers up to 1.4 T (*16*). Also, the results extend the effect of tonicity (with sucrose) to 1.8 T; the relative force in this case is 0.45.

Figure 1C shows the force response

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with KCl. As with NaCl and sucrose, the force decreases with increasing tonicity. The effect was the same up to 2.2 T.

Figure 1D combines the results shown in B and C of Fig. 1. The same linear relation describes the data for permeant and impermeant solutes. Force decreases by a factor of 6 or more over the entire tonicity range. The results show that the effect of tonicity (from 0.6 to 2.2 T) on force is independent of solute permeability. Also, because the same relation between tonicity and force applies throughout, the factors underlying the tonicity effect are probably the same in the hypertonic and hypotonic ranges.

The relation between fiber width and tonicity is shown in Fig. 2. With impermeant NaCl (or sucrose), fiber width decreases progressively with increasing tonicity in the range 0.6 to 1.8 T. In experiments in which KCl was used to change the tonicity, fiber width remained constant up to 2.2 T. Since sarcomere length was fixed at 2.2 μm, these results reflect the influence of tonicity on cell volume. Cell volume as a function of estimated inverse osmolality is plotted in the inset in Fig. 2. The data points for impermeant solutes could be fitted with two lines, suggesting that separate van't Hoff relations apply in the hypotonic and hypertonic solutions (sloping solid lines in the inset in Fig. 2) (*13, 17*). These results show that cell volume decreases with increasing external NaCl or sucrose and that volume is constant with perme-

Table 1. Summary of the effects of tonicity on relative force, relative volume, and radial separation. Numbers in parentheses indicate the number of determinations; other values for volume and force are means ± standard errors.

Measure	Impermeant solute (NaCl or sucrose)		Permeant solute (KCl)	
	1.4 T	1.8 T	1.4 T	1.8 T
Relative force*	0.75 ± 0.01 (12)	0.45 ± 0.01 (7)	0.73 ± 0.03 (5)	0.46 ± 0.04 (5)
Estimated relative volume†	0.74 ± 0.01 (25)	0.49 ± 0.01 (5)	1.00 ± 0.01 (5)	1.00 ± 0.01 (5)
Estimated radial separation‡ (nm)	9.6	6.1 to 8.0§	13.2	13.2

*Ratio of the measurement in a solution of given tonicity to that in standard Ringer solution (1.0 T). †Since the resting sarcomere length was fixed at 2.2 to 2.3 μm, the relative fiber volume in a solution was calculated as the square of the relative width. ‡The surface-to-surface radial separation between thin and thick filaments is taken as 13.2 nm at the resting sarcomere length of 2.2 μm and at 1.0 T. This spacing can be derived from equatorial patterns in the x-ray diffractions and by taking 14 nm and 7 nm as thicknesses for the thick and thin filaments, respectively. It was assumed that a relative change in fiber width squared corresponds to a similar change in the lattice volume for a circular cross section. §The lower limit (6.1 nm) is estimated from the data in this report on single fibers; the upper limit (8 nm) is from x-ray diffraction data on the whole muscle.

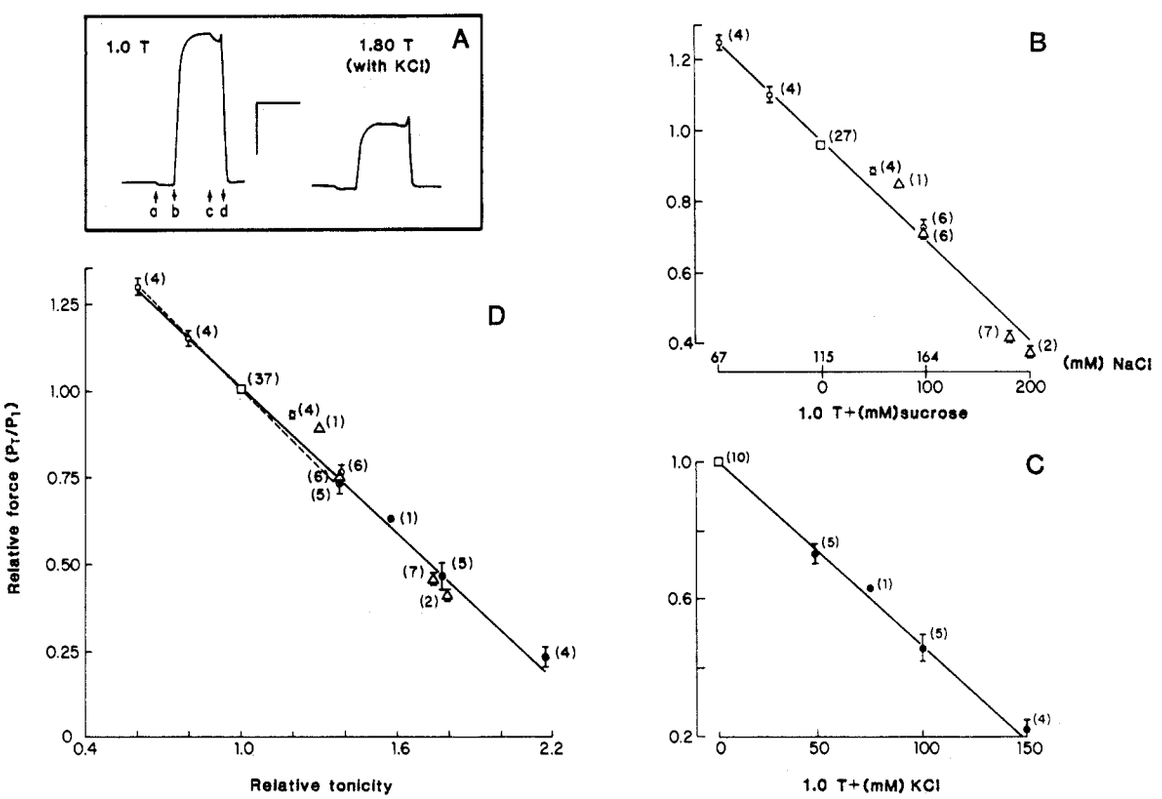

Fig. 1. Effects of tonicity on force development in frog muscle fibers. (A) Activation of a single fiber by a temperature step in the presence of 1 m*M* caffeine in solutions at 1.0 or 1.8 T. After equilibration at 25°C, the fiber (which is attached at one end to an Akers AE 801 force transducer and at the other to a hook) is (*a*) removed from the solution, (*b*) immersed in the solution at 0°C, (*c*) removed from the 0°C chamber, and (*d*) returned to the 25°C chamber. The force level at 1.8 T is 0.43 times that at 1.0 T. The vertical marker is 0.5 mN and the horizontal marker is 5 seconds. Mean force level (P_1) at 1.0 T for all the fibers used in this study was 202 ± 10 kN/m^2 (range, 152 to 302) for *Rana pipiens pipiens* and 231 ± 15 kN/m^2 (range, 168 to 311) for *Rana temporaria*. Fiber diameter ranged from 54 to 98 μm in *R. pipiens pipiens* and from 57 to 184 μm in *R. temporaria*; fiber length between tendons ranged from 9.51 to 16.33 mm. (B) Effect on force development of varying the concentration of NaCl or adding sucrose. Experimental solutions with a tonicity of 1.0 contained 10 m*M* $CaCl_2$, 100 m*M* NaCl, 2.5 m*M* KCl, and 10 m*M* imidazole or 1.8 m*M* $CaCl_2$, 115 m*M* NaCl, 2.5 m*M* KCl, and 3.4 m*M* phosphate buffer. The *p*H was 7.00 ± 0.01 at room temperature. (C) Effect of adding KCl on force development. (D) Combined data from (B) and (C). In (B), (C), and (D), P_1 is maximum tetanic force at 1.0 T and P_T is force in the test solution. The solid lines are least-squares fits to the points. Numbers in parentheses indicate the number of single fibers tested in each case. A total of 37 fibers from the two frog species was used. The dashed line is the ionic strength–relative force relation from an earlier study (*20*). Symbols: (○) NaCl solution, (△) sucrose, (●) KCl, and (□) control.

ant KCl (*10, 18*). Thus, whereas the effects of increasing tonicity on contraction force are independent of solute permeability, the effects on fiber width, and therefore fiber volume, are dependent on solute permeability (Table 1). Therefore the force mechanism in the intact fiber at constant sarcomere length is independent of cell volume.

A given variation in tonicity produces roughly the same change in intracellular ionic strength (*19*), which might explain the observed effect of tonicity on force. This is seen by comparing the present results for intact fibers (solid line in Fig. 1D) with the relation between maximal calcium-activated force and ionic strength for demembranated fibers at 0° to 6°C over an equivalent tonicity range (0.6 to 1.4 T) (dashed line in Fig. 1D) (*20*). The relation between tonicity and relative force is nearly the same for the two preparations. These results suggest that the effect of tonicity on force development in intact muscle fibers is produced by the associated changes in ionic concentrations in the cell. This is additional evidence (*21*) that the cross-bridge force-generating mechanism in frog muscle is sensitive to ionic strength.

The estimates of fiber volume with sucrose and NaCl at a sarcomere length of 2.2 μm are similar to previous estimates from the lattice dimensions in x-ray diffraction studies of toad and frog whole muscles (*22*), suggesting that the gross changes in volume are distributed uniformly throughout the cell (compare the sloping solid and dashed lines with the dotted line in the inset in Fig. 2). Therefore, the present findings for cell volume may be used to compute radial separation distances between the thin and thick filaments at the various tonicities. Interfilament separation at 1.0 T has been estimated from combined x-ray diffraction and electron microscopic measurements as being about 13.2 nm (Table 1) (*3*). Separation distances in impermeant solutes at 1.4 and 1.8 T are estimated at 9.6 and 6.1 to 8 nm, respectively, and at 13.2 nm in permeant KCl at 1.4 and 1.8 T (Table 1). On the basis of these estimates, the conclusion that force development is independent of cell volume can be extended to suggest that the decrease in interfilament separation from 13.2 to ~6 nm has no influence on the force-generating mechanism in frog muscle. The results with the hypotonic solution indicate that the force mechanism is also insensitive to increases in interfilament separation up to 17.9 nm. These findings contrast with results obtained by the use of polyvinylpyrrolidone or other macromolecules on demembranated fibers (*8, 24*). Such agents, however, may have nonspecific influences (as, for example, on ionic strength or viscosity in the lattice) (*23*) besides their presumed osmotic effect.

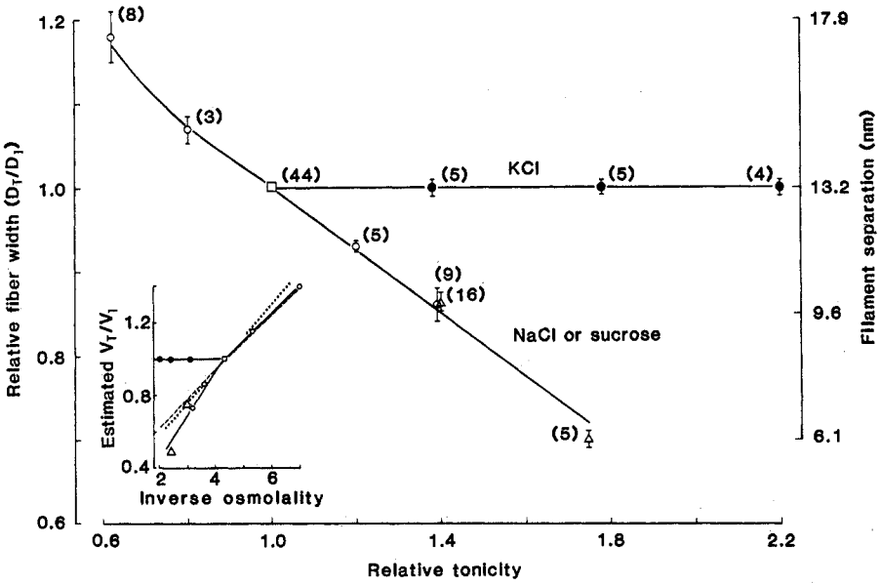

Fig. 2. Effects of tonicity on fiber width. A total of 44 fibers from the two frog species was used; D_1 is mean fiber width at 1.0 T, D_T is mean fiber width at test tonicity, and V_1 and V_T are the respective estimated volumes (assuming a circular cross section). Width was measured with a light microscope (×80) by observing the fiber from above at five locations along its length. In three experiments, additional observations were made at 1.0 and 1.4 T (with sucrose) at a 90° angle from the top. The effects of tonicity were similar in the two planes. The inset shows fiber volume as a function of inverse osmolality. The estimated osmolality of solution at 1.0 T was 0.233 osmole per kilogram. Solid lines in the inset are least-squares fits to the data; dashed lines are those from Blinks (*13*) in the appropriate tonicity range. The dotted line is from x-ray diffraction measurements on whole muscles. Note that the gross volume shifts nearly parallel those occurring in the myofilament lattice.

Since the effect of tonicity on force can be explained almost entirely by the expected changes in intracellular ionic concentrations, these findings show that the force per cross-bridge at a given tonicity is constant over wide changes in separation distances between the thin and thick myofilaments. At all radial distances, the major force component of a cross-bridge probably is parallel to the longitudinal axis of the sarcomere. In conjunction with the known length-force relation, these findings strongly support the idea that cross-bridges act as independent force generators.

A common feature of contraction mechanisms (*3, 25*) is that the attached cross-bridges rotate from 90° to 45° angular orientation (*26*) during the turnover cycle. Therefore, considering the size of myosin subfragment 1 (length, 12 to 19 nm; width, 4 to 6 nm) (*27*), the finding that force per cross-bridge is unaffected by the decrease in radial spacing down to ~6 nm (Table 1) is highly significant. It could suggest that the rotational or azimuthal movement of subfragment 1 is blocked under the isometric conditions. Another possibility is that rotation of the cross-bridges is only a secondary step in the process of force development. A different possibility that might affect the steric hindrance during rotation is a progressive decrease in thickness of the myofilaments with lattice shrinkage in hypertonic solutions. Previous studies of fibers in the state of rigor suggested that the cross-bridge orientation remained unchanged when force was applied externally (*28*). The present results seem to support this; furthermore, they suggest that rotation of a cross-bridge may not be an essential step in isometric force development.

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References and Notes

1. A. M. Gordon, A. F. Huxley, F. Julian, *J. Physiol. (London)* **184**, 170 (1966).
2. G. F. Elliott, J. Lowy, C. R. Worthington, *J. Mol. Biol.* **6**, 295 (1963).
3. H. E. Huxley, *Science* **164**, 1356 (1969).
4. K. A. P. Edman and K. E. Andersson, *Experientia* **24**, 134 (1968).
5. E. W. April and P. W. Brandt, *J. Gen. Physiol.* **61**, 490 (1973).
6. A. F. Huxley, *Reflections on Muscle* (Princeton Univ. Press, Princeton, N.J., 1980).
7. R. E. Godt and D. W. Maughan, *Biophys. J.* **28**, 391 (1979); Y. E. Goldman, I. Matsubara, R. Simmons, *J. Physiol. (London)* **295**, 81P (1979).
8. D. W. Maughan and R. E. Godt, *Pfluegers Arch.* **390**, 161 (1981); Y. E. Goldman and R. M. Simmons, *Biophys. J.* **21**, 86 (Abstr.) (1978).

9. J. Gulati and A. Babu, *Biophys. J.* **37**, 185a (1982).
10. P. J. Boyle and E. J. Conway, *ibid.* **100**, 1 (1941).
11. In Ringer solution the isolated fiber exhibited vigorous all-or-none twitch responses when stimulated with a 40-V, 10-msec pulse across platinum point electrodes separated by a 1-cm gap. The fiber was examined with a Zeiss compound microscope (×430) for sarcomere uniformity along its length.
12. Sarcomere length was adjusted by displaying the first-order laser diffractions of the fiber, near the two ends and in the middle, on a screen 15 cm above the fiber. Length was adjusted to 2.2 to 2.3 μm at 1.0 T and checked in each test solution. The overall intensity of the diffraction pattern decreased reversibly in hypertonic solutions contuining NaCl and sucrose (*13*), but the width of the pattern appeared unchanged in different solutions.
13. J. Blinks, *J. Physiol. (London)* **177**, 42 (1965); R. Rudel and F. Zite-Ferencvy, *Biophys. J.* **30**, 507 (1980).
14. T. Sakai, *Jikeikai Med. J.* **12**, 88 (1965); _____ and T. Yoshiaka, *Jpn. J. Physiol.* **23**, 135 (1973). Our transferring and solution-changing device was similar to that described by Gulati and Podolsky (*21*), but the present unit contained only two chambers and the temperature of each chamber was controlled individually with thermoelectric modules and Cambion bipolar controllers.
15. In these experiments, the fiber was equilibrated in standard Ringer solution with 1 m*M* caffeine for 30 minutes at 25°C and then subjected to the first temperature step. The fiber was allowed to rest for 15 minutes at 25°C before the next temperature step was applied. Three such activation-relaxation cycles were made at 1.0 T. Next, the fiber was equilibrated in NaCl or sucrose (with caffeine) for 30 minutes and then activated. Two additional activations were made in the same solution, with 12- to 15-minute rest periods at 25°C between activations. The fiber was then returned to the control solution. If the force response at 1.0 T was reversible to within 5 percent, the experiment was continued; otherwise, the fiber was discarded. The mean of force levels at 1.0 T was compared with the average value in the test solution, and the ratio was taken as a data point. Reversibility became a problem in NaCl above 1.4 T. This was avoided with sucrose, so the data point at 1.8 T is shown only with sucrose. As an additional precaution, preequilibration at 1.8 T (25°C) was reduced to 2 to 3 minutes; after activation the fiber was returned to the solution at 1.4 T instead of 1.8 T and the cycling was continued as described above. There was an appearance of resting "contracture" tension, lasting 60 to 80 seconds, each time the fiber was placed in sucrose solution at 1.8 T and 25°C [J. Lannergren and J. Noth, *J. Gen. Physiol.* **61**, 158 (1973)]. Fibrillation [T. G. Sato, *Annot. Zool. Jpn.* **27**, 157 (1954)] was always observed in sucrose solution at 1.8 T and only occasionally at 1.4 T; the onset of fibrillation was associated with the resting contracture tension. The fibers that fibrillated at 1.4 T survived less well during further experimentation. With KCl, reversibility may take 2 to 5 hours [R. H. Adrian in (*18*)] and was not attempted; the datum was recorded only if the force level was reproduced to within 5 percent in three consecutive cycles. The successful fiber was subjected to higher tonicity KCl and the same procedure was followed. Every solution in this series contained 1 m*M* caffeine, producing maximal activation. This was checked by measuring contraction force in 2 m*M* caffeine. The results were within 10 percent of those in 1 m*M* caffeine.
16. K. A. P. Edman and J. C. Hwang, *J. Physiol. (London)* **269**, 255 (1977).
17. The data point representing the volume in sucrose solution at 1.8 T deviated slightly from the van't Hoff linear relation describing the other results at lower tonicity. This is not surprising, since the fiber cross section becomes progressively less circular with increasing tonicity (*13*) and the width is a less reliable estimate of the volume.
18. L. G. Palmer and J. Gulati, *Science* **194**, 521 (1976); R. H. Adrian, *J. Physiol. (London)* **151**, 154 (1960).
19. With NaCl and sucrose the change in the intracellular milieu presumably is affected by water movement across the cell membrane. With KCl there is a net movement of potassium and chlorine ions into the cell to maintain the Donnan equilibrium (*10, 18*).
20. J. Gulati and R. J. Podolsky, *J. Gen. Physiol.* **78**, 233 (1981).
21. _____, *ibid.* **72**, 701 (1978).
22. E. Rome, *J. Mol. Biol.* **37**, 331 (1968); B. M. Millman, T. J. Racey, I. Matsubara, *Biophys. J.* **33**, 189 (1981).
23. M. Endo, T. Kitawa, M. Lino, Y. Kakuta, in *Cross-bridge Mechanisms in Muscle Contraction*, H. Sugi and G. H. Pollack, Eds. (University Park Press, Philadelphia, 1979), pp. 365–376.
24. R. J. Podolsky, G. R. S. Naylor, T. Arata, *J. Gen. Physiol.* **78**, 3 (Abstr.) (1981).
25. A. F. Huxley and R. M. Simmons, *Nature (London)* **233**, 533 (1971); E. Eisenberg and T. L. Hill, *Prog. Biophys. Mol. Biol.* **33**, 55 (1978).
26. M. K. Reedy, K. C. Holmes, R. T. Tregear, *Nature (London)* **207**, 1276 (1965).
27. R. Mendelson and K. M. Kretzschmar, *Biochemistry* **19**, 4102 (1980); A. Elliott and G. Offer, *J. Mol. Biol.* **123**, 505 (1978).
28. C. G. dos Remedios, R. C. G. Millikan, M. F. Morales, *J. Gen. Physiol.* **59**, 103 (1972); G. R. S. Naylor and R. Podolsky, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5559 (1981); R. Cooke, *Nature (London)* **294**, 570 (1981).
29. This work was supported by a research grant from the Muscular Dystrophy Association and by grants AM 26632 and HL 18824 from the National Institutes of Health.

28 September 1981; revised 9 November 1981

Different Synaptic Location of Mianserin and Imipramine Binding Sites

Abstract. *The high-affinity binding sites for mianserin and imipramine appear to be located in different neurons of rat brain. Studies in which lesions were produced with 5,7-dihydroxytryptamine and other studies in which the 5-hydroxytryptamine content was decreased with* p*-chlorophenylalanine indicate that some of the imipramine binding sites are on serotonin axon terminals and others are on nonserotonergic synapses. The sites that bind mianserin are on postsynaptic serotonin sites as well as on synapses of other neuronal systems.*

Several clinically active antidepressants, with differing chemical structures, reduce the number of synaptic recognition sites for catecholamines (*1–3*), serotonin (*4*), or other putative neurotransmitters in the rat brain. Because both the down-regulation of recognition sites and the beneficial effects on symptoms of depression are apparent only after about 2 weeks of treatment with antidepressants, a relation between these two drug effects has been proposed (*1, 2, 5, 6*). Some antidepressants block the reuptake of putative neurotransmitters, thereby enhancing the content of neurotransmitters in the synaptic cleft (*7–10*); this increase could participate in the down-regulation of transmitter recognition sites (*3, 11*) because it prolongs the occupancy of neurotransmitter recognition sites by endogenous agonists.

Inhibition of transmitter uptake, however, is not essential to elicit down-regulation or to exert beneficial effects in endogenous depression, since atypical antidepressants, such as iprindole, bupropion, and mianserin, relieve depression and down-regulate recognition sites for neurotransmitters without blocking the reuptake of monoamines by nerve terminals (*12, 13*). Cocaine blocks monoamine uptake (*14*), but does not regulate transmitter recognition sites or elicit beneficial effects in depression. Whatever their action on monoamine uptake, antidepressants bind to crude synaptic membranes stereospecifically and with high affinity (*15–19*).

We investigated the relation between specific synaptic mechanisms and the high-affinity binding of [^{3}H]imipramine and [^{3}H]mianserin. We induced lesions pharmacologically in neurons storing a specific neurotransmitter (serotonin) and compared the binding characteristics of imipramine and mianserin before and after production of the lesion. We confirmed that imipramine binding sites are located in serotonergic synapses (*20, 21*) and demonstrated that there are differences in the brain location of imipramine and mianserin binding sites. Moreover, the binding sites of imipramine and mianserin are not limited to synapses that can be labeled with a specific putative neurotransmitter as a biochemical marker.

Male Sprague-Dawley rats weighing 180 to 220 g (Zivic Miller) were anesthetized with pentobarbital; lesions were produced by injecting 5,7-dihydroxytryptamine (5,7-DHT) stereotaxically either in the lateral ventricle or in the nucleus raphe medianus (A = 0.6; L = 0.0; V = 3.2) (*22*). Sham-lesioned animals were given injections of the vehicle solution, 0.01 percent ascorbic acid in saline. Animals in both groups were injected intraperitoneally with desmethylimipramine (DMI) (25 mg/kg) 40 minutes before the injection of neurotoxin (or vehicle) to prevent the uptake of the neurotoxin into noradrenergic axons and thus minimize the degeneration of these terminals (*23*). Lesioned and sham-lesioned rats were killed 10, 20, and 30 days after operation. In other studies, rats were given intraperitoneal injections of *p*-chlorophenylalanine (PCPA), 300 mg/kg daily for 3 days, then 100 mg/kg every other day for 15 days. At the end of each treatment, rats were killed by decapitation, and the brains were removed, dissected over ice, and stored at −70°C until assayed. Serotonin (5HT)

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