The Regulation of Catch in Molluscan Muscle

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ABSTRACT Molluscan catch muscles are smooth muscles. As with mammalian smooth muscles, there is no transverse ordering of filaments or dense bodies. In contrast to mammalian smooth muscles, two size ranges of filaments are present. The thick filaments are long as well as large in diameter and contain paramyosin. The thin filaments contain actin and appear to run into and join the dense bodies. Vesicles are present which may be part of a sarcoplasmic reticulum. Neural activation of contraction in *Mytilus* muscle is similar to that observed in mammalian smooth muscles, and in some respects to frog striated muscle. The relaxing nerves, which reduce catch, are unique to catch muscles. 5-Hydroxytryptamine, which appears to mediate relaxation, specifically blocks catch tension but increases the ability of the muscle to fire spikes. It is speculated that *Mytilus* muscle actomyosin is activated by a Ca^{++}-releasing mechanism, and that 5-hydroxytryptamine may reduce catch and increase excitability by influencing the rate of removal of intracellular free Ca^{++}.

The morphology of molluscan catch muscle is best understood by comparing it with other known types of muscle. The transverse ordering of filaments characteristic of vertebrate striated muscle is often seen in molluscs (Fig. 1 *A*). Alignment may also be spiral, as diagrammed in Fig. 1 *B*. However, very few mollusc muscles have a Z band. Separate dense bodies are seen in place of a continuous Z line. These dense bodies, like Z, may be associated with abundant vesicular structures as, for instance, in the heart muscle of *Sepia* and *Archachatina* (1, 2). The large filaments can be similar in length and diameter to the large filaments of vertebrate striated muscle (Fig. 1 *A*) or, as in the *Crassostrea* adductor (3), may be thicker and longer (Fig. 1 *B*).

Vertebrate smooth muscle shows no transverse ordering of filaments or dense bodies (Fig. 2 *A*). The muscle fiber diameter is small, approximately 2 μ. Only one size range of filaments is present. These are about 50 A in diameter. A surface reticulum is abundant, and occasional internal vesicles are observed.

The molluscan smooth catch muscle fiber is 5 μ or more in diameter, large compared to the mammalian smooth muscle fiber. There is no transverse alignment of filaments or dense bodies (Fig. 2 *B*). In contrast to mammalian

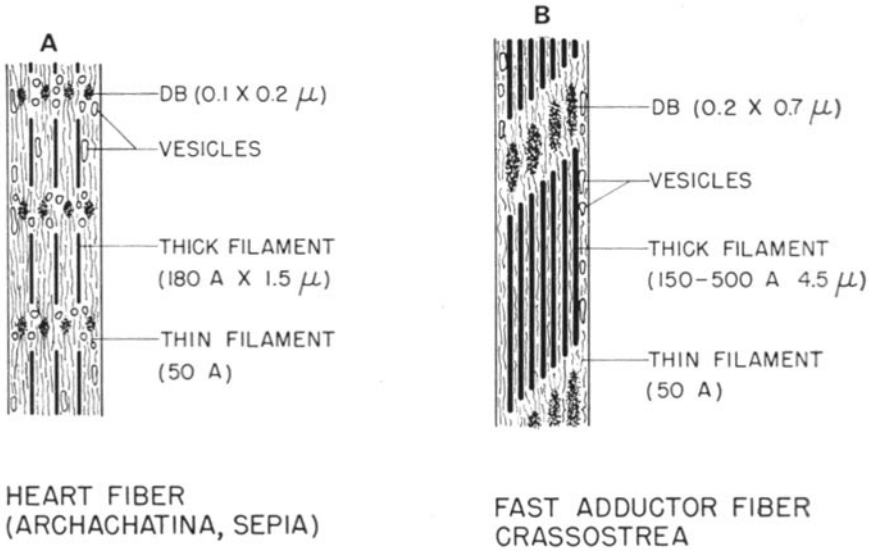

FIGURE 1. Diagram of the morphological features of molluscan "striated" muscle. *A*, transversely "striated" heart muscle fibers of *Archachatina* and *Sepia*. *B*, obliquely "striated" fast adductor muscle fibers of *Crassostrea*. Fiber diameter in both cases, 2 μ. DB = dense body.

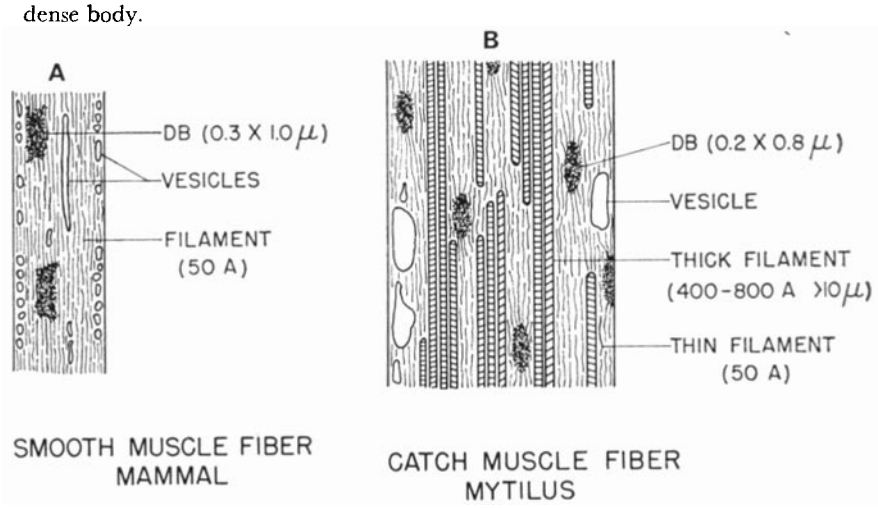

FIGURE 2. Diagram of the morphological features of types of smooth muscle. *A*, mammalian smooth muscle; fiber diameter, 2 μ. *B*, molluscan smooth catch muscle; fiber diameter, 5 μ or larger. DB = dense body.

smooth muscles, two filament types are observed (4).[1] The thick filaments are long compared to all other muscle filaments (up to 30 μ) (4). Only surface vesicles are seen, and there are notably few vesicles relative to the fiber volume.[1]

[1] Z. Gori and K. R. Porter. Unpublished observations.

In Fig. 3, an electron micrograph by Dr. Zina Gori, from the laboratory of Dr. K. R. Porter, fibers of a catch muscle, the anterior byssus retractor muscle of *Mytilus*, are seen in cross-section. The cells are closely packed, with collagen fibers abundant in the intercellular space. Vesicles are at the periphery of muscle cells. The dense bodies are often intimately associated with the

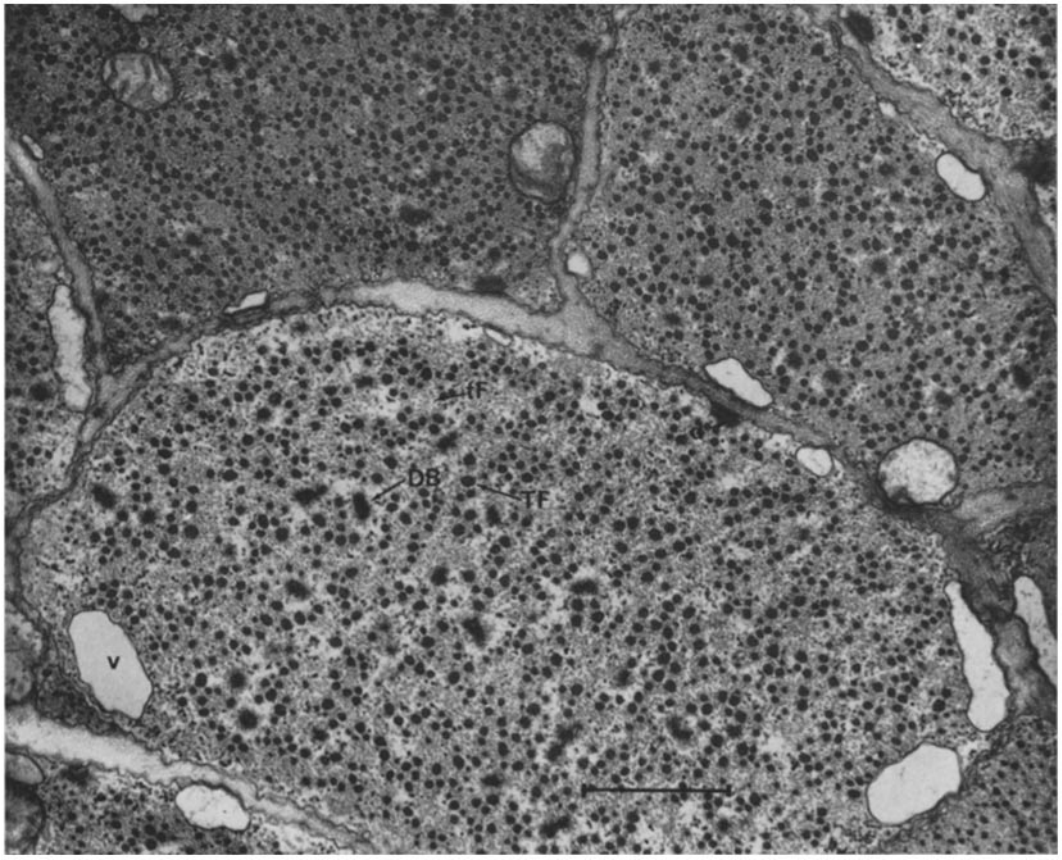

FIGURE 3. Electron micrograph by Dr. Zina Gori of the anterior byssus retractor muscle of *Mytilus*. V = vesicle, DB = dense body, tF = thin filaments, TF = thick filament, d = half-desmosome. Calibration line: 1 μ.

membrane, forming half-desmosomes. Glycogen granules are abundant. Dr. Gori has calculated that 92% of the thick filaments are between 300 and 800 A in diameter. The thick filaments are surrounded by thin filaments. In many areas, only thin filaments are seen.

Dr. Gori's longitudinal section of *Mytilus* muscle in Fig. 4 displays the relationship of thin filaments to the dense body. The half-desmosomes formed by dense bodies at the membrane can also be seen. The length of the thick filaments is noteworthy.

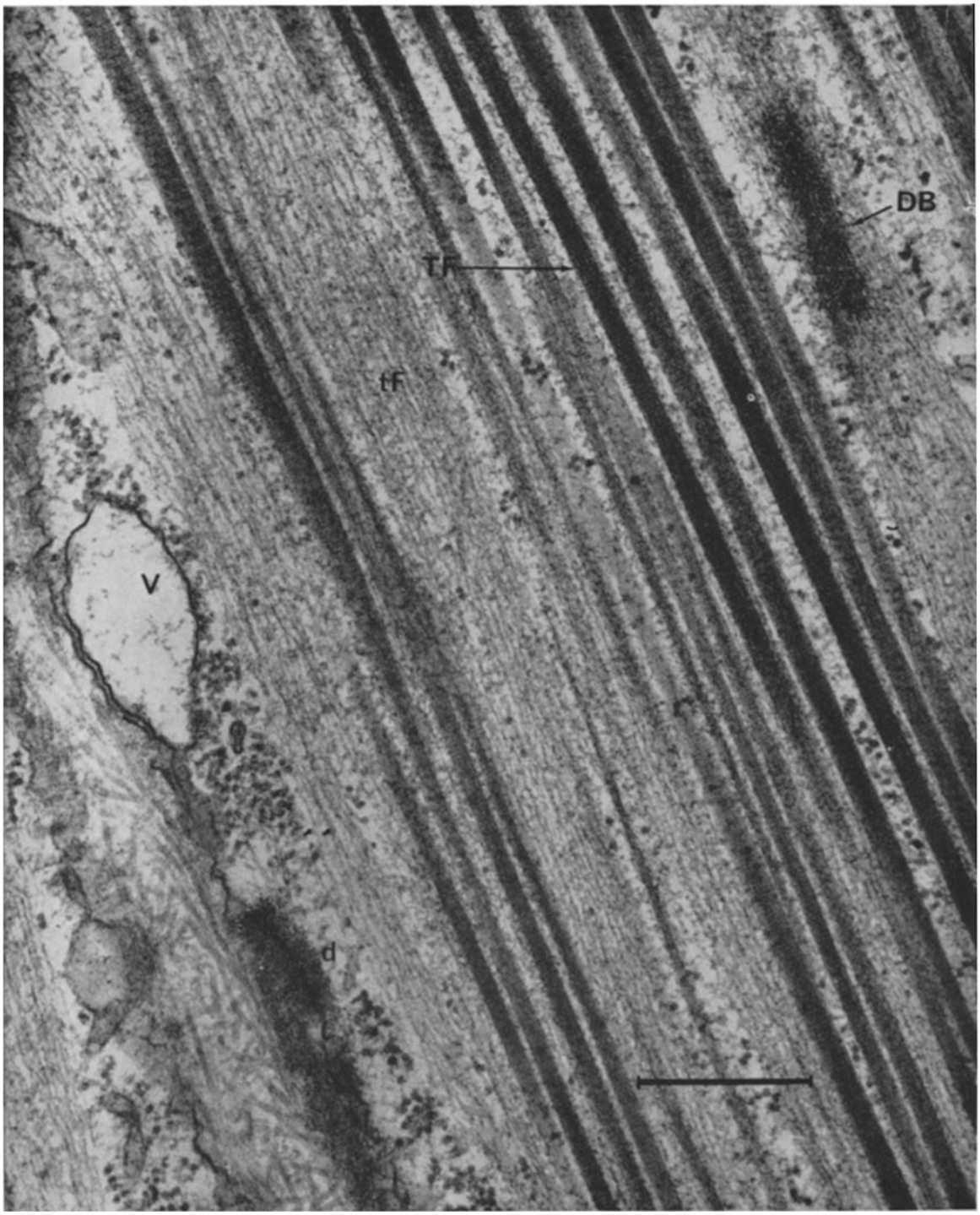

FIGURE 4. Electron micrograph by Dr. Zina Gori of the anterior byssus retractor muscle of *Mytilus*. V = vesicle, DB = dense body, tF = thin filaments. TF = thick filament, d = half-desmosome. Calibration line: 0.5 μ.

The relationship of thin filaments and dense body is seen in detail in Fig. 5; that is, the thin filaments appear to run into and join the dense body. As Hanson and Lowy have suggested, the dense bodies appear to be equivalent to Z (3). The elaborate periodic structure of the thick filaments may be related to the distribution of bridges along them.

The *Mytilus* catch muscle, when small bundles are prepared which are totally isolated from ganglion cells, responds to brief stimulating pulses by a

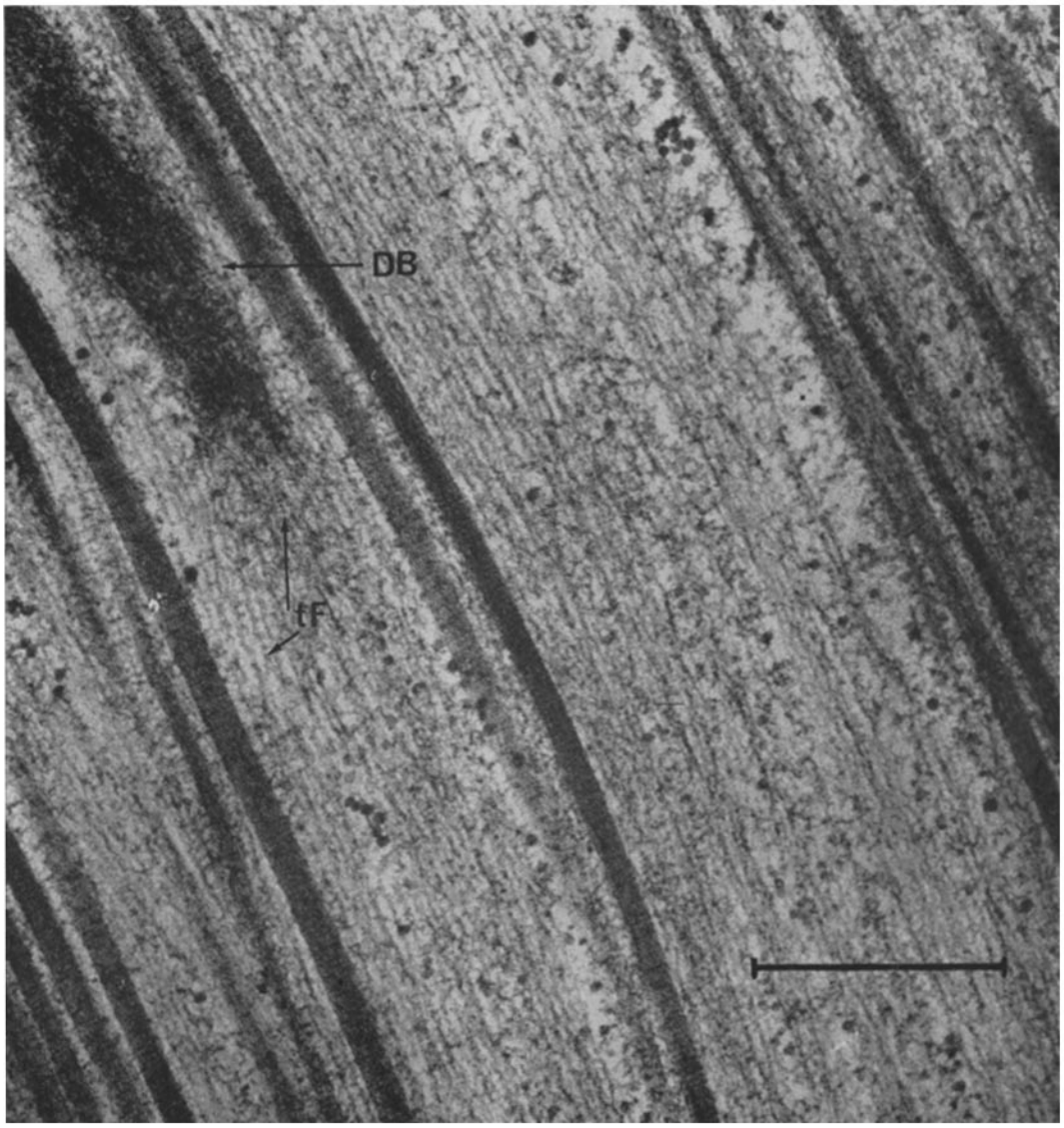

FIGURE 5. Electron micrograph by Dr. Zina Gori of the anterior byssus retractor muscle of *Mytilus*. DB = dense body, tF = thin filaments. Calibration line: 0.5 μ.

brief twitch or tetanic contraction as diagrammed in Fig. 6. The maximum tension developed in tetanus is 10–12 kg/cm², 3 to 4 times as great as in any known noncatch muscle (5). If tension is developed in this muscle by a sliding filament mechanism (4), then the large tension developed is presumably due to the great length of the long filaments and hence the greater number of cross-bridges.

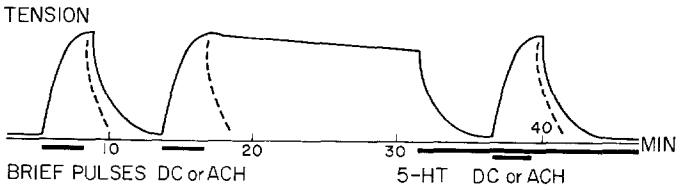

FIGURE 6. Diagram of contractile responses of a small bundle from the anterior byssus retractor muscle of *Mytilus*. In response to repetitive brief pulses (0.5–10 msec duration), a brief tetanus is seen. Prolonged cathodal direct current pulses (DC) or acetylcholine (ACH) produce a contraction which persists after stimulation has ceased: catch. In the presence of 5-hydroxytryptamine (5-HT), contraction no longer persists after stimulation; catch is abolished. Solid line, tension; interrupted line, active state.

When acetylcholine or long cathodal pulses are used to stimulate, tension is sustained after stimulation has ceased. Active state, as measured by quick release, is absent during this sustained tension (5, 6). This is the catch state. 5-Hydroxytryptamine (5-HT) specifically relaxes catch. Subsequent activation in the presence of 5-HT is normal but catch is reduced or abolished, in inverse proportion to the logarithm of the concentration of 5-HT (7, 8).

TABLE I

COMPOSITION OF ANTERIOR BYSSUS RETRACTOR MUSCLE AS A PERCENTAGE OF TOTAL EXTRACTED PROTEIN

Protein	Percentage
Actomyosin	35
Paramyosin	32
Other sarcoplasmic proteins	33

All muscles which, like *Mytilus*, display catch invariably contain large amounts of a specific protein, paramyosin, in addition to actin and myosin (9) (Table I). Paramyosin is a major constituent of the thick filaments.

Some morphological details of the *Mytilus* muscle and the presence of relatively large amounts of paramyosin are typical of catch muscle. The question then arises whether any features of the neural control of the *Mytilus* muscle are uniquely related to catch. The answer is that the activation of contraction seems very similar to activation in mammalian smooth muscles,

and in some respects to frog "fast" striated muscle, while the relaxing system, which turns off catch, is unique. This is best summarized by a brief outline of what is now known about excitation of contraction and relaxation of the *Mytilus* muscle.

The resting potential is 65 mv, somewhat higher and more stable than such mammalian smooth muscles as the vas deferens. Rhythmic fluctuations of membrane potential are absent. Miniature junction potentials are infrequent except just after stimulation (10).

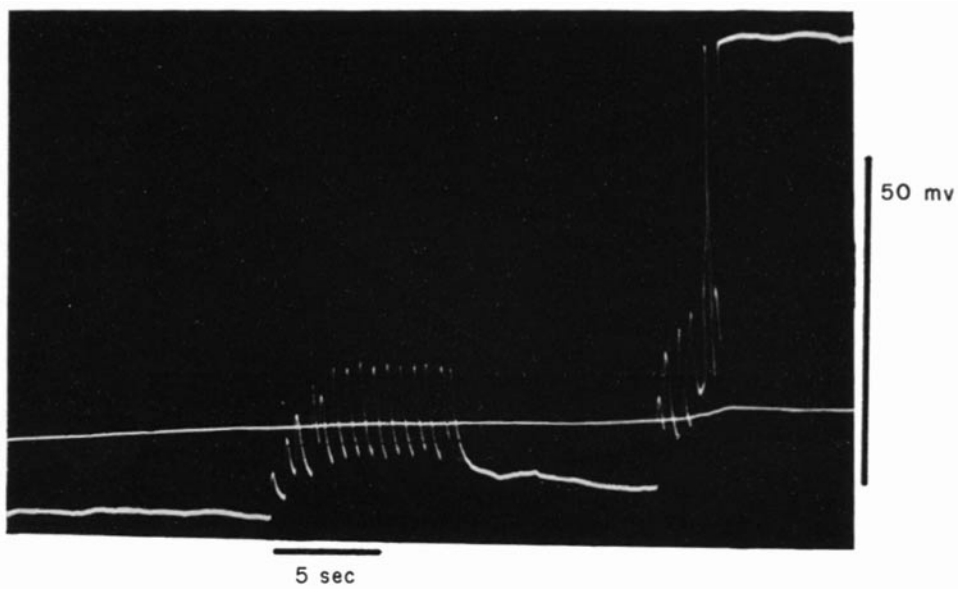

FIGURE 7. Responses of *Mytilus* muscle to repetitive neural stimulation. Upper trace: tension. Lower trace: membrane potential. Pulses 0.5 msec duration, 2.4/sec and 2.6/sec, respectively. *Figure reprinted by permission from The London Journal of Physiology, 1967 b. In press (10).*

Stimulation of the nerve supply gives rise to depolarizing junction potentials with a maximum amplitude of 25 mv (10). The amplitude and rate of rise of the junction potentials increase with stimulus strength. This indicates multiple innervation of individual muscle cells or that mediator liberated along nerves diffuses to many fibers, again similar to the vas deferens.

When stimuli are repeated at frequencies of 1/sec or more, successive junction potentials summate and also show facilitation. When the junction potential depolarizes the membrane to a potential of 35–40 mv, a spike is fired, a strong contraction usually occurs, and most often the microelectrode is pulled out. In Fig. 7 a spike was recorded just before the electrode was dislodged.

The spike is not blocked by high concentrations of tetrodotoxin, suggesting

that, like mammalian smooth muscle and crustacean muscle, it may not depend on a changed sodium permeability (10–12).

Contraction in response to neural stimulation depends on whether a spike is fired. As seen in Fig. 8, when the membrane potential is reduced by increasing K^+ in the bathing medium (in place of Na^+) no tension is recorded until K^+ has been increased above 30 mM. The membrane potential must approach 40 mv before tension is recorded. Only the firing of a spike can bring the membrane potential to the level necessary to produce tension.

Thus *Mytilus* smooth catch muscle closely resembles mammalian smooth muscles and "fast" vertebrate striated muscle in not showing graded responses

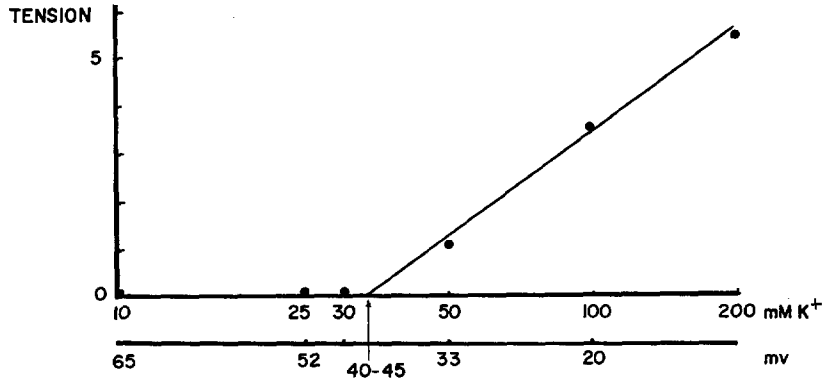

FIGURE 8. Graph of tension in *Mytilus* muscle as a function of applied external K^+ and measured membrane potential. Extrapolation of tension curve indicates that tension arises at a membrane potential between 40 and 45 mv.

to low levels of depolarization. When depolarization exceeds a critical level, tension developed is linearly proportional to the membrane potential, as in frog muscle (13).

There is reasonable evidence that the release of acetylcholine mediates excitation (14, 15). Acetylcholine imitates the action of excitatory nerve stimulation. Anticholinesterases potentiate neural stimulation. Atropine-like agents block neural excitation. An acetylcholine-like substance is present in the muscle.

The action of 5-hydroxytryptamine is to relax catch, rather than to inhibit excitation. This is indicated in Fig. 6 (7, 16, 17). There is evidence that nerves exist which mediate relaxation of catch: Cambridge, Holgate, and Sharp (15) were able to observe a purely relaxing response to nerve stimulation in an isolated muscle bundle when cholinergic excitation was blocked. In addition, there is evidence that 5-HT may be the mediator released by the relaxing nerves: 5-HT in low concentration imitates the action of relaxing nerves (14). 5-HT has been identified both in muscle and in ganglion (18), and an enzyme exists in the muscle which specifically breaks down 5-HT (19). Unfortunately,

it has not yet been possible to block the action of 5-HT or relaxing nerves with known 5-HT–blocking agents.

5-HT does not change the membrane resting potential but it does alter the properties of the membrane. Resistance measurements by Dr. T. Osa indicate that 5-HT decreases the effective membrane resistance to half the resting value (from 45–60 megohms to 23–35 megohms) (17).

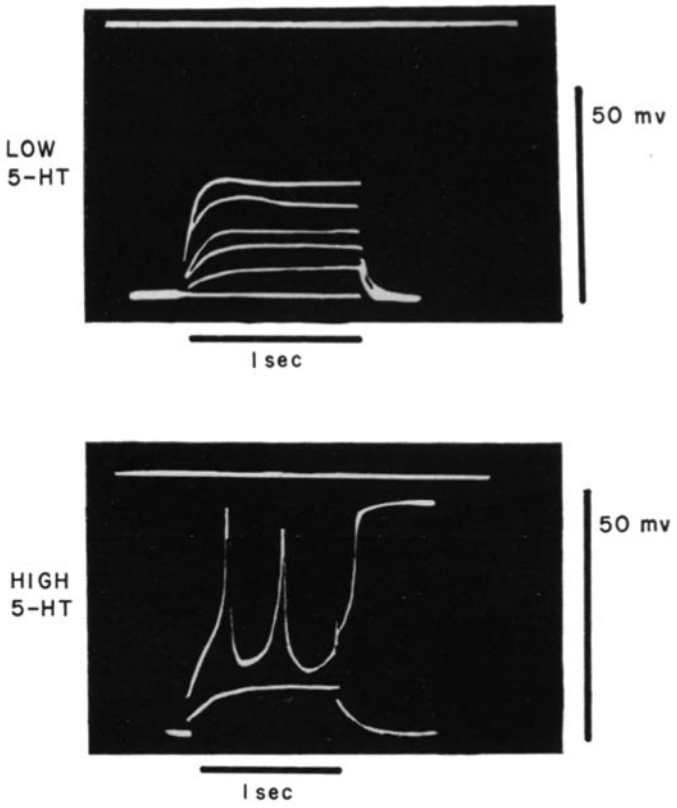

FIGURE 9. The effect of 5-hydroxytryptamine (5-HT) on the response of *Mytilus* muscle to prolonged (1 sec) stimulating pulses. Upper trace: resting membrane potential. Lower traces: responses to stimuli of increasing voltage. *Figure reprinted by permission from The London Journal of Physiology, 1967. In Press (17).*

When the muscle membrane is depolarized by long stimulating pulses in low 5-HT, even strong depolarization fails to evoke spikes (Fig. 9). In high 5-HT, repetitive spikes are fired with less depolarization.

Here, then, is an apparent paradox. 5-HT has an excitatory action on *Mytilus* membrane as it does on many other smooth muscles, but it relaxes catch tension. Furthermore, several factors have been studied, which reduce catch selectively (Table II). These factors all increase muscle excitability (8).

This suggested a possible working hypothesis to explain catch and the mode of control of catch by 5-HT. First, the details of excitation of contraction imply that *Mytilus* muscle is activated by a mechanism very similar to that in mammalian smooth and vertebrate striated muscle. Therefore, with or without 5-HT present, it seems reasonable to suppose that *Mytilus* muscle actomyosin is activated by an increase in intracellular Ca^{++} ion concentration (20). This suggestion is supported by recent experiments of Schadler (21), who demonstrated that glycerinated bundles of *Mytilus* byssus retractor muscle in MgATP develop full tension and display corresponding ATPase activity when Ca^{++} levels are increased from 10^{-7} to 10^{-5} M, at pH 7.0.

Secondly, catch tension varies inversely with the logarithm of the 5-HT concentration from 10^{-8} to 10^{-6} M (20).

Thirdly, the effect of 5-HT on the *Mytilus* membrane, that is, the increased ability to fire spikes, very closely resembles the effect of lowering intracellular Ca^{++} levels in the barnacle muscle (22). Like the barnacle muscle, *Mytilus* is

TABLE II

FACTORS WHICH REDUCE CATCH IN MYTILUS WITHOUT REDUCING ACTIVE CONTRACTION

1. Intact connection with pedal ganglia
2. Temperatures greater than 30°C
3. Increased intervals between stimuli ("rest")
4. 5-Hydroxytryptamine (and related pharmacological agents)

not sensitive to tetrodotoxin and the spike may depend on a change in calcium permeability (10).

A possible speculation is that catch tension and decreased ability to fire spikes depend on maintenance of high levels of intracellular Ca^{++}. 5-HT, then, does not interfere with the Ca^{++}-releasing mechanism in activation but greatly increases the rate of removal of intracellular free Ca^{++} following activation. Active state would be abbreviated, relaxation would be speeded, and the intracellular free calcium would return more rapidly to levels at which a spike can be fired. At any fixed time after activation, intracellular calcium levels would be inversely proportional to the logarithm of 5-HT concentration (20). The paucity of vesicular elements relative to fiber volume suggests that Ca^{++} uptake could be a significant limiting factor.

But catch is not a maintained active state (5). If an elevated Ca^{++} concentration persists, why does not active state persist?

This is a question one cannot answer at present. If intracellular calcium levels are to be implicated, both in activation and in catch, a further speculation is required, namely, that the Ca^{++} released during excitation has an effect over and above activation of actin-myosin, changing the muscle in such a way as to retard breaking of cross-linkages between actin and myosin.

It seems appropriate to consider paramyosin in this regard. A high ratio of paramyosin to actomyosin is found in all catch muscles (9) and is probably of considerable functional significance in catch. If the affinity of paramyosin for Ca^{++} is greater than that of the actomyosin, then, as Ca^{++} levels fall and activation ceases, continued binding of Ca^{++} to paramyosin might prevent the breaking of actin-myosin links. Cohen and Longley (23) have demonstrated that tropomyosins of vertebrate muscle bind calcium and have suggested that molluscan paramyosin may share this property. In recent unpublished experiments, they have confirmed this suggestion.

Still another speculation on a calcium-dependent catch mechanism has been supported by Nauss and Davies (24). It is hypothesized that Ca^{++} participates directly in forming stable actin-myosin linkages and that the linkages are broken when Ca^{++} is removed by 5-HT. This speculation assumes no special function for paramyosin. It will be important to determine whether, in any muscle, Ca^{++} is a direct reactant in actin-myosin interaction, or whether it activates actin-myosin interaction only via "native tropomyosin," as claimed by Ebashi (25).

This work was supported by Grant G-21469 from the National Science Foundation and in addition by Grant NB-00623 to Professor J. H. Welsh from the National Institute of Neurological Diseases and Blindness.

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Discussion

Dr. J. Gergely: If 5-hydroxytryptamine affects the Ca^{++} level, how do you explain its influence on the catch mechanism but not on the twitch contraction?

I would like to add a word of caution. The calcium levels that Cohen and Longley found to cause aggregation of paramyosin were several orders of magnitude higher than those involved in the regulation of the interaction of actin and myosin.

Dr. Twarog: As for the first point, I think that probably the calcium-releasing mechanism is, indeed, separate from the mechanism which takes up calcium. It seems possible that calcium is released by a mechanism which is not sensitive to the same agent which blocks the uptake. This is a possible explanation. Certainly it is not the only one.

As far as the concentrations go, yes, there is a large difference in the concentrations which change the aggregation of paramyosin and those which are postulated to be maintained intracellularly.

Dr. Andrew P. Somlyo: Mammalian smooth muscle does not contain paramyosin. Nevertheless, in the rabbit main pulmonary artery (Abstracts of the Biophysical Society, 1967, p. 113), DC field stimulation elicits a state which resembles catch at least to the extent that it is a sustained contraction lasting up to 35 min. Furthermore, no one so far has been able to demonstrate spike potentials in these fibers, although some preparations may respond to norepinephrine and similar agents with graded depolarization. Thus, repetitive spike electrogenesis can probably be ruled out as the cause of persistent contraction in the rabbit main pulmonary artery. Do you think that the paramyosin filaments may be a greater "red herring," as concerns catch, than we had thought, and have you had experience with other types of either molluscan or other smooth muscle which, like the main pulmonary artery, does not have paramyosin, but goes into a catchlike state?

Note added after symposium

Quick release experiments on rabbit main pulmonary artery (MPA) strips (Fig. 1, Discussion) show that if a quick release is applied during or shortly after the stimulus, the tension lost is redeveloped, as expected, during the active state. In contrast, if a quick release is applied during the catchlike state, the MPA, like the anterior byssus retractor muscle of *Mytilus*, does not redevelop a significant amount of the tension lost during the quick release. Thus the behavior of rabbit MPA smooth muscle, which contains no paramyosin filaments, resembles the molluscan "catch." It remains to be determined whether the mechanisms responsible for the catchlike state of mammalian smooth muscle and for the molluscan catch are identical, and hence both unrelated to paramyosin.

Dr. Twarog: Paramyosin is an interesting problem. In the first place, I don't know of any muscle in which catch has been conclusively demonstrated (that is, sustained tension in the absence of active state or any continued depolarization) where paramyosin is not present.

In other words, I don't believe that paramyosin is a red herring in this case. But I am not absolutely certain that the mechanism of sustained contraction has to be thought of as via paramyosin, in all cases.

Dr. Pringle: I wanted first to say what beautiful electron micrographs those were

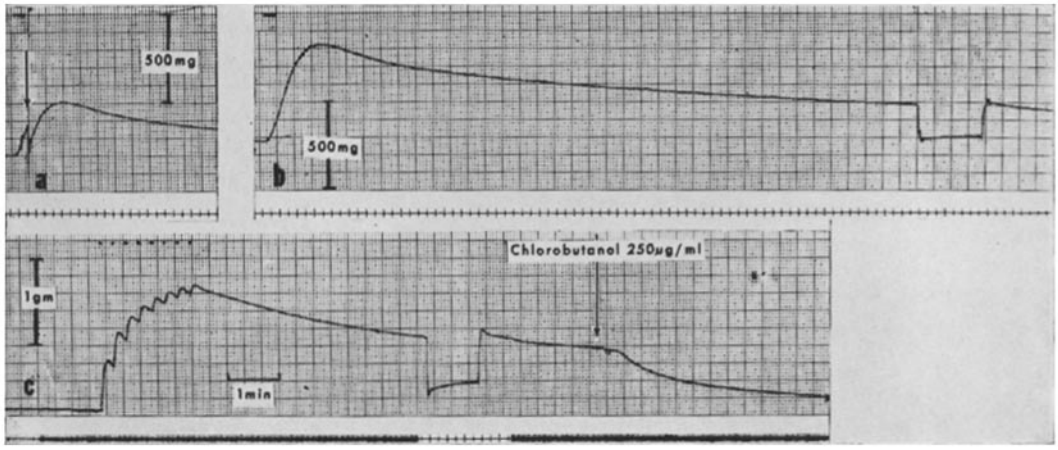

FIGURE 1. Active state and catchlike state during isometric contraction of rabbit main pulmonary artery (A. V. Somlyo and A. P. Somlyo). Helically cut strip; length, 2.3 cm; temperature, 37°C. Dibenamine, 1.5 µg/ml, and atropine, 5.0 µg/ml, added to muscle bath. Horizontal bars, 1 sec. Longitudinal DC field stimuli at 25 v/cm. Interelectrode distance, 3.0 cm. Time marks, 1 sec. *a*, 0.5 mm release at arrow, during active state, indicated by redevelopment of tension. *b*, 0.5 mm release, during sustained contraction, 1.2 min after DC stimulus, then strip restretched by 0.5 mm. *c*, 1.0 mm release during sustained contraction, 4.5 min after last of eight DC stimuli at 15 sec intervals; record speed temporarily increased during quick release and subsequent restretch by 1.0 mm. Chlorobutanol (arrow) released this catchlike state.

and to ask whether they were of muscle in the resting state or in the catch state. Also, is there evidence of any difference in fiber structure, particularly in the degree of association of the actin with the myosin filaments, which appeared in certain parts of those sections to be quite remote?

Dr. Twarog: These pictures of Dr. Gori's were of muscle in the relaxed state. Dr. Gori has studied changes in contraction. She has found that the number of dense bodies per unit area in cross-section increased by about 2.4 times, suggesting a moving together of these units. She has not presented figures on the possible changes in actin-paramyosin filament ratio.

Bridges are occasionally seen, but this is another difficult point. Neither in relaxed nor in contracted electron micrographs is one clearly certain of bridges.