Tension receptors on the closer muscle apodeme in the walking legs of the blue crab, *Callinectes sapidus*

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Summary. 1. Receptors responsive to muscle tension have been located on the closer apodeme of walking legs of the blue crab *Callinectes sapidus*. The axons form a discrete nerve, the closer apodeme sensory nerve (CASN) which joins the PD organ nerve.

2. The receptors are 25–35 bipolar cells whose cell bodies measure between 25–60 μm, located on the distal end of the apodeme. The dendrite of each is inserted through the hypocuticle into the endocuticle of the apodeme. No receptors are found directly associated with muscle fibers.

3. The apodeme receptors exhibit no spontaneous activity at any rest position of the dactyl, and are unresponsive to passive movement of the dactyl.

4. Isometric tension causes the apodeme receptors to fire. Increased tension increases the frequency of output with the recruitment of additional units.

5. Upon isotonic contraction against increasing loads, the receptors show increased output and the recruitment of additional units. Isotonic contraction of the unloaded closer muscle evokes no response by the receptors.

6. The combined anatomical and physiological evidence supports the theory that these sensory cells are series tension receptors.

Introduction

For many years tendon organs in vertebrates were considered high threshold receptors which shut down motor discharge when muscle tension is excessive. This view has since changed completely with the demonstration by Houk and Henneman (1967) that tendon organs have low thresholds to tensions produced by muscle contraction. Tension receptors associated with working muscles of arthropods were first discovered by Macmillan and Dando (1972). These receptors on the flexor apodeme of the meropodite of crab walking legs, in addition to meeting the criteria for tension reception, also evoke reflexes (Clarac and Dando 1973; Macmillan and Laverack 1982; Parsons 1982).

Eagles and Hartman (1975) reported tension receptors associated with the apodemes of the large flexor muscles powering tail spine movement in the chelicerate *Limulus polyphemus*. Tibial flexor muscles of *Limulus* walking legs also contain tension receptors (Eagles 1978) capable of producing reflexes (Eagles and Gregg 1984). Femoral tension receptors evoking reflexes have also been found in insects, specifically *Schistocerca americana gregaria* (Theophilidis and Burns 1979).

In this communication, the physiological and anatomical properties of tension receptors on the closer apodeme of crab walking legs are reported.

Materials and methods

Specimens of *Callinectes sapidus* obtained from Port Aransas, Texas and measuring 12–15 cm across the carapace were used in this research. They were maintained in the laboratory at 18–21° C in 20 gallon aquaria containing artificial sea water and used within three weeks of capture. An animal was caused to autotomize a first or second walking leg, the tip cut from the dactyl, and a hypodermic needle on a large syringe containing cold TRIZMA buffered MBL formula (4) sea water (in *Formulae and methods VI* published by the Marine Biological Laboratory, Woods Hole, Mass. USA) introduced into the dactyl to flush hemolymph from the excised limb. To expose the propus-dactylus organ (PD organ) and the closer apodeme sensory nerve (CASN), a window was cut on both the anterior and posterior faces of the propus, large enough to expose the

opener muscle and the distal end of the closer apodeme, but without disturbing the condyles or the closer muscle fibers. To facilitate access to the receptor nerves, the opener muscle was removed. By carefully exposing the PD organ nerve, a small distinct nerve near the proximal end of the elastic strand could be located. Glass needles were used to separate a long length of this nerve (CASN) from the PD organ nerve.

By cutting windows in the anterior and posterior faces of the carpus, the motor nerve to the closer muscle could be located and isolated for electrical stimulation. The meropodite was then discarded. With the anterior surface upwards, the propus was fastened firmly with stainless steel staples to a Petri dish with a Sylgard (Dow Corning) lined bottom. This arrangement allowed unimpeded movement of the dactyl upon contraction of the closer muscle. The dish containing the preparation and cold oxygenated saline was attached to a Peltier thermoelectric device which maintained the temperature of the contents at 15° C. Glass suction electrodes were used to record individually from the PD organ and CASN and to stimulate the nerve to the closer muscle (Fig. 1).

Manipulations to obtain isotonic or isometric records are described in the Results section. Electrical activity was amplified, displayed, tape recorded, and filmed by conventional means.

The anatomy of the tension receptor cells was determined by backfilling the PD organ-closer apodeme sensory nerves with 300 mmol/1 cobalt chloride for 24 h at 10° C. The cobalt was precipitated with dilute ammonium sulfide and the preparation fixed in Carnoy's. If necessary, following rehydration, lightly-stained fills were intensified using the Bacon and Altman (1977) modification of the Timm's technique. The CASN preparation was again dehydrated, cleared in methyl salicylate, and mounted in Canada balsam in a depression slide. Selected preparations were photographed or drawings made with the aid of a camera lucida.

Results

Physiology

Receptor responses to isometric contraction and release. The responses of the PD organ and the closer apodeme sensory nerve (CASN) to tension development by the closer muscle and to position and movement of the dactyl were compared. To monitor those parameters, one end of a 100 μm stainless steel wire was attached to the tip of the dactyl, and the other end to the shaft of a Sanborn DCDT isotonic transducer (Fig. 1). With each of the two nerves drawn into suction electrodes and an electro-mechanical stop holding the dactyl open (ca. 15°), the motor nerve to the closer muscle was stimulated.

As seen in Fig. 2, low frequency tonic activity from the largest position units in the PD organ nerve could be observed prior to stimulation (smaller position units were also active but cannot be resolved at this amplification). No neural activity was seen from the CASN. Shortly after the onset of stimulation (20 Hz) there was an increase in PD organ activity as low threshold movement units
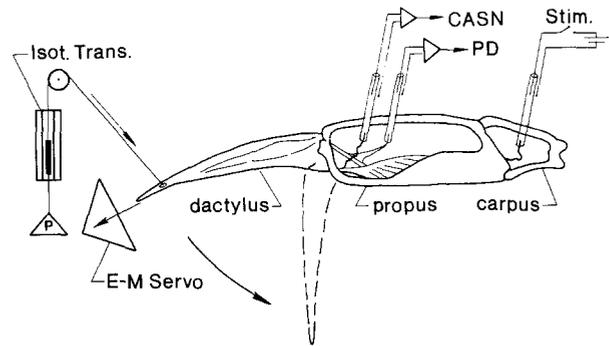
Fig. 1. Arrangement for stimulating the closer motor nerve, recording action potentials from the PD organ (*PD*), the closer apodeme sensory nerve (*CASN*), and monitoring mechanical movement of the dactyl. A rigid probe attached to an electromechanical servomechanism (*E-M Servo*) restrains the dactyl from closing until desired isometric tension of the closer is achieved. Upon rapid withdrawal of the probe, the dactyl closes. Dactyl movement is measured by the isotonic transducer (*Isot. Trans.*)

were excited by closer apodeme displacement upon muscle contraction. These responses then decreased when the dactyl pulled tightly against the stop. When the dactyl-stop was released (upward deflection of the movement trace) the movement units greatly increased their firing rates and larger movement units were recruited as isotonic contraction proceeded and the dactyl closed. The activity exhibited by the CASN followed a different pattern. Shortly after the PD organ detected movement, a few smaller, followed by larger, units of the CASN began to respond, and they continued to fire vigorously during isometric contraction. When the stop was released and the isometric muscle tension dropped to zero, they ceased firing until the dactyl closed and isometric tension developed again. The PD organ was responsive to movement of the dactyl but the CASN was responsive to isometric tension of the closer muscle. When the motor nerve was stimulated at higher frequency (40 Hz), the units of the CASN fired at a higher frequency in response to the resulting greater isometric tension prior to stop-release and again upon reaching the closed dactyl position. The PD organ movement units fired more vigorously to stop-release since the dactyl movement was initially greater, and the greater closed position (note movement trace) recruited an additional large unit.

No spontaneous activity by the CASN was ever observed when the dactyl was held at any position. Imposed passive movement of the dactyl, while causing strong output from the PD organ, never evoked a response from the CASN.

CASN response to isotonic contractions. To qualify as a tension receptor, CASN units should show
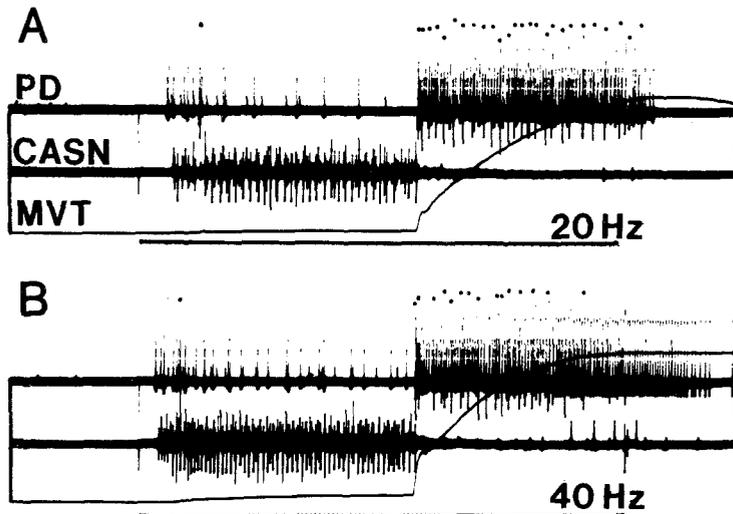
Fig. 2A, B. Records showing the neural responses of the PD organ (*PD*) and the closer apodeme sensory nerve (*CASN*) upon stimulation of the closer motor nerve, isometric contraction, and isotonic contraction. Movement (*MVT*) of the dactyl is monitored by the third trace. The bottom trace is a record of the 2.8 s duration stimulus at 20 Hz (**A**) and 40 Hz (**B**)
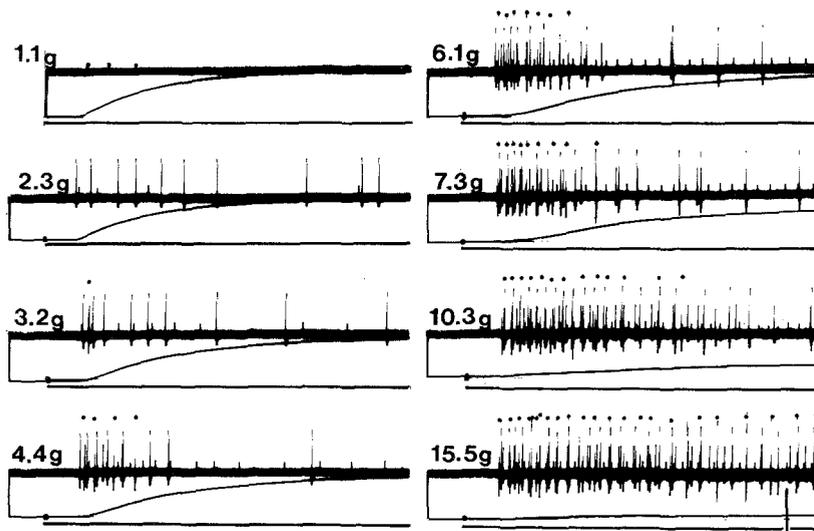
Fig. 3. Neural activity by the CASN (upper trace) to isotonic contraction (middle trace) against increasing loads when the closer motor nerve is stimulated at a standard frequency of 100/s. Time calibration = 1 s; dactyl movement calibration = 90°

increased response to increasing loads upon isotonic contraction. To determine this, small weights were fastened to the fine steel wire attached to the dactyl. The position of the dactyl was monitored by an isotonic transducer (Fig. 1) while the motor nerve was stimulated at 100 Hz. An insect pin held the dactyl near the open position so that the muscle was not directly loaded and each trial began at the same starting point.

When the closer muscle contracted and the dactyl closed lifting a 1.1 g load, one, perhaps two small units fired (Fig. 3). With a load of 2.3 g two additional larger units were recruited. These small units increased in frequency of firing and a larger unit responded when the load was 3.2 g. At 4.4 g loads, all five units responded with brisk outputs. With loads of 6.1 g and beyond, these same recep-

tors increased their outputs, particularly during the isometric latency phase prior to isotonic contraction. A load of 15.5 g produced a nearly isometric contraction and the highest frequency output by the five units. There is a strong suggestion in this record (and replications in four other preparations) that the receptors are being recruited in order of increasing axon diameter. Isotonic contraction of the unloaded closer muscle produced no output by the CASN.

CASN response to isometric contraction. The receptor response to tension developed during isometric contraction was next examined. The muscle fibers attached to the closer apodeme are flaccid when the dactyl is in the closed position (ca. 90°). They are increasingly stretched as the dactyl is opened,
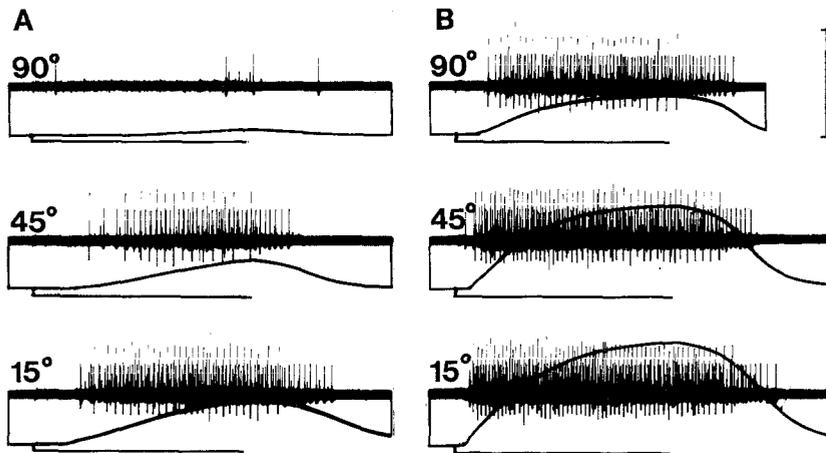
Fig. 4A, B. Output by the closer apodeme sensory nerve (upper trace) to increasing isometric tension (middle trace) upon stimulation of the closer motor nerve for 2 s at 40 Hz (A) and 100 Hz (B). With the dactyl position at 90°, the muscle fibers are flaccid prior to stimulation; at 45° and 15° they assume greater in situ lengths. Tension calibration = 100 g

reaching maximum in situ length when the dactyl is at ca. 15°. It follows then that the muscle fibers develop little tension when they are stimulated to contract while the dactyl is closed, but that greater tensions can be achieved when the dactyl is opened.

In order to record isometric tension the dactyl was attached to a Harvard Apparatus 373 isometric transducer mounted on a manipulator. The dactyl was cut away except for a stub distal to the dactyl-closer apodeme articulation. The stub was then clamped by forceps mounted on the transducer. By adjusting the position of the transducer via the manipulator, the previously determined position (15°, 45°, 90°) of the dactyl could be approximated. The motor nerve was then stimulated at either 40 or 100 Hz for a 2 s duration, and then CASN response recorded.

Stimulation of the motor nerve at 40 Hz for 2 s produced a slow rate of tension development no matter what the position of the dactyl (Fig. 4A). In the closed position (90°) little tension (5 g) was achieved and the few units of the CASN that responded did so weakly. With the dactyl in the midposition (45°) a peak tension of 25 g resulted from stimulation at 40 Hz. The CASN units fired at a higher frequency and a large unit was recruited. The same regime but with the dactyl in the open (15°) position evoked a vigorous response from the same units and a peak tension of 45 g.

When stimulated at 100 Hz for 2 s the rate of tension development increased and the output by the CASN did as well (Fig. 4B). Peak tensions for the closed, mid, and opened positions were 35, 75, and 90 g, and the firing by the CASN showed a corresponding increase in frequency. As the slope of tension development increased, the units also began their response earlier. Similar records were obtained in four other preparations.

In another series of experiments with the dactyl

in the open position (15°), the response by units of the CASN to a wider range of stimuli to the motor nerve was recorded. The isometric tension was monitored by attaching a rigid steel pin fixed to the transducer into the base of the intact dactyl ventral to the condyle. When the motor nerve was stimulated at low frequency (12 Hz) so that little tension developed, one small unit fired at low frequency followed later by a single pulse from a larger unit (Fig. 5). With the stimulus at 15 Hz, greater tension developed, and both the small and larger units fired at higher frequencies. The 20 Hz stimulus recruited another large unit as the frequency of the others increased. Two very small units also responded for the first time. Upon stimulation at 30 Hz and with the development of greater tension, all units fired at higher frequencies and another intermediate unit was recruited. At the maximum frequency utilized (45 Hz) at least six fibers responded to the high tension. The response latency by CASN units decreased with the increase in the slope of tension development.

Anatomy

The closer muscle of the walking leg of *C. sapidus* occupies the ventral half of the propus. Muscle fibers from the anterior and posterior walls of the propus attach to the closer apodeme. Distally the tendon is stout and flattened dorso-ventrally, but narrows laterally while becoming thin, broad, and flattened antero-posteriorly over the proximal three-quarters of the propus. The distal end of the apodeme is hinged and attaches to the dactyl (Figs. 6, 7).

Cobalt backfilling of the PD organ nerve revealed the anatomy of the movement and position cells of the PD organ, as has been described else-
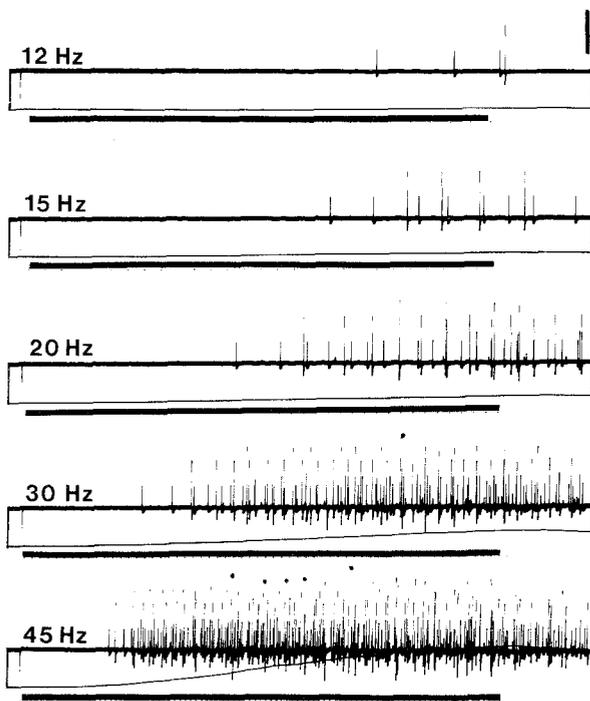
Fig. 5. The effect of varying the frequency of stimulation to the closer motor nerve (bottom trace) upon isometric tension (middle trace) and the output of the closer apodeme sensory nerve (upper trace). The dactyl position for all records was the open position (15°). Tension calibration = 10 g
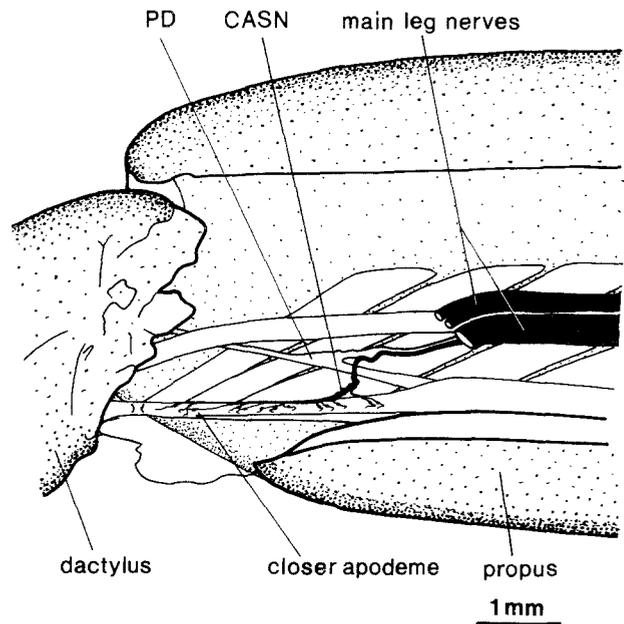
Fig. 6. Propodite-dactylopodite joint of the first right walking leg of *Callinectes sapidus* dissected from the anterior side to show the location of the tension sensory cells on the distal end of the closer apodeme, and the relationships of the CASN with the PD organ nerve. The distal portions of the main leg nerves have been cut away and the opener apodeme and muscles have been removed
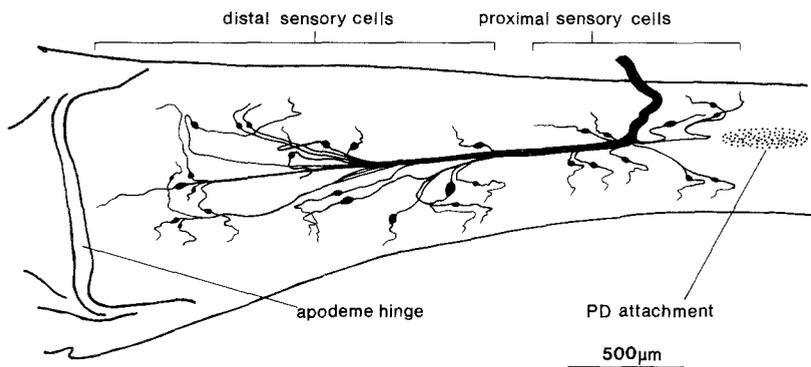
Fig. 7. A camera lucida drawing made of a cobalt chloride backfill of the closer apodeme sensory nerve. The closer apodeme is viewed from above

where (Hartman and Boettiger 1967; Mill and Lowe 1973), but also the presence of numerous bipolar sensory cells ramifying over the distal end of the closer apodeme. Whitear (1962) described a small nerve from the tendon that joins the PD nerve, passing on one side or the other of the proximal part of the elastic strand. She also included the nerve (unlabeled) in her figure of the propodite-dactylopodite joint. The axons of the sensory cells on the closer apodeme form this nerve which in turn joins the PD nerve (Fig. 6). Macmillan and Dando (1972) in their seminal paper on crustacean tension receptors allude to this nerve and to the

fact that '... at least one medium-sized bipolar cell was regularly stained on the nerve'. In keeping with the terminology established by Macmillan and Dando (1972), the term closer apodeme sensory nerve (CASN) has been adopted.

Eight successful cobalt preparations revealed a rather consistent picture of innervation by the CASN of the closer apodeme. Figure 7 is a camera lucida drawing made from one such preparation. Typically twenty-five to thirty-five bipolar cells with cell bodies measuring 25–60 μm could be identified. These cells were divided into a proximal group of 10–15 cells near the attachment of the

PD organ strand to the closer tendon, and a distal group of 20–25 cells inserted over a larger area out to the tendon hinge. The bipolar cells appear to be distributed in about equal numbers on the anterior and posterior sides of the apodeme, and no pattern of cell size to location was apparent. The dendrite of each bipolar cell of the CASN enters the hypodermis and follows a tortuous pathway before tapering to submicron dimensions as it runs through the endocuticle. Very careful examination of the preparations revealed that the dendrites of none of the observed bipolar cells became detached as a result of clearing with methyl salicylate. Furthermore, the dendrites of all the cells that had filled were inserted into the apodeme. This suggests that the dendrites of CASN units are not directly associated with muscle fibers.

Discussion

Receptors responsive to closer muscle tension have been found on the distal end of the closer apodemes of the walking legs of the blue crab *Callinectes sapidus*. The axons from the receptors form a discrete nerve (CASN) which joins that of the propus-dactylus organ. That the CASN carries tension information is supported by the observations that the receptors (1) are unresponsive to passive unopposed movements of the dactyl, (2) respond to isotonic contractions when the dactyl is lifting a load, (3) fire during isometric contractions, (4) abruptly cease firing when isometric contraction is halted by suddenly releasing the dactyl to allow closer muscle shortening against no load. The receptors show a variety of thresholds responding to tensions of less than one gram (Fig. 5) and traversing the physiological range observed for intact animals. In keeping with the observations made by Parsons (1982) for *Carcinus* flexor apodeme sensory nerve (FASN), these receptors respond to the rate of tension development and the total tension achieved (Figs. 2, 4, 5).

The tension sensory cells are divided into distal and proximal groups on the closer apodeme. This arrangement is also evident for cells of the flexor and extensor apodeme sensory nerves although the groups are more widely separated (MacMillan and Dando 1972). Why this division? Do the groups have different thresholds, or are they responsive to different muscle fibers? The results obtained by Parsons (1982) for FASN units in *Carcinus* suggest that proximal cells require more rapid tension development than distal cells, and proximal cells fatigue more rapidly.

The fiber composition and innervation pattern of the walking leg closer muscle of the lobster has recently been studied by electrophysiological and enzyme histochemical techniques. Govind et al. (1981) have shown that the muscle fibers attached to the distal end of the closer tendon (where the tension receptors are located) are tonic, have low ATPase activity, high oxidative capacity, and long sarcomeres. However, the majority (63%) of these fibers are innervated by both the fast closer excitor and slow closer excitor motoneurons, the rest (37%) being controlled by only the slow closer excitor. Overall, they have shown that in functional terms, the whole closer muscle is composed of five different contractile units. Should the closer muscle of *Callinectes* show a similar composition, it would be of interest to observe the output by the individual tension receptors to controlled stimulation of each contractile unit. The tension receptors at this point are amenable to individual recording.

In recordings made from the CASN, the number of tension units responding, based on amplitude of the action potentials, ranged from five to ten, whereas cobalt backfills of the nerve indicated that 25–35 tension receptors are located on the apodeme. Since the axons from these receptors form the CASN, it is obvious that the output of all of them is not being observed in the physiological recordings. Why? It is possible that the saline solution used was not satisfactory and many cells ceased firing. However, as the muscle fibers continued to respond and they are more labile than proprioceptors, this seems unlikely. It also seems unlikely that the thresholds of all the receptors have not been reached. Rapid, careful, and improving dissections should have reduced injury to muscle and nerve fibers so that later preparations might reveal more units, but this was not the case. A possible explanation is that the axons of many of these units are quite small and their output is obscured by the noise level and the larger units responding. Examination of the records from the FASN made by Macmillan and Dando (1972) and Parsons (1982) reveal far fewer tension units responding than axons observed in a cross section of the nerve. Parsons (1980) found eighteen fibers ranging from 9 to 20 μm in diameter and numerous smaller ones.

Burke (1954) observed a sensory discharge from the PD organ during isometric contraction of the closer muscle. Although he attributed the response to the slight movements of the apodeme activating movement units, he was also undoubtedly observing tension receptor activity from the

CASN. Since the tension units of the closer apodeme respond neither to passive unopposed movements of the dactyl nor to stretch of the elastic strand of the PD organ (personal observation), research subsequent to Burkes' on the properties of the movement and position cells of the PD organ does not need amending (see Mill 1976 for a review).

Bush (1962, 1965), working with *Carcinus*, demonstrated that passive opening and closing of the dactyl evokes resistance reflexes. Similar results were obtained for the chela of *Uca* (Spirito 1970) and *Procambarus* (Weins and Gerstein 1976; Lindsey and Gerstein 1977, 1979a, b), and the walking legs of *Cardisoma* (Spirito et al. 1972). Since the above experiments involved unopposed dactyl movements, in which case the tension units of the CASN were not activated, the reflexes were generated by the PD organ afference.

Stimulation of tension receptors in the meropodite has been found to inhibit resistance reflexes (Clarac and Dando 1973; Parsons 1982) and reflex activity evoked by other stimuli (Macmillan and Laverack 1982). Also, under higher stimulation they can excite both the homonymous and antagonistic motor neurons (Clarac and Dando 1973). The experiments involving the movements and manipulations of the dactyl during walking by *Cardisoma* doubtless call into play the tension receptors as well as the PD organ afference. As the reflexes were originally attributed to PD organ input alone, the interpretation of these results is obscured. Recent axon count and profile data of the PD organ nerve of the crayfish claw is confused, should that nerve also contain the CASN units (Lindsey and Brown 1982).

Proprioceptive representation at the propodite-dactylopodite joint includes the PD organ with its movement and position receptors, and tension receptors on the apodemes of the closer muscle and opener muscle (personal observation). The ability to manipulate each of these elements at this simplest of leg joints should allow new insights into the reflexes they generate and how the elements interact during locomotion. Before beginning such reflexive experiments, it would be prudent to first make a thorough search for receptors sensitive to muscle length as such units have been found in *Limulus* leg muscles (Eagles and Gregg 1979).

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