MECHANICAL PROPERTIES AND CONTROL OF NON-MUSCULAR CATCH IN SPINE LIGAMENTS OF THE SEA URCHIN, *STRONGYLOCENTROTUS FRANCISCANUS*

BY MOHAMMAD DIAB AND WM. F. GILLY

*Hopkins Marine Station of Stanford University, Department of Biological Sciences, Pacific Grove, CA 93950, U.S.A.*

Accepted 16 January 1984

**SUMMARY**

1. Catch was induced in the spine ligaments of *Strongylocentrotus franciscanus* (Agassiz) (surrounding muscle and nervous tissue removed) by mechanical agitation.

2. When the catch mechanism is set, the tensile stiffness of urchin ligament can change by nearly an order of magnitude and approaches that of vertebrate tendon.

3. Deflection of a spine in catch through an angle of more than 10° damages the catch mechanism.

4. Setting of catch depends on extracellular calcium.

5. Transglutaminase inhibitors (cadaverine and putrescine) prevent the setting of catch.

6. A model involving Ca-dependent activation of a transglutaminase-like enzyme is discussed as a possible basis for the setting of catch by cross-linking collagen fibrils.

**INTRODUCTION**

The spines of the sea urchin are connected to the animal's test by means of a ball and socket joint. Three concentric cylinders constitute this joint: an outer neuro-epithelial layer, a middle (non-striated) muscular layer, and an inner ligamental layer composed almost entirely of collagen fibrils. In the normal, relaxed state, the ligament is quite extensive, as bundles of collagen fibrils can slide past one another when the ligament is stretched (Hidaka & Takahashi, 1983). Thus, the urchin can move its spines freely in all directions by contracting that portion of the muscular layer facing the direction of deflection. This is accompanied by stretching of the relaxed ligament diametrically opposite and by 'active' (i.e. no kinking) shortening of the ligament facing the direction of deflection. The basis for these length changes is not yet understood, but the ligamental layer also contains a few, very small diameter muscle fibres and nerve endings (Smith, Wainwright, Baker & Cayer, 1981) which probably are involved in the mechanism.

Key words: Sea urchin, catch, transglutaminase.
Extensibility of the ligament and highly movable spines are not static features; however. Agitation of the urchin can cause its spines to 'catch' and their movement due to either active muscular activity or to an externally applied force to be strongly inhibited. The basis for this catch is a rapid and dramatic increase in stiffness of the ligament. Although the mechanisms for setting and maintaining catch are not understood, cross-linking of collagen fibrils which make up the inner ligamental layer seems to be involved (Smith et al. 1981). Another factor may be the ionic composition of the inter-fibrillar matrix of the collagenous tissue (Wilkie, 1978; Smith et al. 1981).

This ligament 'catch' should not be confused with the superficially similar phenomenon which occurs in the non-striated muscle of many molluscs, as first described in *Pecten maximus* by von Uexkull (1912) and more recently studied by Twarog (1954, 1967, 1972). This catch is due to tonic muscular activity which is ultimately controlled by the nervous system, whereas in the sea urchin, catch of the spine ligaments does not involve the outer muscular layer at all (Kawaguti & Kamishima, 1965).

In this paper, we examine the non-muscular type of catch. In contrast to most previous studies (Smith et al. 1981; Eylers, 1982; Hidaka & Takahashi, 1983; Hidaka, 1983) we have studied spine ligaments in situ, that is, attached to the spine and test, in the hope of minimizing mechanical trauma of this complex tissue. Biomechanical properties of the spine ligament in catch are presented, along with considerations of several possible control mechanisms for setting and maintaining catch. Results suggest that catch is triggered neuronally, but that the physical process by which it is set and maintained may be enzymatic in nature.

**METHODS**

*Strongylocentrotus franciscanus* were obtained subtidally in Monterey Bay, CA. Specimens were kept in the laboratory in running, oxygenated sea water (15°C).

The experimental preparation consisted of an urchin test with the Aristotle's lantern removed. The inside of the test was scraped to avoid participation of the internal nervous system (e.g. radial nerves). The ligaments to be studied were mechanically stripped of surrounding muscular and neuro-epithelial layers. The test was held steady by pushing a rubber stopper through that portion previously occupied by the Aristotle's lantern and firmly mounting the assembly in a glass dish filled with sea water. In all cases, the ligament being tested was just above the surface of the water. Room temperature was approximately 16—20°C.

Test spines were connected to a Pixie element force transducer (Endevco Corp.) by means of a rigid cylindrical cap fitted over their tips. The connection between the spine cap and force transducer was of rigid steel wire which could swivel at the spine cap to maintain a horizontal pull on the force transducer. The transducer was attached to a micromanipulator, and lateral movement of the transducer produced spine deflections through a desired angle. Angle was measured with a protractor attached to another micromanipulator and held immediately behind the spine. Resistance to deflection by a spine would bend the semiconductor transducer element, thereby changing its resistance. This resistance change was transformed into a voltage output by an operational amplifier circuit and recorded on a Brush 220 pen oscillograph.
Force measurement was essentially isometric; that is, the transducer bending was a very small fraction of spine deflection (<2%).

Stress-strain data were obtained using a tensometer (Tansey, 1983). The preparation, in this case a ligament in catch with spine and single ossicle of test attached, was held between one clamp mounted on an anchored double cantilever beam and another clamp mounted on a plate moved by a lead screw. Force developed upon stretching was sensed by the deflection of the double beam and was monitored by a linearly variable differential transformer (LVDT) on the anchored plate. Change in ligament length was monitored by another LVDT mounted on the moving plate.

The most effective way to induce catch in the spine ligament was found to be physical agitation by bubbling in sea water for 10 min prior to experimentation. Attempts to trigger catch by electrical stimulation of a stripped ligament were not successful.

All chemicals were applied in drops by means of a Pasteur pipette directly onto the stripped ligament in 2 ml volumes per trial. Solutions containing these chemicals were freshly made up on the day of the experiment and stored in opaque vessels at 4 °C. Ca-free sea water contained (mmol l⁻¹): 445 NaCl, 60 MgCl₂, 10 KCl, 15 EGTA and 10 Tris buffer (pH 7·8). The entire test here was immersed in the solution.

**RESULTS**

*Active muscular force vs ligament strength in catch*

Since the catch ligament of the sea urchin spine is surrounded by muscular and neuro-epithelial layers, it is important to consider the strength of the activated muscles in comparison with that of the ligament in catch. A test spine with the muscular and neuro-epithelial layers intact was connected to the force transducer as described above. Muscular contraction was elicited by mechanical stimulation of the test at the base of the spine diametrically opposite the transducer. This caused the muscle to contract and attempt deflection of the spine in the direction of the stimulus, thus pulling on the force transducer. Records of such contractions are shown in Fig. 1A. Following this experiment, the test spine was stripped of its muscle and set into catch by physical agitation. Force offered upon a 10° deflection of the spine (F-10) is shown in Fig. 1B. Comparison of the magnitude of this force with that in Fig. 1A shows that muscular and catch force are similar in magnitude. Hence, all experiments were run on ligaments stripped of surrounding muscle. This also avoided endogenous control of catch by the neuro-epithelium covering the test.

*Characterization of catch and non-catch states in stripped ligaments*

Fig. 2A shows a record of three F-10 responses from a spine out of catch. Fig. 2B shows responses from the same spine after catch had been induced. Differences in amplitude and time course of the F-10 responses are clear. When out of catch, the spine offers a small, step-like F-10, but when in catch, F-10 shows an initial sharp peak followed by a decay to a steady state value greater than the out-of-catch F-10. This
Fig. 1. Comparison of muscular force and ligament strength. (A) Contractile force developed by the muscular layer surrounding a spine ligament measured upon mechanical stimulation (arrows). (B) Force offered by the same ligament in catch upon a 10° spine deflection (F-10) at time indicated by arrow. Return of force to baseline corresponds to moving spine back to its original position.

decay in catch records was consistently observed, but its extent was variable as illustrated in Fig. 2C. This spine, from the same test, shows a more marked decay. The basis of this decay is not completely understood, but it seems to be characteristic of the catch state. Presumably, the deflection of the spine and the concomitant stretching of the ligament occur quickly relative to the relaxation time of the viscoelastic material comprising the ligament. In all further experiments, F-10 is defined as peak amplitude of the force transient accompanying a rapid 10° deflection. This represents an apparent 'instantaneous' stiffness at the moment of stretch.

Differences in peak F-10 amplitude for catch and non-catch records are further illustrated in the histogram of Fig. 3, where F-10 values from 80 spines, some agitated and some not, are plotted. From this, a quantitative indication of catch vs non-catch is evident. All non-agitated spines offered F-10 of less than 0.1 g. Spines which we
Ligament catch in sea urchin spines

Stated fell mainly into the F-10 range of 0.34–0.64 g. The former group (F-10 = 0.062 ± 0.027 g, mean ± s.d.) was deemed to be out-of-catch, and the remainder of the population in catch (F-10 = 0.464 ± 0.085 g, mean ± s.d.). Cases of F-10 values substantially greater than the mean, e.g. 0.7 g in Fig. 3 and up to 1.5 g (not illustrated), were also occasionally observed in agitated spines. Whether this is due to an unusually strong catch state or reflects small errors in angular deflection measurements, differences in spine length, or some other variable is not clear.

Fig. 2. Characteristic F-10 responses for ligaments in and out of catch. (A) Spine was deflected 10° (three trials at arrows) with ligament out of catch. (B) Same spine was deflected twice through 10° after setting catch. (C) Examples of F-10 responses for a spine from the same test with its ligament in catch. Extent of sag in force during response is more pronounced than in (B). In each case the sudden decrease in force to the baseline marks the return of the spine to its initial position.
Critical deflection angle for ligament damage

A spine that is set in catch cannot be deflected through a large angle without suffering a drastic change in the properties of its ligament. Repeated spine deflections of 10° or less over the same arc yield resistive forces of similar magnitudes (connected triangles in Fig. 4), whereas repeated deflections of greater extent (20°, squares) result in a large decrease in resistive force. These results indicate that a ligament in catch begins to exhibit plastic flow for deflections of between 10 and 20°.

This phenomenon appears to reflect true damage of the ligament, in that it is irreversible on a time scale of days. Furthermore, the damaged portion of the ligament is restricted to the span covered by the original deflection angle. Thus, if damage occurs between 0 and 20° as in Fig. 3, then a deflection from 20 to 30° would still exhibit an F-10 characteristic of the normal catch state.

Resistive force vs deflection for small angles

Spines in catch were deflected incrementally to different angles and the resistive force offered at each angle was recorded. Data from a typical experiment are plotted in Fig. 5. Most noteworthy is that for deflections of approximately 10°, there is a dramatic increase in the slope of the curve relating force and angle. This angle coincides with the region of deflection in which the critical angle for damage was found to lie.
The large increase in resistive force may be due to application of deflective force directly to the inter-fibrillar cross-links which are hypothesized to be the mechanical basis of catch in the collagenous ligament (Smith et al. 1981). Consequently, excessive force applied here might break or damage some of these cross-links and thereby impair the ability to set catch.

As another assessment of resistive force vs deflection for small angles, measurement of stress (force/cross-sectional area) at varying strains (change in ligament length/original length) was carried out using a ligament in catch connected to its spine and a single ossicle (see Methods). Data obtained were plotted as the stress-strain curve in Fig. 6. From the slope of the stress-strain curve at 0.5% strain, Young’s modulus, a measure of tensile stiffness, was found to be $2.8 \times 10^8$ N m$^{-2}$. This value agrees with that given by Hidaka & Takahashi (1983) for ligaments in catch from a different urchin and is close to that for vertebrate tendon at similar strain (Wainwright, Biggs, Currey & Gosline, 1976). Maximum stiffness of tendon, measured at considerably higher strain, is $1-2 \times 10^9$ N m$^{-2}$ (Ker, 1981).

As demonstrated in the Appendix, the stress-strain curve and the resistive force vs angle of deflection curve are physically equivalent. They differ simply in the extent of strains applied in these experiments. For example, the maximum strain to which the spine ligament was subjected in Fig. 6 (2.5%) can be shown to correspond to an

![Graph](image-url)
angular deflection of about 5.4°. Using the formula for Young's modulus derived in the Appendix (equation 10), and the data of Fig. 5, a value of $1.1 \times 10^8$ N m$^{-2}$ at 0.5% strain was calculated, in satisfactory agreement with the more direct method. Stiffness thus estimated for greater strain, e.g. 10%, would be somewhat larger, but still less than the maximum value for tendon.

**Effects of calcium on catch**

Wilkie (1978) proposed that the ionic composition of the extracellular matrix surrounding the collagen fibrils in echinoderm connective tissue is responsible for the maintenance of catch. Variations in inter-fibrillar ionic composition might, therefore, influence catch in the spine ligaments of sea urchins, and Smith et al. (1981) have shown that the absence of all divalent cations (i.e. Ca- and Mg-free sea water) do
prevent the setting of catch. Hidaka (1983) further showed that Mg could not substitute for Ca in sustaining catch. This has been confirmed in the present study. A ligament out of catch was bathed in Ca-free sea water for 5 min and then agitated in that medium for another 10 min. Pre- and post-agitation F-10 responses in two spines thus studied were identical and of the non-catch sort, indicating that the setting of catch is Ca-dependent.

Smith et al. (1981) also reported that the prevention of catch by divalent cation-free sea water was reversible, but they gave no indication of the time course for reversal. Following the above experiments with Ca-free sea water, the preparations were bathed in running natural sea water (10–15 mmol l$^{-1}$ Ca) at 15°C and periodically agitated to determine the time course of catch recovery. Results from the most complete experiment are shown in Fig. 7. F-10 responses in Ca-free sea water (time zero) and in natural sea water for up to 3 h are characteristic of spine ligaments out of catch. After 12 h, recovery F-10 shows both the amplitude and time course (not illustrated) typical of ligaments in catch. Thus, recovery of catch-setting ability following Ca-free treatment is very slow.

**Are disulphide bonds involved in catch?**

Collagen from the nematode *Ascaris* contains a high density of cysteine residues and
is known to form cross-links by means of disulphide bonds (Wainwright et al. 1976). The possibility of disulphide cross-linking in sea urchin spine ligaments was tested by applying sulphydryl reagents to spine ligaments. Dithiothreitol (DTT, 5 mmol l⁻¹), which breaks disulphide bonds, was applied to two ligaments in catch. No abolition of catch occurred, as shown by similar F-10 responses before and after DTT treatment. As another test, N-ethylmaleimide (NEM, 5 mmol l⁻¹), which prevents formation of disulphide linkages, was applied to two ligaments out of catch which were then agitated to induce catch. F-10 responses before and after agitation in the presence of NEM were typical of those for normal spines (e.g. Fig. 2). Results with both of these reagents suggest that disulphide linkages do not play a significant role in the setting or maintenance of catch, but this conclusion should be regarded as tentative.

![Graph](image)

Fig. 7. Recovery of the ability to set catch following treatment of a ligament with Ca-free sea water. Point at zero time was obtained in the Ca-free solution. The preparation was then placed in natural sea water for recovery. Each point thereafter was obtained following 10 min agitation. Recovery time is very slow; F-10 characteristic of catch is developed only after many hours.
**Transglutaminase inhibitors prevent the setting of catch**

In vertebrates the enzyme transglutaminase catalyses the *in vitro* formation of cross-links between fibronectin and collagen in cultured fibroblasts (Folk, 1980). Cadaverine (1,5-diaminopentane) and putrescine (1,4-diaminobutane) act as substrates for this enzyme and thereby inhibit its action when added to a system in excess (Curtis & Lorand, 1976). As a test of transglutaminase-like cross-linking in sea urchin ligaments, cadaverine and putrescine were applied to ligaments out of catch in an attempt to prevent catch setting. Two experiments with each substance at 0·01 mmol I$^{-1}$ concentration gave F-10 responses of the non-catch type before and after agitation (see also Table 1). Thus, catch setting was prevented by these transglutaminase inhibitors. Application of cadaverine and putrescine to spines already in catch (two each) did not abolish or significantly weaken catch. These results strongly suggest that cross-link formation in the spine ligament may involve a chemical mechanism similar to that catalysed by transglutaminase, but that once formed, cross-links do not require enzyme activity.

**Classical neurotransmitters do not appear to affect catch-setting**

The results described so far have dealt with the mechanical and chemical properties of the catch mechanism. In the living urchin, catch appears to be rapidly turned on through sensory input to the nervous system (Maeda, 1978). Neural processes have also been found in the ligament itself and appear to be secretory in nature (Smith *et al.* 1981; Hidaka & Takahashi, 1983). Furthermore, in single-spine ossicle preparations, both ligament ‘tonus’, measured as length change under a constant load (Takahashi, 1966), and apparent stiffness (Hidaka & Takahashi, 1983) are reported to be increased by 0·1 mmol I$^{-1}$ acetylcholine (ACh) and decreased by 0·01–0·1 mmol I$^{-1}$ epinephrine. The extent of these changes relative to non-catch control spines is unclear however.

**Table 1. Action of various substances on spine ligaments in and out of catch**

<table>
<thead>
<tr>
<th>Agent applied</th>
<th>Applied (N)</th>
<th>Time (mmol I$^{-1}$)</th>
<th>Test spine in/out of catch</th>
<th>Release of catch</th>
<th>Induce catch</th>
<th>Prevent catch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>(4)</td>
<td>0·1</td>
<td>10</td>
<td>Out</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>(4)</td>
<td>0·1</td>
<td>10</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>d-tubocurarine</td>
<td>(2)</td>
<td>1</td>
<td>3</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>(4)</td>
<td>0·01</td>
<td>10</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Dopamine</td>
<td>(2)</td>
<td>0·1</td>
<td>3</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Serotonin</td>
<td>(2)</td>
<td>0·1</td>
<td>3</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>DTT</td>
<td>(2)</td>
<td>5</td>
<td>3</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>NEM</td>
<td>(2)</td>
<td>5</td>
<td>3</td>
<td>Out</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>(2)</td>
<td>0·01</td>
<td>10</td>
<td>Out</td>
<td>---</td>
<td>++</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>(2)</td>
<td>0·01</td>
<td>10</td>
<td>In</td>
<td>---</td>
<td>++</td>
</tr>
<tr>
<td>Putrescine</td>
<td>(2)</td>
<td>0·01</td>
<td>10</td>
<td>Out</td>
<td>---</td>
<td>++</td>
</tr>
<tr>
<td>Putrescine</td>
<td>(2)</td>
<td>0·01</td>
<td>10</td>
<td>In</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

DTT and NEM represent dithiothreitol and N-ethylmaleimide, respectively.

Only cadaverine and putrescine showed any effect on the ligament catch mechanism. Both substances prevented the setting of catch.

Each symbol represents a single trial: (−) indicates no effect, (+) indicates an effect.
Attempts were made in the present study to obtain more detailed information on the controlling role of the nervous system using in situ stripped ligaments and F-10 responses as indicators of the state of catch. No effects were obtained for several suspected neurotransmitters, and results are summarized in Table 1.

ACh (0·1 mmol l⁻¹) was applied to ligaments out of catch, to see if catch could be induced, and to ligaments already in catch, to see if some extreme state of catch, normally not attainable by physical agitation, could be achieved (cf. Fig. 3). In every case, ACh had no effect on F-10. As another test of cholinergic control, 1 mmol l⁻¹ d-tubocurarine was applied to a ligament in catch. Again, there was no effect on F-10.

Hidaka (1983) suggested that the ACh-induced increase in stiffness reported by Hidaka & Takahashi (1983) is transient in nature and begins to disappear after 1–2 min of exposure. Thus, a 'desensitization'-like process may have masked any ACh effect in the present experiments where 10-min exposures were used.

Several catecholamines were also tested on ligaments already in catch. Epinephrine (0·01 mmol l⁻¹) had no inhibitory effect on catch as judged by the unchanged F-10 response. This is in agreement with Hidaka & Takahashi (1983) who also found no inhibitory effects when quick stretches were used. Similarly, 0·1 mmol l⁻¹ serotonin and 0·1 mmol l⁻¹ dopamine had no effect. These latter substances cause release of catch in the non-striated muscle of various molluscs (Florey, 1966).

As a check on the efficacy of the method for application of these substances, methylene blue sea water was applied in exactly the same manner as above. One minute following application, the ligament was cut open to expose its innermost surface; this was found to be well stained. Therefore, there does not appear to be a significant diffusional barrier to substances applied to the outer surface of a stripped ligament.

**DISCUSSION**

This paper presents data on the mechanical properties of the spine ligaments in the sea urchin and considers some possible physiological control mechanisms for the setting and releasing of catch. It confirms and extends on another genus of urchin some basic findings of Smith et al. (1981) and Hidaka (1983) and the stiffness measurements for ligaments in catch by Hidaka & Takahashi (1983). It also offers suggestive evidence for a Ca-dependent enzymatic mechanism of collagen cross-linking as the chemical basis for setting catch.

One important result described here is the occurrence of structural damage or weakening when a spine in catch is deflected through an angle of greater than 10°. A similar alteration in ligament constitution may have occurred at approximately the same amount of stretch in the 'movable' spines described by Smith et al. (1981).

These ligaments showed a dramatic decrease in extensibility at a stretch of 0·3–0·4 mm (longitudinally applied), and this amount of stretch can be converted to an equivalent angle of deflection by a method employing the same principles as that described in the Appendix. Using a ligament radius of 1·0 mm (estimated from their micrographs) and other parameter values cited in their report, the 'critical' 0·3–0·4 mm stretch is equivalent to 16·9–21·8° of deflection, which corresponds to that region where the critical angle for damage lies. Hidaka & Takahashi (1983) als
Ligament catch in sea urchin spines

Reported that the apparent stiffness of a ligament in catch began to decrease at stretches of \( >10 \% \), and that tensile strength was exceeded by stretches of \( \sim 15 \% \). In fair agreement with these data, we observed ligament damage to begin at approximately 8% stretch (\( \sim 15^\circ \), cf. Figs 5, 6). Exactly what happens at this angle requires a more detailed mechanical model for the ligament system and the mechanism of catch maintenance by collagen fibrils. According to the ideas presented by Smith et al. (1981), this critical angle may represent the limiting amount of stretch which the cross-links between collagen fibrils can withstand before breaking. Since this breakage appears not to be readily reversible (cf. Fig. 4), it follows that the cross-links are not capable of rapid reformation once broken by excessive stretch.

This type of structural damage due to excessive stretch does not appear to be related to the force decay phenomenon in F-10 responses described in conjunction with Fig. 2. Decay in force following a quick stretch to a maintained angle of deflection corresponds to a slow stress relaxation due to the viscoelastic properties of the ligament. This does not reflect damage to the catch mechanism, as F-10 responses of nearly identical magnitude and time course can be repeatedly obtained over the same arc of spine deflection. Fast and slow components of F-10 decay were seen in every ligament in catch studied. This behaviour may involve the sliding of bundles of collagen fibrils past one another that Hidaka & Takahashi (1983) observed in relaxed ligaments under stretch. The extent of sliding during catch is not known. A similar phenomenon may occur in holothurian dermis, where stretch under a load produces fast and slow creep rates (Motokawa, 1982).

Another new finding in this work is that the transglutaminase inhibitors cadaverine and putrescine at very low concentration can prevent the setting of catch. This suggests a mechanism involving transglutaminase or a transglutaminase-like enzyme (TGL) for the formation of collagen cross-links. In support of this hypothesis is the observation that the setting of catch is Ca-dependent, since Ca is required to activate vertebrate transglutaminase (Curtis & Lorand, 1976). Thus, if a Ca-dependent transglutaminase or TGL controls catch, then the absence of Ca should block its setting, as was observed.

What triggers the actual setting of catch remains unclear. All attempts to activate catch in stripped ligaments with electrical stimulation or suspected neurotransmitters were unsuccessful. The anatomical basis exists, nevertheless, for activation via secretion of some substance from axonal processes inside the ligament itself (Smith et al. 1981), and this remains the most likely possibility.

These postulated mechanisms for triggering and setting catch are both critically dependent on Ca ions. Thus, Ca might support both the neurosecretory release of TGL from its intracellular storage site in the intra-ligamental nerve endings and the subsequent activation of TGL which results in catch. This simple model is thus consistent with both the pharmacological and anatomical data.

One problem with this, however, is the very long recovery time for the ligament to regain catch-setting ability when its Ca supply is replenished following treatment with Ca-free sea water (many hours). Several explanations can be offered. One is that Ca-free sea water in some unknown way causes loss of inactive TGL from the intracellular storage sites. Catch regaining would then require regeneration of the lost enzyme, presumably a slow process.
A second explanation requires modification of the simple model. In this case, the TGL storage site may not be intracellular, and TGL must then be bound extracellularly in some Ca-dependent manner and activated by an additional cofactor. It would then be this cofactor which is stored in the axonal processes and released by nervous activity to activate TGL and trigger catch. Depletion of Ca would result in the inhibition of neurosecretory release of cofactor and the loss of inactive TGL from its binding sites to the medium surrounding the spine. Again, the limiting step in determining recovery time following treatment with Ca-free sea water would be regeneration of lost enzyme.

Summarizing, results described here suggest that nervous activity and the action of neurotransmitters are not the sole elements involved in the mechanism of setting catch. Nor does it seem likely that catch is directly controlled in vivo by changes in extracellular ionic strength, pH (Hidaka, 1983) or tonicity (Eylers, 1982). These factors modify stiffness by two- to three-fold, considerably less than that observed in the present study, and proposed mechanisms for in vivo changes are lacking.

Rather, we propose that catch in the spine ligaments of the sea urchin is mediated by neurosecretory activation of a Ca-dependent enzymatic process involving transglutaminase or a similar enzyme. The sea urchin spine ligament is a complex composite material of apparently discontinuous collagen fibres in an unusual and very interesting matrix. Our model of TGL-activated cross-linking of collagen with itself

Fig. 8. Schematic diagram of spine-ligament system (see Appendix). (A) Representation of spine deflection through angle $\theta$ which results in ligament stretching through a distance $x$. (B) Cross-section through the ligament. Spine deflection is about the dashed line, with the lower half of the ligament as drawn being compressed (thus not contributing to the F-10), and the upper half being stretched (thus resisting stretch).
Ligament catch in sea urchin spines

and possibly with other matrix components not only provides a basis for setting and maintaining catch that is consistent with available morphological and physiological data, but also suggests how interactions between collagen and matrix may change in the TGL-activated catch process.

APPENDIX

In this appendix we derive a relationship between horizontally applied force on a spine and longitudinal stretch of the ligament as based on the schematic diagram in Fig. 8. From this, a calculation of Young’s modulus, \( E = \delta / \epsilon \), is possible.

First, consider the strain (\( \epsilon \)) to which the ligament in catch is subjected:

\[
\epsilon = \frac{x}{L}.
\]

From Fig. 8A it follows that:

\[
x \approx X \sin \theta
\]

for small \( \theta \) and from Fig. 8B that:

\[
X = r \sin \phi.
\]

Thus, a new expression for strain can be written:

\[
\epsilon = \frac{r \sin \phi \sin \theta}{L}.
\]

Related to this strain is the stress (\( \delta \)) of the ligament:

\[
\delta = \epsilon E.
\]

Incorporating equations 4 and 5 into one then gives:

\[
\delta = \frac{Er \sin \phi \sin \theta}{L}.
\]

Stress is also related to the resistive force \( F_{\text{res}} \) of the ligament through cross-sectional area (\( A \)):

\[
F_{\text{res}} = \int \delta A,
\]

where \( A = rd \phi \) is that element of ligament area at each \( \phi \). Expanding this, we get

\[
F_{\text{res}}X = \int \epsilon E A X
\]

or

\[
F_{\text{res}}X = \frac{E \sin \Theta}{L} \int r^3 \sin^2 \phi drd\phi.
\]

Now, magnitude of the torque applied on a spine when deflected through a small angle \( \theta \) is met by the resistance to stretch of the ligament in catch along \( x \). Thus,

\[
F_{\text{appl}} Y = F_{\text{res}} X
\]

or

\[
F_{\text{appl}} Y = \frac{E \sin \theta}{L} \left( \frac{r^4}{4} \right) \left[ R_2 \left( \frac{1}{2} \phi - \frac{1}{4} \sin 2\phi \right) \right]_0^\pi.
\]
Rearranging and evaluating integrals yields the final expression for Young's modulus, E:

\[
E = \frac{F_{\text{appY}}}{\sin \theta (r^4) [\frac{R_2}{(2\phi - \sin 2\phi)]^0}}
\]  

or

\[
E = \frac{F_{\text{appY}}}{\pi (R_2^2 - r_1^4) \sin \theta}.
\]  

We thank Dr Mark Denny for thoughts on Young's modulus and comments on the manuscript, Dr David Epel for bringing transglutaminases to our attention, Freya Sommer for collecting urchins, and Keith Tansey for use of the tensometer.

REFERENCES


