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THE ACTIONS OF PROCTOLIN, OCTOPAMINE AND SEROTONIN ON CRUSTACEAN PROPRIOCEPTORS SHOW SPECIES AND NEURONE SPECIFICITY

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Summary

A comparative survey is presented of the responsiveness of crustacean mechanoreceptors to the neurohormones proctolin, octopamine and serotonin. Seven identifiable primary afferents were examined in the crayfish *Cherax destructor* and the lobster *Homarus americanus*: three from the oval organ (OO) of the second maxilla, two from the non-spiking stretch receptor (NSSR) of the swimmeret and two from the muscle receptor organ (MRO) of the abdomen. Proctolin modulation was observed in 10 of the 14 fibres tested and was invariably potentiating, resulting in enhanced receptor potential amplitudes and increased firing. Octopamine and serotonin each modulated 8 of the 14 fibres and their effects were excitatory or depressive depending upon the target fibre. In the latter case the receptor potentials became attenuated, often to subthreshold levels, with loss of spiking.

A comparison of results from *Cherax* with those of *Homarus* shows that there is species specificity in the responses of homologous neurones. Neurohormones that are excitatory in one species may be ineffective or depressive in the other.

The broad distribution of modulatory effects observed here suggests that sensitivity to biogenic amines and peptides is a general property of proprioceptors.

Introduction

In recent reviews of the importance of neurohormones in facilitating the expression of particular types of behaviour in animals (Kravitz, 1988; Bicker and Menzel, 1989), emphasis was placed upon the multiplicity of loci at which neuromodulation can occur. A neurohormone, usually a biogenic amine or neuropeptide, may bring about concerted changes in activity in interneurones (Glanzman and Krasne, 1983), central pattern-generating circuits (Dickinson, 1989), subsets of motoneurones (Harris-Warwick and Kravitz, 1984) and muscle fibres (Bishop *et al.* 1987) in such a way as to promote a certain posture or behavioural sequence. In several invertebrate examples, key modulators have

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been identified and their effects on motor components well characterized down to the cellular level (reviewed recently by Calabrese, 1989). Less well documented is the influence neuromodulators may exert by their effects on proprioceptive feedback.

Pasztor and Bush (1987, 1989) showed that the biogenic amines octopamine and serotonin and the peptide proctolin, known to be significant in biasing behaviour in crustaceans (Kravitz, 1988), modulate responses of a simple proprioceptor in the ventilatory appendage of *Homarus*. Receptor potentials, and the consequent bursts of spikes in two of the three primary afferents, were enhanced by proctolin and depressed by the amines. Arthropod receptors have several advantages for neuromodulator studies. Many of them have identifiable, large, robust afferents suitable for both extracellular and intracellular recording. Some are non-spiking and send only analogue signals to the central nervous system (for a review see Bush, 1981). The present study used crustacean examples to address these basic questions. Is responsiveness to neurohormones a general property of mechanoreceptors? If so, do individual neurohormones evoke the same effects in all responsive afferents? Finally, we were interested in determining whether there was species specificity in the response patterns of sensory fibres to neuromodulators.

Three proprioceptors were tested in this survey: the oval organ (OO) of the second maxilla, the non-spiking stretch receptor (NSSR) of the swimmeret, and the muscle receptor organ (MRO) from the tonic extensor muscle layer of each abdominal segment. They well illustrate the diversity of organization found in crustacean stretch receptors, ranging from simple strand receptors (OO and NSSR) to the complex muscle receptor organs with efferent motor and inhibitor fibres. They also provide the full range of afferent fibre types with typical spiking units in the MRO and oval organ (Y and Z fibres), sparsely spiking units like the X fibre of the oval organ, and non-spiking fibres in the swimmeret receptors. Interspecific comparisons are made between a large, robust Australian freshwater crayfish, *Cherax destructor* (Clark), and the lobster, *Homarus americanus* (Milne-Edwards).

Three neuromodulators, known to be present in decapod crustaceans, were applied: two monoamines, octopamine and serotonin (5-HT), and the pentapeptide proctolin (RYLPT).

A preliminary report of some of these data has been published (Pasztor and Macmillan, 1988).

Materials and methods

Australian freshwater crayfish *Cherax destructor* were obtained by a local supplier from the Murray River system. They were kept in groups of 3–5 in outdoor aquaria exposed to their normal rhythms of temperature and ambient light. Provisions of ample shelter material and dry pellet cat food maintained the crayfish in good health for several months. Seventy intermoult male and female

specimens (carapace length 6–10 cm) were used in these experiments which were all made during the summer.

North Atlantic lobsters *Homarus americanus* were purchased from commercial sources and kept in artificial sea water at 15 °C. Thirty-six intermoult or pre-moult male and female specimens (carapace length 10–14 cm) were used in this series of experiments. Oval organ data from 47 additional *H. americanus* experiments described by Pasztor and Bush (1989) are included for comparative purposes.

The animals were chilled in iced water until anaesthetised, then dissected under cold aerated saline. All preparations were well washed prior to experimentation to flush away residual neurohormones.

The oval organ preparation

This stretch receptor lies at the base of the scaphognathite at the fulcrum between the blade and the basal segments of the second maxilla. Its three sensory afferents run in the scaphognathite levator nerve to centrally located somata within the suboesophageal ganglion. The anatomy of the oval organ in *Cherax* is very similar to that already described for the lobster, *Homarus* (Pasztor and Bush, 1983*a*), and the same procedures were used in preparing it for recording.

The ventral half of the cephalothorax was cut out and washed thoroughly to remove any digestive or excretory fluids. Ventral dissection of the sternites and endophragmal skeleton exposed the suboesophageal ganglion and maxillary nerve roots. Removal of the coxal and endite muscles then revealed the scaphognathite nerve and the oval organ. The mechanoreceptive dendrites are supported by an elliptical cone of connective tissue strands extending across the appendage. The base of the cone originates in a well-defined oval of flexible arthrodial membrane on the dorsal face, and its apex inserts onto a ventral, calcified infolding at the central end of the endites. The intact receptor assemblage, connecting nerve and suboesophageal ganglion were lifted out into a small experimental bath. The oval organ was oriented vertically with the arthrodial membrane firmly secured to the Sylgard bath floor with minuten pins, while the remaining fragment of sclerite at the apex of the cone was pierced by a fine hook attached to a puller assembly. Thus, upward pull on the cone of connective tissue imposed longitudinal stretch on the sensory dendrites.

For extracellular recording, the scaphognathite nerve was sectioned and a suction electrode applied to the distal cut end (see Fig. 1A). For intracellular recording, the nerve was stretched over a small Sylgard platform and the nerve sheath split lengthwise to reveal the three sensory afferents within (see Fig. 2A). Each could be identified first by its characteristic size (mean diameters in *Cherax*: X, 28 μm; Y, 24 μm; Z, 19 μm) and, later, physiologically. The flaps of cut nerve sheath were pinned back with cactus spines to immobilize the afferents during mechanical stimulation of the sensory endings.

The swimmeret preparation

There are several proprioceptive units responsive to movements of the

swimmeret base. In both *Cherax destructor* and *Homarus americanus*, two afferent fibres are readily identified by their extremely large diameter (approximately 60 μm) and non-spiking responses to swimmeret retraction. These were used in the neuromodulator trials. The dissections were identical in the two species.

The abdomen was bisected horizontally and the phasic musculature was removed from the ventral half to expose the ganglionic chain, first nerve roots to the swimmerets and the swimmeret orbits within the expanded ends of the sternal ribs. One hemisegment (usually the left fifth) was then isolated and prepared for recording. Early experiments used an intact swimmeret preparation mounted over a small trough sculpted into the Sylgard lining of the bath, allowing the appendage to hang down in the normal posture. Threads connected the protopodite to a motor-driven pulley system which moved the swimmeret smoothly back and forth in a close approximation of the natural beat pathway. However, the orbit of the swimmeret was packed with adipose tissue which was particularly viscous in *Cherax*. With the *in situ* preparation, the danger existed that the proprioceptive endings would be insulated from the saline flow in the bath, and thus deprived not only of oxygen but also of introduced neuroactive substances. Accordingly, we developed an isolated strand preparation which completely exposed the sensory endings (see Fig. 4A).

Both extrinsic and intrinsic coxal muscles were dissected out and all fat remaining in the swimmeret orbit was carefully removed exposing the central, orbital origin of the elastic connective tissue strand bearing the dendrites. This lies near the abdomino-coxal condyle. Excision, first of the pleural plate, then of the lateral and anterior rim of the orbit, revealed the distal insertion of the strand onto the arthrodial membrane near the proximal margin of the basipodite. A tongue of basipodite and membrane bearing the insertion was freed for attachment to the puller. In most crayfish, the receptor strand is too delicate for such procedures, but the large, robust *Cherax* specimens yielded reliable preparations.

For intracellular recording, with both the intact swimmeret and isolated strand preparations, the swimmeret nerve was held under slight tension over a Sylgard platform and transilluminated. Careful adjustment of the angle of incident light usually enabled both large afferents to be distinguished from the smaller motor axons. Desheathing was not necessary in *Cherax* but was helpful in *Homarus*.

The muscle receptor organ preparation

In both *Cherax* and *Homarus* a medial strip of the abdominal roof was stretched out, ventral side up, under saline, and first the phasic then the superficial tonic extensor muscles were removed, leaving the pairs of receptor muscles, RM1 and RM2, still attached to the dorsal carapace. A square of cuticle bearing the rostral origins of both RM1 and RM2 was excised, as was the border of the next posterior segment bearing their caudal insertions. The pairs of sensory receptors, with intact muscle strands and lengths of second nerve root attached, were removed to a Sylgard-lined experimental chamber.

MROs were taken from the second to the fifth segments and mounted in one of

four different configurations. (1) For experiments involving static stretch alone, the receptor muscles were held under tension by pins through the skeletal fragments. (2) In trials requiring both static stretch and dynamic pull stimuli, the receptor muscles were held in forceps mounted on a micromanipulated penmotor (see Fig. 6A). (3) To eliminate any tension changes contributed by receptor muscle contractions, the sensory neurones were anchored to the Sylgard floor by small steel staples immobilizing the receptor strands immediately to either side of the central dendritic zone. (4) To monitor drug-evoked contractions, the muscles were disposed vertically with their ends attached to a finely balanced Harvard heart lever bearing an opaque vane positioned in the light path of a phototransducer system.

Neuromodulator trials

The 10 ml experimental bath was perfused with aerated salines at constant temperature (20 °C for *Cherax*; 15 °C for *Homarus*). In *Cherax* trials, constant flow and bath level were maintained by gravity feed from an array of reservoirs at equal heads of pressure. In *Homarus* trials, a peristaltic pump provided continuous flow at 2.5 ml min^{-1}. Saline compositions were for *Cherax* (in mmol l^{-1}): 205 NaCl, 5.4 KCl, 13.5 $CaCl_2$, 2.6 $MgCl_2$, 10 Tris, 5 maleic acid, pH 7.4; and for *Homarus* (in mmol l^{-1}): 462 NaCl, 16 KCl, 26 $CaCl_2$, 8 $MgCl_2$, 11 glucose, 10 Tris, 5 maleic acid, pH 7.4. 5-hydroxytryptamine creatine sulphate (5-HT), DL-octopamine hydrochloride (Sigma Chemical Co.), proctolin (Peninsular Laboratories) and neuropeptides of the FMRFamide family (Cambridge Research Biochemicals) were applied at 10^{-9}–10^{-5} mol l^{-1} as pulses in the perfusate. Each trial consisted of 5 min of recording in normal saline and 5 or 10 min of modulator application, followed by a minimum of 20 min of washout in normal saline. At least 90 min elapsed before any modulator was tested a second time. The data presented here are taken from 224 trials using 10^{-6} mol l^{-1} modulators. This concentration is commonly used in comparable studies appearing in the literature, and was selected here to facilitate direct comparisons with them and with the *Homarus* oval organ data (Pasztor and Bush, 1989).

Controlled trapezoidal or sinusoidal pull stimuli were provided, either by a d.c. penmotor for *Cherax*, or by a loudspeaker coil for *Homarus* trials, driven by a VIC-20 microcomputer and triggered by a Grass S48 stimulator. Pull amplitudes ranged from 0.5 to 2.0 mm; durations were 0.25–1.25 s; single or repetitive pulls of 0.05–2 Hz were used.

Afferent sensory responses from these three mechanoreceptors were recorded either extracellularly with polyethylene suction electrodes applied to freshly sectioned nerve trunks, or intracellularly with 3 mol l^{-1} KCl-filled glass micropipettes (15–25 MΩ) inserted into afferent fibres 3–5 mm proximal to the sensory dendrites. Recordings were taped on instrumentation recorders for subsequent display and analysis. Permanent records were made on chart recorders (Gould 8000S for *Cherax*, Gould 220 for *Homarus*), filmed from the oscilloscope screen, or plotted on a Hewlett Packard digitizing plotter (7090A). Intracellular records of non-spiking units were inspected for changes by superimposing images on a light

table. Blocks of five consecutive responses recorded under control, pre-trial conditions were compared with responses recorded during and after drug presentation. Changes in amplitude or waveform were only included in the data when at least four of a block of new responses could not be fitted by the control responses.

The *t*-test was used to determine the significance of changes in spike frequency or receptor potential amplitude induced by individual neuromodulators. Comparisons between neuromodulators were analysed with the single classification, model I analysis of variance (ANOVA) (Sokal and Rohlf, 1981).

Results

Cherax *oval organ*

Methylene Blue staining and cobalt backfilling revealed that the innervation of the oval organ in *Cherax* is very similar to that described in *Homarus* (Pasztor and Bush, 1983*a*). In both species, each oval organ sends three large afferent fibres to central somata lying within the respiratory neuropile of the suboesophageal ganglion. The three peripheral arborizations of their sensory dendrites distribute branches uniformly throughout the cone of connective tissue strands.

Extracellular recordings from *Cherax* oval organ nerves (Fig. 1B) showed similar burst structure in response to pull stimuli to those of *Homarus* (see Pasztor and Bush, 1989, Fig. 11), with two slowly adapting spiking units dominating the response.

Intracellular recordings confirmed that the three afferents in *Cherax* are homologous to X, Y and Z fibres in *Homarus* and have similar response properties to pull stimuli. Receptor potentials are conducted with little decrement along the afferents, and may be observed underlying regenerative impulses in intracellular recordings made 3–5 mm from the transducer region (see Fig. 2B and C). As in *Homarus* (Bush and Pasztor, 1983), fibres X, Y and Z, though anatomically similar, have characteristically diverse response patterns. The thickest afferent, fibre X, has the largest graded potential but spikes only sparsely (<4 spikes per response) with firing limited to the dynamic phase of trapezoidal pull stimuli. In several *Cherax* preparations, spiking was absent in the X fibre, even when well rested (see Fig. 2B). Fibres Y and Z showed smaller graded potentials but spiked readily throughout the pull.

Neuromodulator trials on *Cherax* oval organ were carried out under the same two stimulation regimes that had proved useful for *Homarus* (Pasztor and Bush, 1989). In the first, the amplitude and low repetition rate of 1 s pulls were selected to produce brisk bursts of spikes that would remain stable throughout extensive control series of stimuli in normal saline. Modulation of such burst patterns is shown in the extracellular recordings of Fig. 1B. In this proctolin trial, fibre X was not firing, but spikes from Y and Z could be differentiated by amplitude, and their numbers of spikes per burst are plotted separately in Fig. 1C. Typically, 1.75 min after the introduction of 10^{-6} mol l^{-1} proctolin the number of spikes per pull

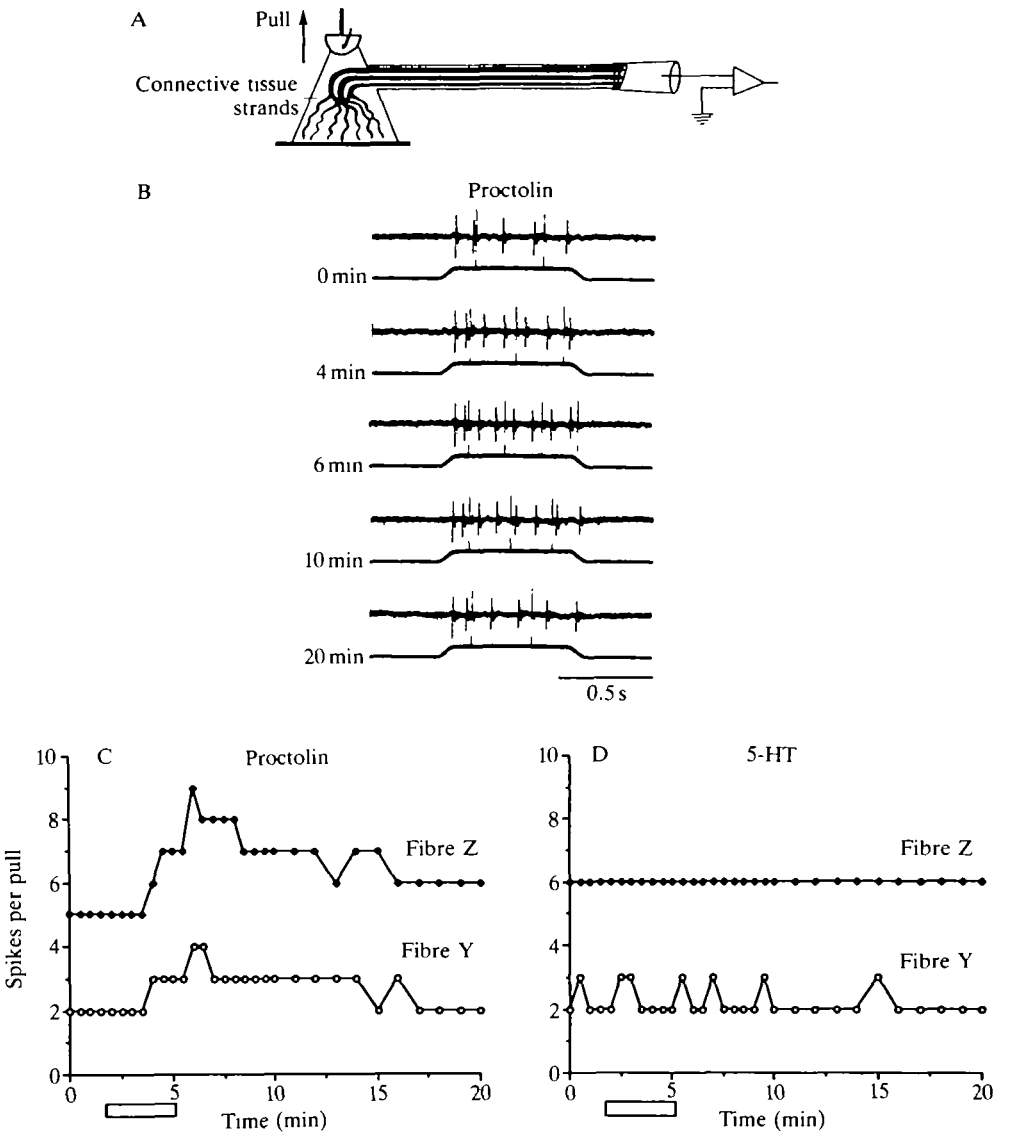

Fig. 1. Contrasting effects of proctolin and serotonin on spiking fibres in *Cherax* oval organ. (A) Diagram (not to scale) of the preparation used for extracellular recording of sensory afferent discharge. The suction electrode was applied to the distal stump of the scaphognathite nerve sectioned 4 mm from the oval organ. 0.75 s pull stimuli at 30 s intervals were applied to the connective tissue strands supporting the sensory dendrites. (B) Excerpts from a continuous extracellular recording showing the enhancement of Y fibre (large spikes) and Z fibre (small spikes) responses after the application of 10^{-6} mol l^{-1} proctolin. (C) Time course of the modulatory action of proctolin during the trial recorded in B. (D) 10^{-6} mol l^{-1} serotonin is ineffective in modulating the *Cherax* oval organ.

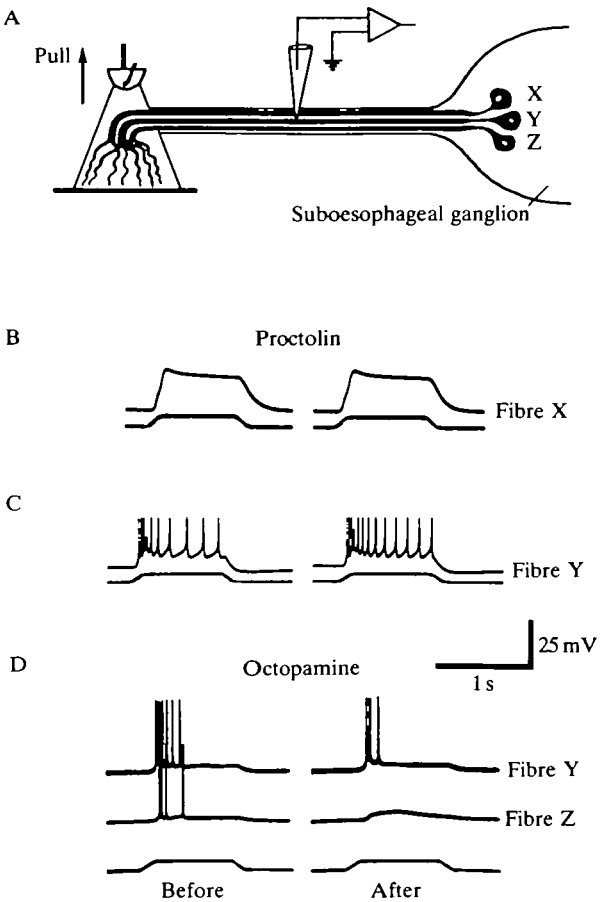

Fig. 2. Modulation of oval organ responses to pull stimuli by proctolin and octopamine (10^{-6} mol^{-1}). (A) The oval organ prepared for intracellular recording (not to scale). The micropipette was inserted 3 mm (6 mm in *Homarus*) from the confluence of the sensory dendrites. 1 s pulls were delivered at 1 min intervals. (B) Proctolin is ineffective in modulating a *Cherax* X fibre. (C) Additional spikes are generated in a *Cherax* Y fibre after the introduction of proctolin. (D) The receptor potentials of *Homarus* Y and Z fibres are attenuated by octopamine with consequent loss of spikes.

increased in both fibres, remained elevated for approximately 4 min and slowly returned to baseline during a 15 min washout period. Octopamine (not shown here) produced a similar enhancement, whereas serotonin (Fig. 1D) had no effect.

Complementary intracellular recordings of individual, identified fibres confirmed these findings and established that, as with *Homarus*, X fibres were not affected by any of the modulators tested (Fig. 2B) while Y and Z fibres both showed 20–30% increases in the number of spikes generated per pull in the presence of proctolin (Fig. 2C) or octopamine. In *Homarus*, Y and Z responses

are significantly depressed by both octopamine (Fig. 2D) and serotonin; such effects were never observed in *Cherax*.

The second stimulus regime used consisted of short, low-amplitude pulls at faster repetition rates, mimicking the natural scaphognathite rhythm. This stimulation induces a decline in response magnitude in *Cherax*, as has already been described for *Homarus* (Pasztor and Bush, 1983*b*). The number of spikes generated per response diminishes progressively, correlated with a decreasing receptor potential amplitude, in the time-dependent modulation associated with sensory habituation. Careful selection of the pull amplitude and repetition rate produces trains of receptor potentials which, when habituated, just bring the membrane to the threshold for impulse initiation. At this stage, any drug-induced modulation of receptor potential amplitude causes significant changes in the number of responses generating spikes, so the preparation provides a very sensitive assay for neuromodulation. Typical trials of this nature are shown in Fig. 3. The initial response to the first pull in each train generated 2–3 spikes but, as habituation proceeded, the probability of generating spikes fell as the receptor potential amplitudes declined (see the samples of intracellular records in Fig. 3Di and ii). In the control trial, the number of spikes generated per 10 pulls stabilized to a constant level (Fig. 3A). In octopamine (Fig. 3B) and proctolin (Fig. 3C) trials, addition of the modulator enhanced the rate and extent of response depolarization and significantly increased the number of spikes generated per 10 pulls (Fig. 3Diii and iv), whereas addition of serotonin (not shown) had no effect. This series of experiments ($N=7$) indicated that octopamine was a less potent modulator than proctolin as its effects were smaller and took longer to develop.

In summary, proctolin (14 trials) and octopamine (12 trials) both potentiated sensory responses in *Cherax* Y and Z fibres, whereas serotonin (11 trials) was ineffective.

Cherax *and* Homarus *swimmeret non-spiking stretch receptor*

The *Cherax* studies reported here confirm and extend the work of Macmillan and Deller (1989): the two large afferents, which together innervate the elastic strand S1 at the base of the swimmeret, are non-spiking under all normal conditions. Anatomically and physiologically they appear to be identical to those described in *Pacifastacus leniusculus* by Heitler (1982) and his nomenclature is used: NSSR-Ant. for the anterior member of the pair and NSSR-Post. for the posterior one.

Comparable non-spiking, large-diameter afferents were also observed in *Homarus americanus* and they are considered to be homologous to crayfish NSSRs. Their peripheral dendrites insert onto fine elastic strands like the crayfish ones, quite separate from the broad ligaments designated strands A and B by Davis (1968). These proprioceptors are also clearly distinct from the twisting muscle receptors (TMRs) described by Miyan and Neil (1986) in *Nephrops norvegicus* and *Homarus gammarus*. TMRs also monitor swimmeret retraction, and have centrally located cell bodies like the NSSRs, but they have spiking

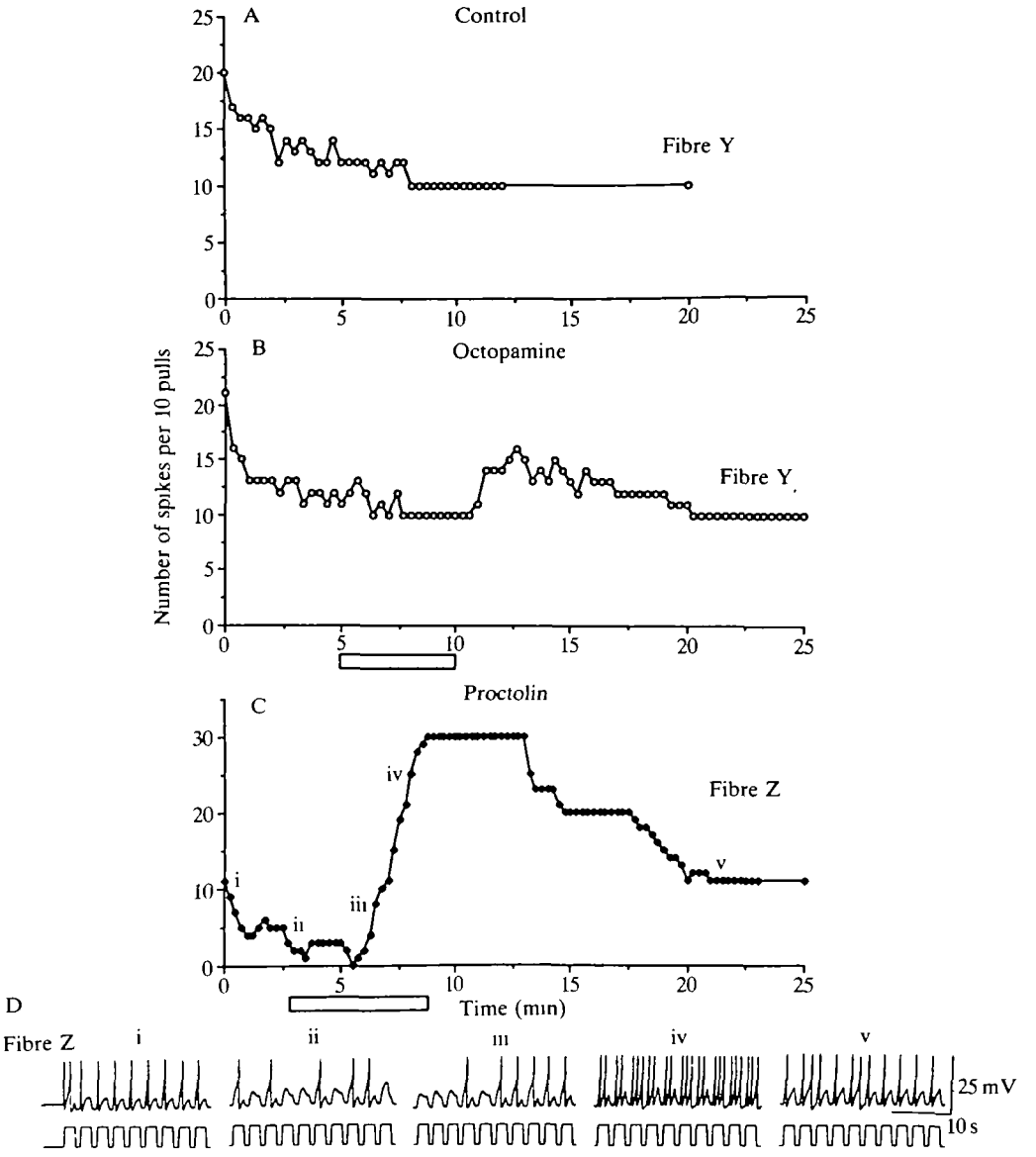

Fig. 3. Potentiation of *Cherax* oval organ afferents by octopamine and proctolin, assayed as increases in the number of impulses generated by 10 responses to repetitive 0.4 Hz pull stimuli. Each trial started with a well-rested preparation and was stimulated repetitively until fully habituated (explanation in text) before the modulator was introduced. Pull amplitudes and repetition rates were selected to give a stable habituated response pattern of zero or one spike per pull. (A) Control series for a Y fibre. No modulator added. (B) Octopamine trial on the same Y fibre. 10^{-6} mol l^{-1} octopamine potentiated spike generation. (C) Proctolin trial on a Z fibre (different preparation). (D) Excerpts from the intracellular recordings which provided the data for the graph in C. The samples i–v correspond to the times indicated on the graph. Note the augmentation in spiking from 0 to 3 impulses per response after the addition of 10^{-6} mol l^{-1} proctolin.

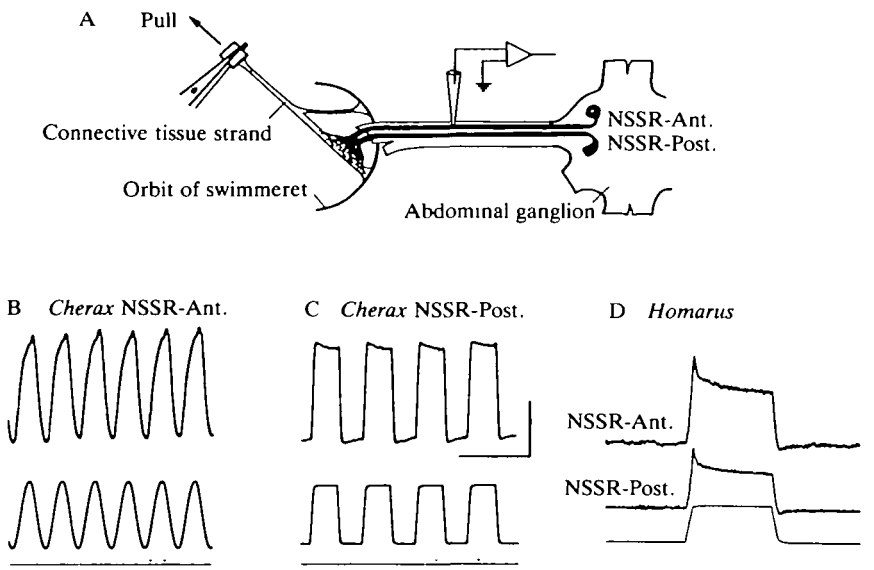

Fig. 4. Two large stretch receptor units at the base of each swimmeret are non-spiking and give precise analogue responses to strand extension. (A) Diagram (not to scale) of the isolated strand preparation used for intracellular recordings of *Cherax* NSSRs. The *Homarus* preparation was essentially similar. (B) Typical intracellular recordings of NSSR-Ant. from an intact *Cherax* swimmeret preparation subjected to sinusoidal displacements. (C) Responses of NSSR-Post. from a *Cherax* strand preparation stimulated with repetitive trapezoidal pulls. Recording site for B and C was 5 mm from the confluence of the sensory dendrites. (D) Simultaneous recordings of *Homarus* NSSR afferents made 10.0 mm (Ant.) and 10.5 mm (Post.) from the sensory dendrites. Calibration: B and C 5 s, 4 mV; D 1 s, 6 mV.

responses and short receptor strands inserting onto a muscle base. TMRs were not observed in *Homarus americanus*.

Intracellular recordings from NSSRs of *Cherax* and *Homarus* were similar whether the receptor strands were left *in situ* and the units stimulated by imposed swimmeret retraction, or whether the strands were exposed, freed distally and stretched directly with a puller (Fig. 4). At recording loci 3 mm from the transducer region of *Cherax*, maximal depolarizations of 23 mV were observed using isolated strands and somewhat smaller potentials were obtained from intact preparations. Presumably the swimmeret articulation set limitations on S1 extension *in situ*. In both species the two units are faithful monitors of strand length and there were no significant differences in the responses of the two afferents NSSR-Ant. and NSSR-Post. (Fig. 4D). Spiking was never observed. There was little adaptation during maintained static pulls and no habituation in response to repetitive stimuli.

We conducted 30 modulator trials on *Cherax* NSSR fibres. The intracellular records were inspected carefully for changes in amplitude, waveform, resting potential, adaptation, habituation or for any induction of spiking which might

Table 1. *Amplitude changes in NSSR receptor potentials following bath application of putative modulators*

Modulator	No. of trials*				Range of changes observed† (mV)	Mean change†‡ (%)	*P*§
	Positive changes	Negative changes	Zero change	Total trials			
Cherax							
Proctolin	4	4	3	11	0.6–2.0	7.8	NS
Octopamine	3	3	1	7	0.8–2.5	13.2	NS
5-HT	4	4	4	12	0.2–2.5	7.2	NS
Homarus							
Proctolin	10	–	–	10	0.4–1.6	26.1	<0.0005
Octopamine	7	–	–	7	0.2–1.0	17.3	<0.005
5-HT	9	–	–	9	0.2–1.5	14.4	<0.005

* Trials on both fibres included; no significant fibre-specific differences.
† All trials; sign of change ignored.
‡ Calculated from percentages of control amplitude for each trial.
§ *t*-test.

have occurred at times related to the arrival of the drugs in the perfusate. Changes in receptor potential amplitude did occur during the test periods, but not in any consistent way. As listed in Table 1, trials producing increases in receptor potential amplitude, trials producing depressed amplitudes and those producing no changes at all occurred with equal frequency for each of the modulators used. The order of drug presentation had no bearing upon the results obtained in any one trial, nor did any preparation show a propensity for one type of result. In no trial were the results biphasic. The potentiated responses that did occur were clearly related to application of the drugs, but the onset of modulation had approximately twice the latency that was usual for modulator effects in other stretch receptors and occurred after the re-introduction of normal saline perfusate. The *Cherax* NSSR data do not support the notion that proctolin, octopamine or serotonin are effective neuromodulators when applied separately *in vitro*. Fig. 5A illustrates a representative proctolin trial during which the responses to pull were not modulated.

In contrast, 27 *Homarus* NSSR trials showed consistent amplitude enhancement during proctolin, octopamine and serotonin application (Fig. 5B–D). The changes, recorded 10 mm from the sensory endings, were small but represent 19 % of pre-drug receptor potential amplitudes and are highly significant ($P<0.005$, see Table 1). They all occurred with latencies characteristic for stretch receptor modulation. We conclude therefore that, for this species, the three substances are effective NSSR modulators.

The abdominal muscle receptor organ

Crayfish and lobster MROs show differences in the number and distribution of

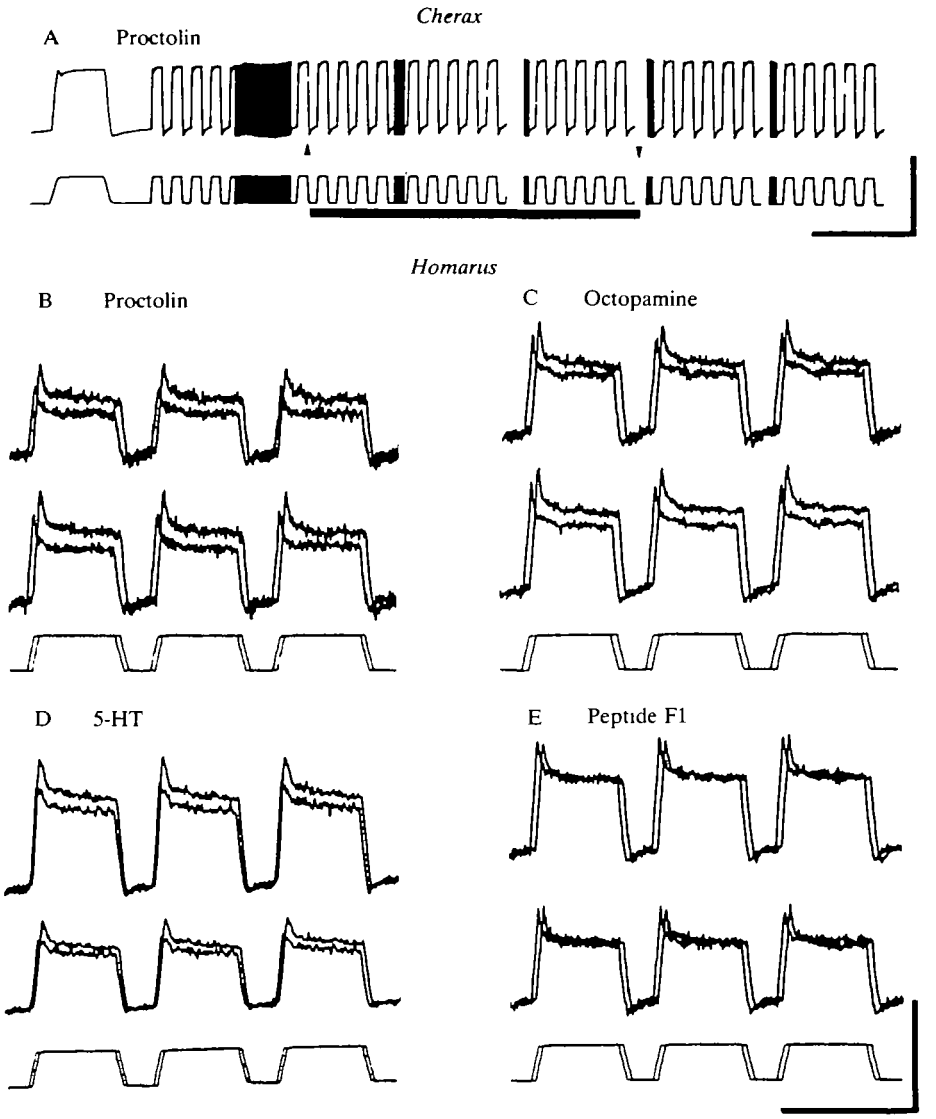

Fig. 5. Potentiation of *Homarus* but not *Cherax* NSSR responses by proctolin, octopamine and serotonin (10^{-6} mol^{-1}). (A) Samples of intracellular recordings of *Cherax* NSSR-Post. from a proctolin trial. Three paper speeds are shown. 1 s pulls presented at 0.5 Hz are monitored in the lower line. No change in receptor potential amplitude or waveform was observed in this trial. In panels B–E the recordings made in the presence of modulator were offset by about 100 ms before being superimposed on pre-modulator recordings. Samples, recorded 2–6 min after the arrival of modulator, were selected to show maximal modulation. Recording sites as in Fig. 4D. (B) Proctolin, (C) octopamine and (D) serotonin caused increases in receptor potential amplitude of 17–35 % (see also Table 1). (E) Neuropeptides of the FMRFamide family were ineffective in modulating NSSR responses. A representative trial using neuropeptide F1 (TNRNFLRFamide) is shown here. No significant changes in resting potential were observed during the *Homarus* NSSR trials (N=33). Calibration: A 1 s, 5 s, 5 min, 20 mV; B–E 2 s, 5 mV.

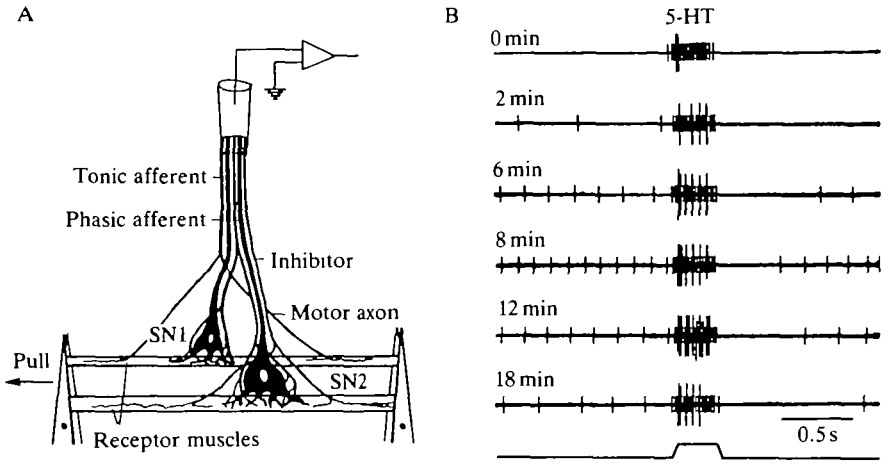

Fig. 6. Serotonin potentiates both tonic and phasic MRO units. (A) Diagram of the preparation used for extracellular recording of sensory afferent discharge. The receptor muscle strands were held under tension in micromanipulated forceps and brief trapezoidal pulls were delivered at 20 s intervals. (B) Excerpts from a representative serotonin trial on *Cherax* MRO showing increases in tonic firing (small spikes) and additional spikes per pull (large spikes) after the addition of 10^{-6} mol l^{-1} 5-HT. Complete recovery to pre-drug levels took about 20 min.

motor and inhibitory efferents, but the sensory afferents have very similar properties in the two groups (for a review, see Fields, 1976). Extracellular recordings such as those in Fig. 6 confirm that in *Cherax* SN1 is a typical tonic unit and SN2 is a typical phasic unit and they respond to maintained stretch and brief pulls with firing patterns similar to those of *Homarus*. The tonic firing rates of the SN1 and the number of spikes in the phasic burst of SN2 were the parameters chosen to assay modulator effects, since they probably reflect the most significant components of the sensory signals *in vivo*.

Proctolin (28 trials), octopamine (17 trials) and serotonin (42 trials) all enhanced spiking responses in *Cherax* SN1 and SN2. Recordings of a typical serotonin trial are shown in Fig. 6B. In this example, the static tension was adjusted to a low level just below the threshold for tonic firing, and the velocities of the pulls were selected to ensure that the phasic unit continued to respond even when fully habituated. In the presence of 10^{-6} mol l^{-1} serotonin, tonic firing in the SN1 reappeared and rose to 9 Hz, and the SN2 response generated nine additional impulses per 350 ms pull. With *Homarus* MRO, proctolin (11 trials) and serotonin (16 trials) produced similar results to those seen in *Cherax* and significant enhancement was observed in both tonic and phasic units. However, with octopamine modulatory effects were weak or absent. In six trials on the tonic unit, firing was unchanged in three trials and showed only insignificant increases with long latency in the remaining three trials. The phasic unit showed no significant changes in burst structure (N=3).

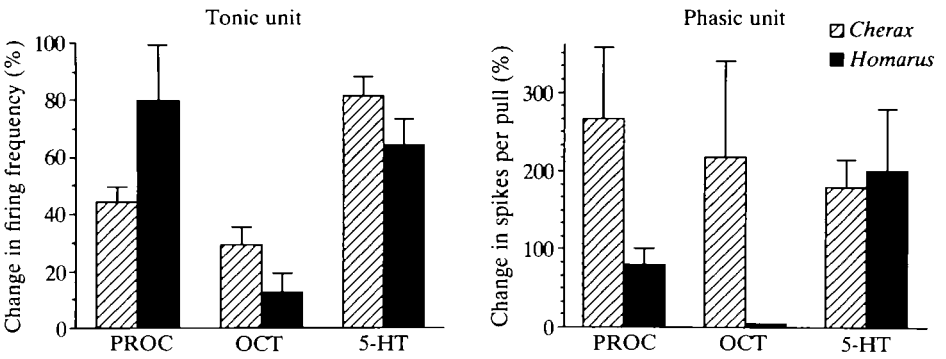

Fig. 7. Histograms showing interspecific comparisons of modulator actions on tonic and phasic units of the abdominal MRO. Maximal increments in tonic firing rates and numbers of phasic spikes per pull were used in calculating mean percentage increases resulting from modulation. (Numbers of trials are given in Table 2.) The bars represent means for each fibre and the line segments show 1 S.E.M. The modulated values of spike generation are different from their saline controls at $P<0.05$ (paired t-test) with the exception of the octopamine effects in *Homarus*. PROC, proctolin; OCT, octopamine; 5-HT, serotonin.

The histograms in Fig. 7 compare, for *Cherax* and *Homarus*, the mean increments in tonic unit firing (Fig. 7A) and phasic unit bursts (Fig. 7B) produced by the three modulators at 10^{-6} mol l^{-1}. Statistical comparisons indicate that, for *Cherax*, serotonin is the most potent modulator of the tonic unit, whereas proctolin has a significantly greater effect on the phasic unit than the other two neuromodulators ($P<0.05$). In *Homarus*, the pattern is reversed: the tonic unit responds best to proctolin and the phasic unit to serotonin.

Earlier studies on the time course of sensory modulation in the lobster oval organ (Pasztor and Bush, 1989) had indicated that, while proctolin effects were shortlived, with recovery commencing after 2–5 min, serotonin and octopamine effects far outlasted the presence of modulator in the bath and were sometimes irreversible. Such prolonged aminergic modulation was not observed in either *Cherax* or *Homarus* MROs. Comparisons of the time courses of proctolinergic and aminergic modulation in individual MRO fibres showed no significant differences in latency to onset, persistence or recovery rates. Spike data sampled at 1 min intervals and averaged for each set of modulator trials ($N=5$–22), plotted in Fig. 8, show that elevated spiking performance persisted for less than 8 min with all three modulators, and in most cases appreciable recovery had occurred after 10 min of washout.

The MRO is a complex sense organ with several possible loci for modulation. Preliminary experiments on *Cherax* suggest that modulator effects involving tension changes in the receptor muscle contribute little to the increased firing observed. Pairs of modulator trials ($N=4$) were performed on receptors before and after immobilization or deletion of the contractile portions of the receptor strands. The isolation of the dendrites from any possible influence of contracting

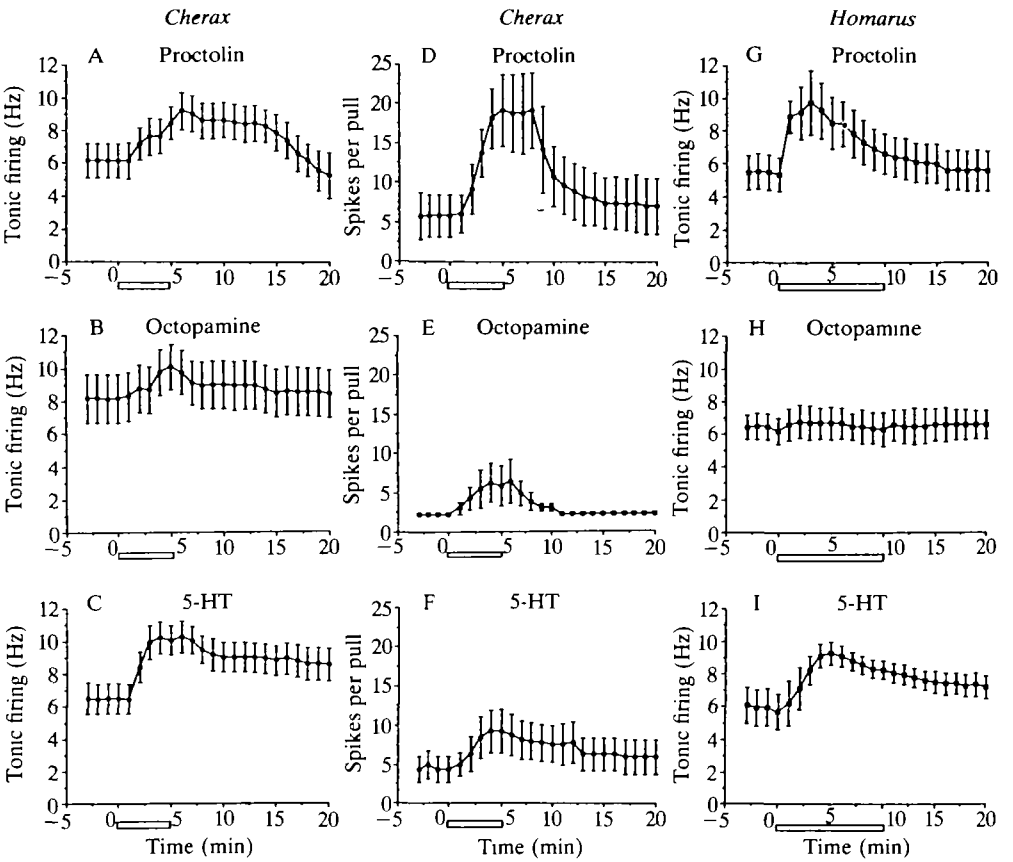

Fig. 8. Proctolin and serotonin potentiate sensory afferent responses of tonic and phasic units in both *Cherax* and *Homarus* MRO. Octopamine is ineffective in *Homarus* MRO. Graphs summarizing the time courses of modulator actions on *Cherax* tonic firing (A–C), *Cherax* phasic bursts (D–F) and *Homarus* tonic firing (G–I). Means from 5–22 trials (±S.E.M.) are given at 1 min intervals. Horizontal bars indicate the presence of 10^{-6} mol l^{-1} modulator in the bath.

muscle had no effect on the modulation of firing. In complementary experiments shown in Fig. 9, the receptor muscles were induced to contract by the insertion of a bolus of 10^{-4} mol l^{-1} glutamate into the superfusate. Contractions were monitored photometrically while recording tonic firing from the T fibre. Glutamate-evoked contractions were correlated with replicable increases in the tonic firing rate. By contrast, application of serotonin that modulated the firing rate to a similar extent did not itself cause any observable muscle contracture.

The FMRF-like neuropeptides

The molluscan cardioacceleratory neuropeptide, FMRFamide, was tested on both *Cherax* and *Homarus* and neuropeptide F1 (TNRNFLRFamide) was applied to the three *Homarus* proprioceptors. In no case was sensory modulation

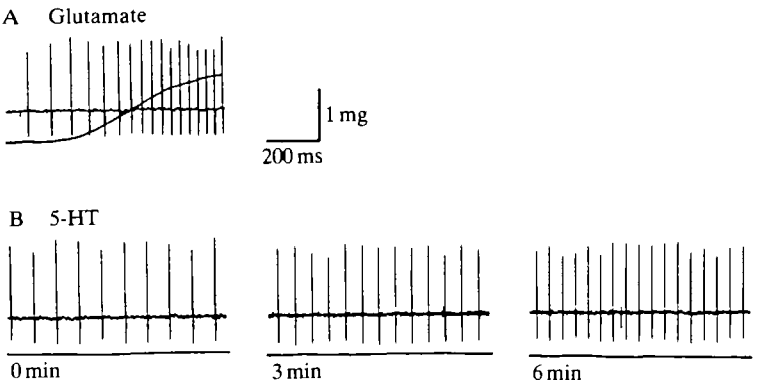

Fig. 9. The serotonergic modulation of tonic firing in *Cherax* MRO is not caused by receptor muscle contracture. Upper trace: afferent discharge of the tonic unit held at moderate, constant stretch by a photometrically monitored isotonic lever system. Lower trace: photocell output. (A) Receptor muscle contraction (upward deflection of monitor trace) evoked by a bolus of 10^{-6} mol l^{-1} glutamate results in increased afferent discharge. (B) Excerpts from a continuous recording of tonic firing and receptor muscle length during a serotonin trial. Increased afferent discharge occurs in the absence of muscle contracture (no deflection of monitor trace).

observed. Other members of this family of neuropeptides occurring in crustaceans are currently being tested.

Discussion

Table 2 summarizes the effects of the neurohormones proctolin, octopamine and serotonin on the *Cherax* and *Homarus* proprioceptors tested in the current study and adds data from previous *Homarus* work (detailed by Pasztor and Bush, 1989) for comparison. Cooper and Hartman (1989) found that serotonin enhanced chordotonal responses in the crab leg whereas octopamine and proctolin were ineffective. Ramirez and Orchard (1990) and Ramirez *et al.* (1990) report that several insect mechanoreceptors, providing important feedback during flight, were potentiated by amines, notably octopamine, but not by neuropeptides. Our survey of seven individual, identifiable mechanoreceptive afferents from two crustacean species, together with these recent reports of sensory modulations from other species, suggests that selective responsiveness of mechanoreceptors to neuromodulators is general throughout the Arthropoda. This implies that the quality of sensory input arriving at the central nervous system can be varied by changes in the concentrations of circulating neurohormones.

It is important to note that some afferents are not modulated by these major crustacean neurohormones. For example, the X fibre of the oval organ in both *Cherax* and *Homarus* may represent a class of mechanoreceptor which sends invariant feedback signals whatever the circulatory neurohormone levels. Presum-

Table 2. *Interspecific comparisons of modulator effects in three crustacean stretch receptors*

		Proctolin		Octopamine		Serotonin	
		N	Effect	*N*	Effect	*N*	Effect
Oval organ							
Fibre X	*Cherax*	3	–	3	–	2	–
	*Homarus**	16	–	9	–	13	–
Fibre Y	*Cherax*	8	↑	5	↑	5	–
	*Homarus**	23	↑	16	↓	18	↓
Fibre Z	*Cherax*	3	↑	4	↑	4	–
	*Homarus**	16	↑	9	↓	18	↓
Swimmeret NSSR							
NSSR-Ant.	*Cherax*	6	–†	4	–†	6	–†
	Homarus	4	↑	3	↑	4	↑
NSSR-Post.	*Cherax*	5	–†	3	–†	6	–†
	Homarus	6	↑	4	↑	6	↑
Abdominal MRO							
Tonic unit	*Cherax*	22	↑	12	↑	30	↑
	Homarus	11	↑	6	–	16	↑
Phasic unit	*Cherax*	6	↑	5	↑	12	↑
	Homarus	3	↑	3	–	4	↑

* Data from Pasztor and Bush (1989).
† Non-significant changes are given in Table 1.

ably their roles demand that they function with a fixed calibration for stimulus parameters.

Individual neuromodulators can have different effects at different sensory loci. For example, serotonin has a powerful depressive action upon *Homarus* oval organ Y and Z fibres, where it can reduce the amplitude of the receptor potential to sub-threshold levels and eliminate spikes from the sensory response (Pasztor and Bush, 1987). More frequently, however, serotonin potentiates the sensory response, as in *Cherax* and *Homarus* MROs and *Homarus* NSSRs. Octopamine can also be either depressive or excitatory depending upon its target. This implies that peripheral mechanoreceptor membranes bear a diversity of modulator receptors (capable of activating various enzyme cascades and resulting in the reduction or potentiation of several ion currents) comparable to that found in central neurones.

Our comparison of sensory modulation in *Cherax* and *Homarus* suggests that species specificity in neuromodulator actions must be expected even in closely related species. For example, in the oval organ comparison, aminergic modulation is depressive in *Homarus* but excitatory (octopamine) or absent (serotonin) in *Cherax*. *Homarus* NSSRs consistently showed augmented responses in the presence of each of the three modulators, whereas in *Cherax* NSSR modulation

was capricious and not significant under the conditions of this study. Finally, the MRO comparison provides an example of a species-specific pattern of relative effectiveness of modulators. In the modulation of the postural proprioceptors, serotonin is more potent in *Cherax* and proctolin is more effective in *Homarus*. For the fast phasic system, the reverse is true. Octopamine had a small effect on the two *Cherax* MRO units but was without any significant action in *Homarus* MRO. Clearly, homologous fibres from different species, which appear to be physiologically similar when observed *in vitro* in normal saline, may behave in subtly diverse manners *in vivo* when subject to varying neurohormone levels. Such species specificity has also been demonstrated for other, non-sensory neuromodulator targets, such as molluscan hearts (Painter and Greenberg, 1982) and stomatogastric neurones (Marder and Hooper, 1985).

In simple stretch receptors, like the oval organ and NSSR, there are no contractile elements or efferent fibres, and evidence suggests that the major target for modulation is the primary afferent fibre (Pasztor and Bush, 1989). The abdominal stretch receptor, by contrast, is a complex structure where afferent firing is a function of both passive and active tension in the muscular receptor strand plus efferent inhibitory control by one or more accessory fibres. This complexity opens the possibility of multiple targets for neuromodulator control, as is the case for *Limulus* eyes (Barlow *et al.* 1980, 1987). While our preliminary experiments tend to discount non-sensory neurone sites as the primary source of the modulation we have described, additional neurohormones or other experimental designs might also reveal secondary modification in receptor output resulting from modulation of the efferent systems. Experiments are currently being directed to identifying the loci and nature of neuromodulator effects in the MRO.

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