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Lunar-Modulated Geomagnetic Orientation by a Marine Mollusk

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Behavioral experiments indicated that the marine opisthobranch mollusk *Tritonia diomedea* can derive directional cues from the magnetic field of the earth. The magnetic direction toward which nudibranchs spontaneously oriented in the geomagnetic field showed recurring patterns of variation correlated with lunar phase, suggesting that the behavioral response to magnetism is modulated by a circa-lunar rhythm. The discovery of a magnetic sense in a mollusk with giant, reidentifiable neurons provides a unique opportunity to study the cellular mechanisms underlying magnetic field detection.

ALTHOUGH THE MAGNETIC FIELD OF the earth is known to influence the orientation of a variety of organisms (*1*), the neurophysiological mechanisms underlying magnetic field detection in metazoans have not been established. It has been hypothesized that ferrimagnetic material functions as a transducer for a magnetic sense in several animals (*1, 2*). However, primary magnetoreceptors have proven difficult to isolate, and direct neurophysiological evidence implicating ferrimagnetic particles in the detection of magnetic fields has not been obtained.

Electrophysiological analysis of the neural mechanisms underlying magnetoreception in vertebrates has been impeded by the small size of vertebrate neurons, difficulties in reidentifying individual cells, and the complexity of the central nervous system. Here we report a behavioral response to ambient magnetic fields by the marine mollusk *Tritonia diomedea,* a nudibranch that has large, reidentifiable neurons and a relatively simple nervous system accessible to electrophysiological studies (*3*). In addition, we present evidence of a novel pattern of orientation based on magnetic field detection and related by an unknown mediator to lunar phase.

When tested in the laboratory, a variety of invertebrates spontaneously orient to context-irrelevant stimuli (*4, 5*). Experimental manipulation of such spontaneous orientation has been used to demonstrate sensitivity to ambient magnetic fields (*5*) and to polarized light (*4*). In an initial 4-day experiment, we therefore examined the orientation of *T. diomedea* maintained in darkness under two ambient magnetic field conditions. On nights 1 and 3 of the experiment, nudibranchs were tested in the magnetic field of the earth (*6*). On nights 2 and 4 the horizontal component of the earth's magnetic field was canceled (*7*) by using a Rubens' coil system (*8*) to generate a field equal in intensity to the horizontal component of the geomagnetic field but opposite in direction (*9*).

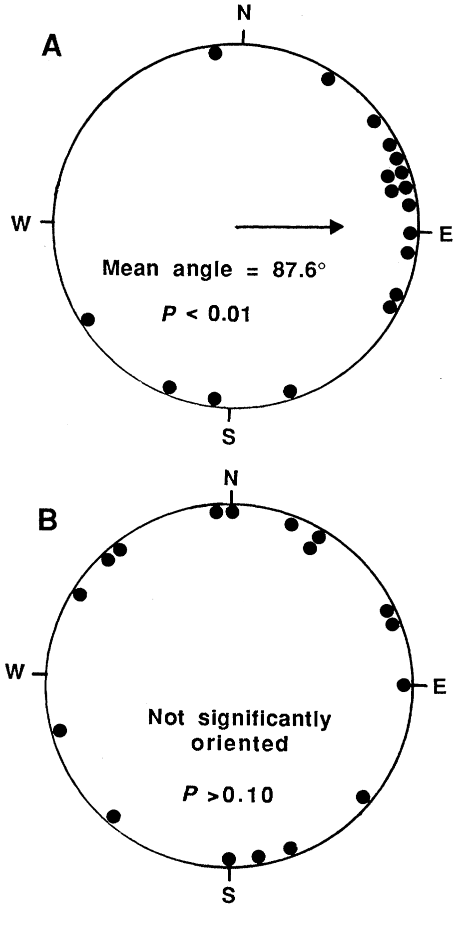

Fig. 1. Orientation of *Tritonia diomedea* under two ambient magnetic field conditions. (**A**) Orientation in the geomagnetic field. The group is significantly oriented [$n = 18$, $r = 0.58$, mean angle = 87.6°, $P < 0.01$, Rayleigh test (*10*)]. (**B**) Orientation in a field with a canceled horizontal component. The distribution is indistinguishable from random [$n = 17$, $r = 0.19$, mean angle = 29.0°, $P >> 0.10$, Rayleigh test (*10*)].

In the earth's field, the orientation of the animals was significantly [$P < 0.01$, Rayleigh test (*10*)] nonuniform with a mean angle of 87.6° (Fig. 1A). In contrast, animals tested in the canceled horizontal field showed orientation statistically indistinguishable from random (Fig. 1B). A comparison of the two distributions with the Watson test (*11*) indicated that they are significantly different ($U^2 = 0.199$, $P < 0.05$). These data suggest that the eastward orientation observed in the geomagnetic field was mediated by magnetic field detection and that eliminating the horizontal component of the geomagnetic field impaired the ability of the animals to orient. We emphasize that our measurements were of body axis alignment only and not of directional movements [an animal on the western side of the tank could be oriented eastward (*12*)].

The results of this initial experiment encouraged us to examine in greater detail the orientation of *Tritonia* in the natural magnetic field of the earth. Measurements of body axis alignment conducted sporadically over a period of 4 months, however, indicated that the animals did not always orient toward the east (*13*).

Initially we did not perceive any pattern in the directional variation and unsuccessfully sought to identify variations in experimental procedure that could account for it. Several reports, however, have suggested a relation between geomagnetic orientation and lunar phase. Homing pigeons, for example, utilize geomagnetic cues in orientation and show an apparent lunar rhythm in their initial bearings from some release sites (*14*). Mud snails and flatworms show behavioral responses to magnetic fields that reportedly vary subtly with lunar phase (*15*), as does the fly *Drosophila* (*16*). In view of these reports, we plotted the mean angles of orientation for groups of nudibranchs as a function of day of the lunar month (Fig. 2). The results of circular correlation analysis (*17*) indicated the two parameters were sig-

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Table 1. Results of the initial Y-maze experiment. Numbers represent the total numbers of left turns and right turns under the geomagnetic field and an ambient field rotated 90° clockwise. All experiments were conducted between 0 and 144 hours before full moon.

Field	Magnetic direction corresponding to		n	n turning	
	Left	Right		Left	Right
Geomagnetic	East	South	21	17	4
Rotated	North	East	20	9	11

nificantly ($P < 0.05$) related. A second analysis (*18*) treating each of the 170 animals tested as an independent data point (*10*) also indicated that orientation angles and lunar day were related ($P < 0.02$).

We reasoned that if the relationship had predictive value and if body axis alignment reflected preferred direction of movement, it might be possible to achieve a simple, unambiguous demonstration of lunar-correlated variation in geomagnetic orientation by presenting animals with choices between two directions in a Y-maze. In an initial experiment, the maze (*19*) was oriented in the geomagnetic field so that nudibranchs placed in the stem of the Y were initially directed toward 135° southeast. Thus, upon reaching the branch point, animals turning left moved eastward while those turning right went south. Data from Fig. 2 suggested that the mean angle of orientation during the 6 days preceding full moon is usually between north and east. We therefore predicted that animals tested in the geomagnetic field at this time would show a clear preference for left-turning (toward east) over right-turning (toward south). However, a 90° clockwise rotation of the ambient field was expected to eliminate the left-turning tendency, since the choice would become one of north (left) or east (right), with the "preferred direction" between.

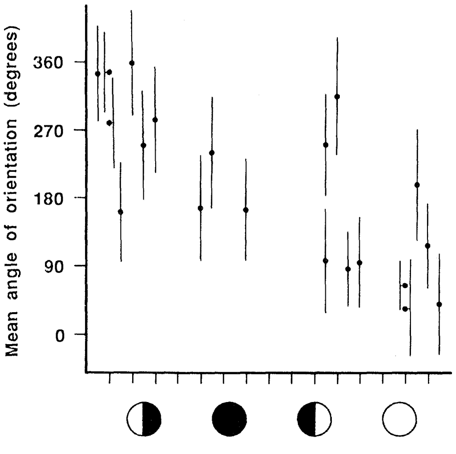

Fig. 2. Mean angles of orientation for nudibranch groups in the geomagnetic field as a function of day of the lunar month. Each data point represents the mean angle of a different group of animals tested once on a single night between 16 October 1983 and 23 April 1984. Brackets denote angular deviation, the circular statistical equivalent of the standard deviation in linear statistics (*29*). Hash marks on the *x*-axis delineate 2-day intervals. To present the data in "linear" form with conventional angles (0° to 360°) we have arbitrarily started and ended the graph with a data point from lunar day 19 [the new moon marks the start of day 1 (*14*)]. North = 0°, east = 90°, south = 180°, and west = 270°. Circle-circle correlation analyses (*17*) indicate that

Results of the experiment were consistent with the predictions (Table 1). About 80% of the responding nudibranchs turned left in the geomagnetic field, and the turning preference vanished altogether when the ambient magnetic field was rotated 90° clockwise. The turning ratios in the two field conditions were significantly different ($P = 0.019$, Fisher's exact probability test), indicating that ambient magnetic fields influenced turning behavior in *Tritonia.*

In a second series of Y-maze experiments (*20*), the maze was oriented as in the initial experiment; the Rubens' coil, however, was arranged so that activating it reversed the horizontal component of the geomagnetic field, rotating magnetic north 180° to geomagnetic south (Fig. 3).

Trials were conducted during only two 5-day periods of the lunar month. One of these 120-hour periods was centered on full moon; the other centered on new moon. On the basis of Fig. 1A, Fig. 2, and the initial experiments (Table 1), we predicted that most animals tested at full moon in the geomagnetic field would again turn left (east) in the Y-maze rather than right (south). In the reversed-field full moon trials, we predicted one of two outcomes. (i) The majority of animals might turn right (north) since this is closer to east than left (west) is. (ii) The animals might not exhibit any preference if both north and west are outside the range of preferred directions and are thus both equally unacceptable (or acceptable) to the animals.

On the basis of an expectation of approximately westward movement at new moon (*21*), we predicted different results for trials at that time. Animals tested in the geomagnetic field were expected either to show no turning preference or to turn right (south) rather than left (east) (*22*). In contrast, we predicted that animals tested in the reversed field would show a clear preference for left (westward) turning.

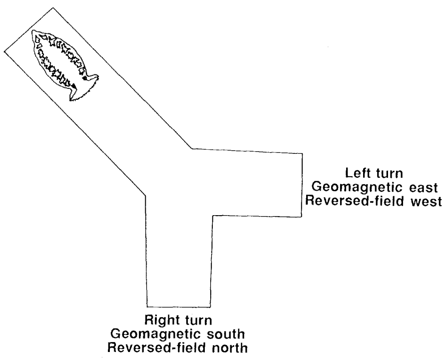

Fig. 3. Experimental design for the second series of Y-maze experiments. In the geomagnetic field, a left turn led toward east and a right turn toward south. When the Rubens' coil system was activated, reversing the horizontal component of the magnetic field, a left turn led toward magnetic west and a right turn toward magnetic north.

the predictions. In the full moon geomagnetic field trials, 76.3% of the animals turned left (east) rather than right (south). This turning preference vanished in the full moon reversed-field trials when the choice became one between north (right) and west (left). In contrast, nudibranchs tested in the geomagnetic field at new moon showed no significant turning preference. In the new-moon reversed-field trials, 83.0% of the animals turned left (west).

The cyclical nature of the behavioral response is evident when responses under each field condition are plotted for each of the three full moons and two new moons occurring during the experimental period (Fig.

Table 2. Summary of the results of Y-maze experiments indicating total left turns and total right turns under two ambient magnetic field conditions in experiments conducted within 60 hours of full or new moon. Since multiple comparisons of the left-right turning ratios under the four experimental conditions were made in the analysis, significance levels reported below are expressed as experimentwise type I error rates [to be significant at the experimentwise level of $P < 0.05$, two ratios had to be significantly different at the $P < 0.01274$ level when treated as an independent comparison (*30*)]. The left-right turning ratios in the geomagnetic field and reversed field at full moon are significantly different ($G_1 = 7.20, P < 0.05$); similarly, the ratios in the geomagnetic field and reversed field at new moon are different ($G_1 = 10.58, P < 0.01$). Results in the geomagnetic field at full moon are marginally different from results in the geomagnetic field at new moon ($G_1 = 6.04, P < 0.055$). Results in the reversed field at full moon and those in the reversed field at new moon are different ($G_1 = 14.05, P < 0.01$).

Field	Full moon		New moon	
	Left	Right	Left	Right
Geomagnetic	45	14	33	27

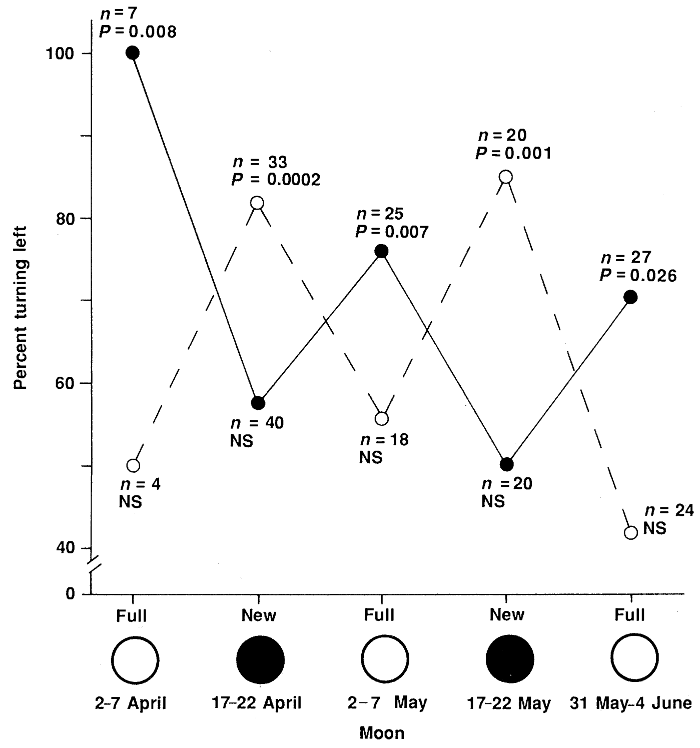

Fig. 4. The proportion of *Tritonia* turning left in the Y-maze in the geomagnetic field and reversed field during a 10-week experiment (Fig. 3). On the basis of initial experiments, we predicted animals tested in the geomagnetic field would turn left at full moon, and that those tested in the reversed field would turn left at new moon. The solid line and solid data points represent results of trials in the geomagnetic field. Broken lines and open data points represent results obtained in reversed magnetic field trials. Data points under each magnetic field condition are connected for clarity and do not imply sampling at times other than at new and full moon. *n* is the number of responses obtained under each set of conditions. Values of *P* represent the binomial probability of the observed result had it been drawn from a population showing random orientation (50% expected in each maze arm). NS (not significant) indicates that no significant preference for either arm was demonstrated under the test conditions ($P \geq 0.20$ in all such cases).

4). In the geomagnetic field, a statistically significant majority of animals turned left during each of three consecutive full moons, but there was no significant turning preference during either of two new moon periods. In the reversed field, animals tested in the geomagnetic field did not demonstrate a significant turning preference during any of the full moon periods; however, a statistically significant majority turned left during both new moon experiments.

These data indicate that ambient earth-strength magnetic fields influence the turning behavior of *Tritonia*. Moreover, the behavioral response to magnetism exhibited a consistent pattern of variation through time that correlated with lunar phase. The data are not sufficient to determine a precise period length for this apparently cyclical behavior. Nevertheless, the results are consistent with the interpretation that the response to magnetism is modulated by a lunar or a circa-lunar rhythm (*23*).

We do not know if the patterns of orientation we have observed in the laboratory reflect behavior that is significant to animals under natural field conditions. To our knowledge, *T. diomedea* has never been studied systematically in its natural habitat; thus nothing is known of its orientation and directional movements there. If animals in the natural habitat show directional movements that follow the pattern suggested by Fig. 2, they could move in a slow counterclockwise circle or spiral over the course of the lunar month. Although such a pattern of locomotion could play a role in foraging (*24*), reproduction (*25*), or both, it is equally plausible that the laboratory results represent orientation to detectable but context-irrelevant stimuli (*4, 5*).

Regardless of adaptive considerations, the results indicate that *Tritonia* derives directional information from the magnetic field of the earth. The presence of a magnetic sense in an opisthobranch mollusk with large, reidentifiable neurons (*3*) represents a unique opportunity to examine the neurophysiological mechanisms underlying magnetic field detection in metazoans. We have initiated electrophysiological studies designed to identify neurons involved in the detection of the geomagnetic field. One neuron (left pedal neuron 5) consistently shows enhanced electrical activity in response to changes in earth-strength magnetic fields (*26*). This neuron has no apparent motor function (*3*), does not respond to conventional tactile or chemical stimuli (*3*), and is inhibited during escape swimming (*27*). In addition, the whitish pigmentation of left pedal neuron 5 is characteristic of cells that contain neuroactive peptides and function in modulating behavioral state (*28*).

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5. The horizontal dance of the honeybee is the best-known example of such "nonsense" orientation to ambient magnetic fields [J. L. Gould, *Annu. Rev. Physiol.* **46**, 585 (1984)]. Other examples were described by G. Becker [*Z. Angew. Entomol.* **54**, 75 (1964)].
6. At about 0230 on each of the 4 days (16 to 19 February 1984) of the experiment, a different group of 8 to 12 nudibranchs was placed in a plastic bucket, gently swirled, and then poured into a circular orientation tank (0.9 m in diameter) to ensure random orientation at the start of each trial. In all four trials the animals remained in the tank in complete darkness in the magnetic field of the earth for 17 hours (until 1930). At this time they were exposed to one of two field conditions (geomagnetic field or canceled horizontal component field) for an additional 90 minutes. Orientation was assessed at the conclusion of the 90-minute period. When *Tritonia* are transferred into a new tank in which water currents are absent, the animals often remain motionless for hours in the positions in which they land. We thus sought to promote movement in the initial stages of each session by providing weak water currents. Care was taken to ensure that these currents could not be used in orientation or account for differences in orientation observed under the two field conditions. A rubber hose delivered 1000 to 1100 ml of seawater per minute directly downward to a fixed position in the center of the circular tank. The water was turned on when the animals were put in the tank (0230) and turned off 60 minutes before orientation was assessed (at 2000, when the animals had been in the experimental field for 30 minutes). Dye injections indicated that the water, when flowing, dispersed gently outward in all directions from the center of the tank.
7. The intensity of the net horizontal field was less than 0.01 Oe at the cube coil center in contrast to the natural horizontal geomagnetic component of about 0.16 Oe at Friday Harbor, Washington (both measured with a single-axis fluxgate magnetometer).
8. M. Rubens, *Rev. Sci. Instrum.* **16**, 243 (1945). The cube coil was 130 cm on a side.
9. To record orientation, a sheet of plexiglass was placed over the tank. Looking down at the nudibranchs through the plexiglass, an observer drew a line on the plexiglass, connecting bases of the two rhinophores on each animal, and an arrow indicating the anterior direction. At the conclusion of the experiment a north-south magnetic axis was drawn on the plexiglass. Body axis orientation was defined as the angle formed between the north-south axis and a line perpendicular to the line connecting the rhinophores; the angle corresponded to the direction the animal would be expected to move if it continued to crawl straight ahead. We estimate a margin of error of 5° using this technique.
10. E. Batschelet, *Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms* (American Institute of Biological Sciences, Washington, DC, 1965). Although animals were tested in groups, we think it reasonable to regard the orientation of each individual as a statistically independent data point for the following reasons: (i) although some mollusks follow mucous trails of conspecifics, we know of no evidence suggesting such an ability in *Tritonia* and have been unsuccessful in generating such evidence ourselves; (ii) earlier experiments have failed to indicate that the directional movements of *Tritonia* are influenced in any way by the presence of conspecifics [A. O. D. Willows, *Mar. Behav. Physiol.* **5**, 115 (1978)]; (iii) the eyes of *Tritonia* are beneath the integument, and the visual sense is extremely limited [R. Chase, *J. Exp. Biol.* **60**, 707 (1974)]; (iv) the experiments were conducted in darkness, which eliminates the remote possibility of visual interactions between individuals; and (v) none of the animals were in physical contact with any others when the measurements were taken; the angles recorded represent

body axis orientations rather than positions within the arena. Accordingly, we regard the assumption of independence as reasonable and the application of circular statistics appropriate.

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12. There was no obvious clustering of nudibranchs in any part of the tank under either field condition, although spatial distribution was not rigorously examined.
13. Procedures utilized in these experiments were the same as in the previous experiments except that the length of time animals remained in the tank varied. A variety of analyses have failed to reveal any relation between time in the tank and orientation angle.
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17. E. Batschelet, *Circular Statistics in Biology* (Academic Press, London, 1981). Data were analyzed with circle-circle rank correlation procedures. Days of the lunar month were calculated according to the procedures of (*14*). Ties in ranking of lunar day (occurring when more than one trial was assigned to the same lunar day) were broken by determining which of the two tests was performed earlier in the lunar day.
18. This analysis used the Jupp-Mardia circular correlation coefficient (*17*).
19. The Y-maze has been described by A. O. D. Willows [in (*10*)]. *Tritonia* is strongly rheotactic and will locomote into water currents in the maze. Water flow into each arm was 1000 ± 50 ml/min in all Y-maze experiments reported here.
20. Nudibranchs in experiment 1 (conducted in May and June of 1984) were tested in small groups to facilitate rapid data collection (many animals do not move in the 1-hour time period allotted). In experiment 2 (April to June of 1985), animals were tested one at a time; after each trial, the walls and floor of the Y-maze were scrubbed thoroughly with a nylon bristle brush to reduce the possibility that residual chemical cues or slime trails would influence subsequent trials. Throughout both experiments we alternated between trials in the geomagnetic field and rotated or reversed field (a trial under one field condition was followed by a trial in the other).
21. We had few data points from new moon trials (Fig. 2), but predicted westward movement at new moon because (i) at full moon the animals seem to orient approximately eastward (for example, Figs. 1A and 2); (ii) Fig. 2 suggests that the mean angle of orientation rotates at an essentially constant rate through 360° over the course of the lunar month.
22. Both choices could be outside the range of "preferred" directions (resulting in no preference) or the animals might turn south because this is closer to west than east is.
23. Since experiments in the circular tank were conducted at essentially the same time each solar (24-hour) day and therefore at different times in the lunar (24.8-hour) day, it is hypothetically possible that the relationship in Fig. 2 reflects variation attributable to a lunar daily rhythm rather than a lunar monthly rhythm. Y-maze experiments were conducted continuously for 120 hours at new and full moon, however, and there was no apparent variation in response over the course of the lunar day. At present the data are thus most consistent with the circa-lunar rhythm hypothesis (but other possibilities cannot be ruled out).
24. *Tritonia* feeds on sea pens and sea whips (pennatulacean cnidarians), which are often found in patches [C. Birkeland, *Ecol. Monogr.* **44**, 211 (1974)]. A circular pattern of movement could keep nudibranchs in an area of high food concentration, or a gradual outward spiraling from the last known prey item might enhance search effectiveness.
25. Numerous marine invertebrates have reproductive rhythms related to lunar phase. All of our nudibranchs were trawled from a location near Olga, Orcas Island, Washington, where the nearest body of land is almost directly eastward. Eastward movement at the time of full moon could facilitate reproduction by bringing dispersed groups of animals together on shoals at a specific point in the lunar month. Westward movement around the time of new moon could represent an offshore migration back to deeper water.
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29. See references in (*10*) and (*17*).
30. Multiple-comparison procedure for four unplanned comparisons [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, ed. 2, 1981)]. Although our comparisons were planned, we used this analysis as a conservative statistical treatment for our multiple comparisons.
31. We thank T. Quinn, J. Palka, W. Wright for critically reading the manuscript; M. Njegovan for technical assistance during preliminary experiments; and S. Adolph for assistance with the statistical analyses. Supported by NIH grants 18658-01 and 22974-01 to A.O.D.W., GM 07108-10 (instructional training grant) to K.J.L., and funding from the Western Society of Malacologists to K.J.L.

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Myosin Rod Phosphorylation and the Catch State of Molluscan Muscles

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"Catch" is a prolonged state of tension in molluscan smooth muscles shown by mechanical measurements to be associated with the level of protein phosphorylation. Myosin isolated from these muscles is unusual in being phosphorylated in the rod portion by an endogenous kinase, like certain nonmuscle myosins. These findings suggest that the myosin rod is a target for phosphorylation and that this reaction may control the transition from catch to relaxation.

CERTAIN MOLLUSCAN SMOOTH MUSCLES display a specialized stretch-resistant state called "catch" in which tension is maintained for long periods. In the catch state, although adenosine triphosphate (ATP) is present, the myosin cross-bridges are attached to actin but cycle very slowly [for review, see (*1*)]. Catch muscles are characterized by distinctive thick filaments containing a large core of paramyosin and an outer layer of myosin (*2–4*). Aspects of the packing of the paramyosin core are understood (*3, 5–7*). In contrast, the organization of myosin in the thick filaments of catch muscle is not yet known, although the myosin array in a related molluscan noncatch muscle has been visualized (*8*). The myosin of both catch and the more rapidly relaxing striated adductor muscles of molluscs is directly activated by the binding of Ca^{2+} to the head portion of the molecule (*9, 10*). Several hypotheses for the mechanism of catch involve phosphorylation of paramyosin, with possible effects on paramyosin-paramyosin and paramyosin-myosin interactions (*11–14*). We report that the maintenance of catch is associated with dephosphorylation and also show that the rod portion of a catch muscle myosin can be phosphorylated in vitro. These findings suggest new possibilities for the molecular basis of catch.

We have confirmed the reports of Cornelius (*15, 16*) that detergent-skinned fiber preparations of the anterior byssus retractor muscles (ABRM) of *Mytilus edulis* display mechanical properties similar to those of the intact muscle (Fig. 1). Contraction is activated by addition of 10^{-6} M Ca^{2+}, producing an "active state." The muscle is locked into catch by removal of Ca^{2+}, and relaxation from catch is obtained by the addition of cyclic adenosine monophosphate (cAMP). This cyclic nucleotide, however, does not inhibit development of active tension by Ca^{2+}. This permeable fiber preparation therefore allows the direct investigation of the role of second messengers in the contractile process.

We have studied the mechanical response of these muscles to the catalytic subunit of cAMP-dependent protein kinase [for review see (*17*)]; to ATP-γ-S, a nonhydrolyzable ATP analog often used as a substrate for kinases (*18*); and to NaF, an inhibitor of phosphatases. Skinned ABRM fiber bundles preincubated with the catalytic subunit of the cAMP-dependent kinase develop active tension comparable to the control (Fig. 2), but removal of Ca^{2+} produces rapid relaxation instead of locking the muscle into catch. This finding suggests that the cAMP-dependent kinase is the target protein of cAMP in this system.

Relaxation from the catch state can also be induced by addition of 20 mM NaF (Fig. 3). The presence of NaF does not affect activation by Ca^{2+}, but it inhibits the development of catch even after its removal by extensive washing.

When ATP-γ-S is substituted for ATP during catch, rapid relaxation is observed

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