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The Relationship Between Sensory Activity and Muscle Tension in the Anterior Flexor Muscle of Horseshoe Crab Walking Legs

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A population of series tension receptors associated with the anterior tibial flexor muscle in the walking legs of the horseshoe carb was studied. Active contraction of the muscle readily elicited receptor activity but the receptors were largely insensitive to imposed extension or flexion ~~of the passive muscle~~. The anterior flexor muscle inserts on the tibia. One of the three heads of the muscle originates in the patella and the other two originate in the femur. For a given contraction, a higher frequency sensory discharge was observed when the muscle was fixed at greater lengths. This relationship held whether muscle length was changed by manipulation of the femero-tibial joint, the patello-tibial joint, or both. For most of the units studied (12 of 15) the relationship between receptor spike frequency and whole muscle tension was highly linear. The results of this study suggest that the sensory endings measure the tension in apodemes common to the three heads of the muscle, define the relationship between muscle tension and receptor activity, and show that the tension in both of the flexor muscles operating the rotatable patello-tibial joint is monitored by appropriate sensory activity.

INTRODUCTION

Two basic groups of force-sensitive sensory elements have been

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recognized in arthropods. The first group consists of receptors sensitive to externally applied forces, such as cuticular stresses and strains. Among the insects, this class is represented by the campaniform sensilla, which have been associated with walking and postural reflexes in the cockroach (Pearson, 1972, Zill and Moran, 1981a, b; Zill *et al.*, 1981), the locust jump (Heitler and Burrows, 1977a, b) and eliciting the fast reflex activation of appropriate wing muscles during locust flight (Wendler, 1978). Similar information concerning forces impinging upon the exoskeleton is provided by the slit sensilla of arachnids (Pringle, 1955) and the cuticular stress detectors in Crustacea (Shelton and Laverack, 1968; Clarac *et al.*, 1971; Wales *et al.*, 1971).

Sensory receptors composing the second group of tension receptors are intimately associated with the working musculature, directly monitoring the forces developed within these muscles. These receptors also represent a wide range of activities, including, for example: shell closing in the mollusc, *Mytilus* (LaCourse and Northrup, 1978); contraction of the cibarial pump muscles of the blowfly and tsetse fly (Rice, 1970); forces in the tailspine muscles of the horseshoe carb, *Limulus* (Silvey, 1973; Eagles and Hartman, 1975); and walking leg activity in both the decapod Crustaceans (Macmillan and Dando, 1972) and in *Limulus* (Eagles, 1978).

Although tension receptors have been described in a number of invertebrate systems, comparatively little attention has been devoted to characterizing, in detail, the relationships between sensory activity and muscular force. This information is vital to understanding the strategy employed by invertebrates in assessing muscle loading and, hence, in load compensation. We have addressed this control problem using the tension receptor system of the walking legs in *Limulus* (see Eagles, 1978). In this report, evidence is presented for the presence of true series tension receptors in the anterior tibial muscles. In addition, the relationship between receptor spike discharge rate and whole muscle tension is defined quantitatively.

MATERIALS AND METHODS

Horseshoe crabs, *Limulus polyphemus*, were purchased from the Gulf Specimen Co., Panacea, Florida. These animals (16 ± 2 cm in prosomal width) were maintained in artificial seawater (Instant Ocean, Aquarium Systems, Eastlake, Ohio) at 15–18°C in a 50-gallon aquarium without feeding and were used within one month of arrival. Third and fourth legs from either side were used, and legs exhibiting any anomalies were rejected. The legs were removed from the animal by severing the femur.

Suction electrodes were used for stimulating and recording nervous activity from severed nerves using standard electrophysiological techniques.

The tibial flexor muscles were exposed by cutting through the medio-ventral exoskeleton of the patella (terminology of Snodgrass, 1965) and pinning the leg segment open onto a Sylgrard (Dow Corning) coated dish containing *Limulus* saline (Eagles, 1978). This mounting procedure did not allow extension of the patello-tibial joint beyond 180° but the P-T joint was otherwise free to rotate. All experiments were performed at ambient temperatures of approximately 25°C. The average duration of an experiment, from dissection to termination, rarely exceeded one hour.

why

Whole muscle tension was measured by attaching a Grass FTO3 force transducer to the tibial index (Wyse, 1971) with 000 surgical silk. The electrical output of the FTO3 was displayed on the oscilloscope and stored on magnetic tape. The potential generated by the FTO3 prior to induced muscle contraction was defined as the zero baseline against which all subsequent forces were compared.

Movements of the tibia relative to the patella were controlled in two different ways. Precisely controlled movements in the flexion-extension plane were produced and monitored by the electromechanical system described by Eagles (1978). For movements in other planes and for more complex movements, such as circular rotation of the tip of the tibia, a glass rod attached to the tibial index with a small section of pliable plastic tubing was manipulated by hand. During manual manipulation, the position of the tibia was estimated to within ±5° by reference to a protractor. The protractor was mounted on a mirror, and, as an aid in minimizing parallax, the limb was aligned with its virtual image.

In data analysis, linear regression analysis was used, where indicated, to calculate the "best fit" line via least square analysis. Linear coefficient (r) values ≥ 0.900 were accepted as indicative of a linear relationship between the X and Y values.

effect of F-P joint status on the activity of the tension receptors associated with the anterior tibial flexor. As the F-P joint is extended, muscle fibers in head III are stretched. Thus, during an active bout of muscular contraction, extension of the F-P joint should result in an increased whole muscle tension. The responses of a total of 38 identified tension units were recorded while manipulating the planar F-P joint. As expected, when the F-P joint was extended (with the P-T joint fixed at 150°), anterior tension unit activity increased and when the F-P joint was flexed, the anterior tension unit activity decreased. Although the frequency of unitary activity varied during F-P joint manipulation, no change in the force vs. activity relationship was noted (Figure 7).

DISCUSSION

Most of the receptors (33 out of 36) associated with the anterior tibial flexor muscles responded selectively to muscular tension in the anterior tibial flexor muscle. The anterior flexor tension receptors characteristically showed higher thresholds in their responses to passively imposed stretch than to tension generated by active contraction of the muscle with which they are in series. Rapid removal of tension via a sudden release of the leg from an extended position (during which the flexors were excited by stimulation of motor nerves to the anterior flexors) resulted in immediate cessation of sensory activity, indicating that tension was the adequate stimulus. The anterior tension unit response to cyclic flexion and extension of the tibia was consistent with the expected changes in tension in the flexor muscles as the tibia was moved. Flexion of the tibia, leading to decreased tension in the flexors, caused a decrease in tension receptor activity, while extension of the tibia, which increased tibial flexor tension, led to increased activity in the tension receptors. The tension receptors associated with the anterior flexors, therefore, appear to be physiologically similar to those described in association with the posterior tibial flexors (Eagles, 1978). Thus, the level of tension within both tibial flexor muscles is represented by a bilateral tension system.

Parsons (1982) has suggested that decapod tension receptors respond mainly to the rate of increase rather than the level of muscle tension. Our results did not permit resolution of rate vs. level as the major determinant of tension receptor activity. Compared to those of other arthropods, the muscles of *Limulus* receive and abundant motor supply (Fourtner and Sherman, 1973). The results of several studies of tension responses in *Limulus* suggest that the receptors are in series with some muscle fibers and in parallel with others (Eagles and Hartman, 1975; Eagles, 1978; Fig. 5d, e of this report). Variations in the functional relationships between tension receptors and the muscle fibers they innervate may account for the differences in the level of tension adequate to saturate the responses of individual receptors, noted below.

Unlike the posterior tension nerve, the anterior tension nerve was sometimes inhomogeneous for tension receptors, as evidenced by the presence of the few "position" sensitive elements. Whether these position sensitive elements represented, perhaps, joint receptors, or tension receptors whose behavior was altered by experimental procedures, or other proprioceptors, was not clear.

Close inspection of the individual plots of force vs. frequency for anterior tension units revealed that individual receptors shared the common property of a linear response to muscle tension but differed in the level of tension at which the response reached a plateau spike frequency. This relationship is common among tension receptors and is likely a consequence of an anatomical arrangement in which receptors are in series with some muscle fibers and in parallel with others (Houk and Henneman, 1967; Eagles and Hartman, 1975; Reinking *et al.*, 1975). Since the calculation of '*r*' incorporated all points in the plot, the median linear coefficient of 0.976 is, if anything, an underestimate of the highly linear relationship that exists between anterior tension unit activity and whole muscle tension throughout the physiological range of receptor sensitivity.

Although the majority of units studied exhibited such a linear relationship, the slopes derived for individual receptors (# spikes/sec/gm) differed, indicating that, for the population, no single relationship existed between whole muscle tension and a particular frequency. It would seem likely, then, that the central representation of muscular force is a complex process involving the recognition of the properties of individual receptors.

It shall be noted that, in the plots of whole muscle tension, vs. anterior unit activity, not all of the regression lines extrapolated *back* to the zero intercept on the Y-axis. This observation may *reflect* receptor threshold and/or force components lost due to muscle head rotation prior to the actual shortening of the muscle, to visco-elastic components, or an inability to impose strict isometric contractions at these low levels of tension. Since none of the receptors were spontaneously active in the relaxed leg, it appears that the receptors respond non-linearly at very low tensions.

The tibial flexors of the horseshoe crab leg are rather unusual in having their sites of origin (head III) and insertion separated by a planar (F-P) and a non-planar (P-T) joint. This system is further complicated by the fact that extension of the F-P joint is entirely passive (Ward, 1969). It would appear that the tension units participate in the sensory representation of the status of both the P-t and the F-P joints (both of which play important roles in locomotory behavior), with additional information provided by tibial length receptors and F-P joint proprioceptors. It is particularly interesting that, while extension of the F-P joint led to increased whole muscle tension in the tibial flexors, this extension did not alter the linear nature of the receptor response to changing tension. Since one of the three heads is affected by F-P joint movement, a reasonable conclusion is that tension units innervate the apodeme at a location distal to the site of muscle insertion.

Since two joints are known to affect the status of the tibial flexor muscles, the tension receptor system, alone, cannot provide unambiguous information about the origin of the force exerted at the tendon. This finding implies that, in order for the CNS to ascertain the full set of circumstances which caused the loading of the flexor muscles, collaborative information detailing the status of the F-P and P-T joints is required. In antennae of Decapod Crustacea, differential activity in two (Hartman and Austin, 1972) or three (Rossi-Durand and Vedel, 1982) groups of sensory neurons associated with different regions of branched elastic strands in a single chordotonal organ spanning two joints provided unambiguous information about antennal position. In *Limulus*, the presence of information pertaining to tibial muscle length (Eagles and Gregg, 1979) and of the position of each leg joint (Hayes and Barber, 1967) provides similarly unambiguous information to the CNS in analyzing the parameters leading to the achievement of a given level of muscle tension.

The major conclusion of this study is that tension reception in the horseshoe crab primarily involves the assessment of whole muscle tension summed at the tendon rather than representing a sample of the tension developed within anatomically discrete muscle heads or groups of fibers. At present, the tension receptor neurons have not been identified morphologically. Based on our observations, we speculate that the receptors most likely reside toward the insertions of the two most distal heads of the anterior flexor, the receptor population thus being ideally located to sample whole muscle tension. Such a system would resemble the tension receptors at the flexor apodemes of the merus-carpus joints of decapods, as morphologically described by Parsons (1980).

The overall strategy of whole muscle tension being described linearly by tension elements has also been postulated in the vertebrates by Reinking *et al.*, (1975). Muscle tension information is provided in the vertebrates by the Golgi tendon organs, which are in series with muscle fibers from different motor units within a given muscle (Stuart *et al.*, 1972). Although the activity of a single Golgi tendon organ is not necessarily related in a linear fashion to the tension generated by any of the muscle fibers attached to the organ (Gregory and Proske, 1975; Fukami and Wilkinson, 1977; Proske and Gregory, 1980), the collective behavior of a population of Golgi tendon organs was suggested by Reinking *et al.* (1975) to be linearly related to whole muscle tension, provided that the CNS randomly integrated the tension summed at the tendon. The differences in the behaviors of the individual receptors may stem from the heterogeneity of the motor units inserting upon the Golgi tendon organ capsule (see Binder *et al.*, 1982, for review). In *Limulus*, since the skeletal muscles are composed of relatively homogeneous muscle fiber types (Fourtner and Sherman, 1973), a linear response by series tension receptors may be a consequence of a greater degree of muscle fiber homogeneity.

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