Swimming in the pteropod mollusc Clione limacina has been described in terms of the electrophysiology of component neurons and muscle cells and of the morphology of the neuromuscular apparatus (Arshavsky et al. 1985a–d, 1986, 1989b; Satterlie, 1985, 1989, 1991a,b, 1993; Satterlie and Spencer, 1985; Satterlie et al. 1985, 1990). The pattern generator is relatively simple, producing a two-phase output that is translated into dorsal and ventral flexions of a pair of wing-like parapodia. Two forms of swimming, slow and fast, have been described which reflect plasticity in the organization of the pattern generator since the change from slow to fast swimming involves the activation of additional interneurons (Arshavsky et al. 1985d, 1989b). All but one type of swim interneuron and all swim motor neurons are restricted to the pedal ganglia. The swim musculature includes slow-twitch fatigue-resistant and fast-twitch fatigable fibers, the former being activated by a population of small motor neurons during slow swimming, while the latter are recruited into activity by a pair of large motor neurons during fast swimming (Satterlie, 1991a, 1993; Satterlie et al. 1990). This change of swimming speed can be abrupt and dramatic or it can be gradual and more subtle. Additionally, changes in swimming speed can occur within slow or fast ‘gears’ so that the distinction between slow and fast swimming is sometimes difficult to appreciate.

Owing to the simplicity of the pattern generator, which is almost entirely restricted to the pedal ganglia, the two-speed organization of the neuromuscular system, and our current anatomical and physiological understanding of swim system components, the Clione limacina swimming system represents an excellent model system for investigation of neuronal mechanisms of control and modulation of locomotory speed. One trigger for acceleration of swimming in Clione limacina is bath application or local application of serotonin (5-hydroxytryptamine, 5-HT), which produces both an increase in the frequency of swimming movements and an increase in the force of wing contractions (Arshavsky et al. 1985a; Satterlie, 1989; Kabotyansky and Sakharov, 1990). These behavioral changes are characteristic of fast swimming, suggesting that serotonergic inputs to the swimming system may be involved in at least some forms of swim acceleration. Similarly, serotonin

SEROTONERGIC MODULATION OF SWIMMING SPEED IN THE PTEROPOD MOLLUSC CLIONE LIMACINA

I. SEROTONIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM AND WINGS

RICHARD A. SATTERLIE, TIGRAN P. NOREKIAN, SHERYL JORDAN AND CHARLES J. KAZILEK

*Department of Zoology, Arizona State University, Tempe, AZ 85287-1501, USA and Friday Harbor Laboratories, Friday Harbor, WA 98250, USA

Accepted 12 December 1994

Summary

Serotonin-immunoreactive somata in the pteropod mollusc Clione limacina were restricted to the cerebral and pedal ganglia. 10–14 pairs of cells were consistently found in the cerebral ganglia, including one large pair that had soma positions and axon branching patterns reminiscent of those of the metacerebral cells of other molluscs. Two clusters of somata were found on the midline near the cerebral commissure, one on the anterio-lateral margin and one posterio-laterally. A distinct paired cluster of up to nine somata was found on the dorso-lateral margin of the pedal ganglia, near the emergence of the pedal commissure. Up to five of these cells innervated the ipsilateral wing via the wing nerve. Dye-fills of these cells showed that they branch repeatedly in the ipsilateral wing and innervate the swim musculature. Double-labelling experiments indicated that the filled neurons were also serotonin-immunoreactive. Neurobiotin fills that were processed for electron microscopy revealed two types of terminals associated with the swim musculature: direct contacts and reactive terminals adjacent to non-labelled presynaptic terminals. Additional immunoreactive neurons in the pedal ganglia included the asymmetrical heart excitor neuron of the left pedal ganglion and up to nine ventral somata.

Key words: serotonin, swimming, modulation, muscle, Clione limacina, immunoreactivity, mollusc.

Introduction

Swimming in the pteropod mollusc Clione limacina has been described in terms of the electrophysiology of component neurons and muscle cells and of the morphology of the neuromuscular apparatus (Arshavsky et al. 1985a–d, 1986, 1989b; Satterlie, 1985, 1989, 1991a,b, 1993; Satterlie and Spencer, 1985; Satterlie et al. 1985, 1990). The pattern generator is relatively simple, producing a two-phase output that is translated into dorsal and ventral flexions of a pair of wing-like parapodia. Two forms of swimming, slow and fast, have been described which reflect plasticity in the organization of the pattern generator since the change from slow to fast swimming involves the activation of additional interneurons (Arshavsky et al. 1985d, 1989b). All but one type of swim interneuron and all swim motor neurons are restricted to the pedal ganglia. The swim musculature includes slow-twitch fatigue-resistant and fast-twitch fatigable fibers, the former being activated by a population of small motor neurons during slow swimming, while the latter are recruited into activity by a pair of large motor neurons during fast swimming (Satterlie, 1991a, 1993; Satterlie et al. 1990). This change of swimming speed can be abrupt and dramatic or it can be gradual and more subtle. Additionally, changes in swimming speed can occur within slow or fast ‘gears’ so that the distinction between slow and fast swimming is sometimes difficult to appreciate.

Owing to the simplicity of the pattern generator, which is almost entirely restricted to the pedal ganglia, the two-speed organization of the neuromuscular system, and our current anatomical and physiological understanding of swim system components, the Clione limacina swimming system represents an excellent model system for investigation of neuronal mechanisms of control and modulation of locomotory speed.

One trigger for acceleration of swimming in Clione limacina is bath application or local application of serotonin (5-hydroxytryptamine, 5-HT), which produces both an increase in the frequency of swimming movements and an increase in the force of wing contractions (Arshavsky et al. 1985a; Satterlie, 1989; Kabotyansky and Sakharov, 1990). These behavioral changes are characteristic of fast swimming, suggesting that serotonergic inputs to the swimming system may be involved in at least some forms of swim acceleration. Similarly, serotonin

*Address for correspondence.
Materials and methods

Animals were collected from the breakwater of Friday Harbor Laboratories and held in 1 gallon (3.79l) jars partly submerged in a sea table (Friday Harbor) or in 1 l beakers held in a refrigerator (Arizona State University). Animals were anesthetized in a 1:1 mixture of sea water and MgCl₂, and dissected in a Petri dish coated with Sylgard (Dow Corning). For both immunohistochemical and electrophysiological measurements, have been used to describe serotonin-containing neurons using immunohistochemical techniques. Similar techniques, in addition to direct biochemical measurements, have been used to describe serotonin-containing neurons in other opisthobranchs, most notably two species of *Aplysia* (Goldstein et al. 1984; Ono and McCaman, 1984; Longley and Longley, 1986; McPherson and Blankenship, 1991), whose organization of central ganglia is at least superficially similar to that of *Clione limacina*. Here we describe serotonin-immunoreactivity in the central ganglia and parapodia (wings) of *Clione limacina*. In the following papers (Satterlie, 1995; Satterlie and Norekian, 1995), the role of some of these immunoreactive cells in producing swimming speed changes is described in detail.

For immunohistochemical staining, reduced preparations were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 2–4h followed by a 12h wash period with PBS. The tissue was then incubated overnight in PBS with 0.1% Triton X-100 and sodium azide (PTA). Following a 4h exposure to 5% goat serum in PTA, the tissue was incubated in serotonin antibody (INCSTAR, 1:200–1:500 dilution in PBS) for 24–48h. Following 12h of washing in PTA, the tissue was placed in secondary antibody (goat anti-rabbit in either PTA or PTA minus sodium azide for peroxidase preparations) for a minimum of 12h. For fluorescent preparations, the tissue was mounted on glass slides in a 1:9 mixture (v/v) of 50mmol⁻¹ Tris buffer and glycerol. Fluorescent tags to secondary antibodies included FITC, Rhodamine (Kirkegaard and Perry, Sigma) and AMCA (Vector Labs). In peroxidase preparations, the secondary antibody system was an avidin–biotin–peroxidase complex (Vectastain elite, Vector Labs). Following incubation in the biotinylated secondary antibody for 12h, the preparation was washed, placed in the ABC reagent for 12h, washed, and reacted with diaminobenzidine and H₂O₂ until a brown precipitate was visible in immunoreactive neurons. To avoid labelling of endogenous peroxidases, preparations were soaked for 30min in 0.2% H₂O₂ in PBS prior to primary antibody labelling. For Lucifer Yellow antibody (Chemicon: 1:500 dilution) immunohistochemistry, the techniques were identical to those for serotonin with the peroxidase secondary system.

Controls consisted of parallel preparation of ganglia/wing preparations, omitting the primary antibody incubations. No staining was observed in these preparations. Attempts to eliminate staining by pre-incubating the primary antibody in 10⁻⁴mol⁻¹ serotonin creatine sulfate were only partially successful, greatly reducing, but not eliminating, immunoreactivity. Since the antibody was produced against a serotonin/bovine serum albumin conjugate, complete elimination of staining with serotonin alone might not be expected. The antibody specification sheet from INCSTAR Corporation indicates that serotonin immunoreactivity in vertebrate neural tissue was completely blocked by pre-incubation of the primary antibody with a serotonin/bovine serum albumin conjugate.

Immunohistochemical preparations were scanned with an argon laser Sarastro 2000 confocal microscope (Molecular Dynamics), with 10μm optical sections presented individually or reconstructed with the Sarastro software. Stereo pairs were photographed with a 7° angle shift.

Intracellular dye-fills were made with microelectrodes filled with the following solutions: 4% Lucifer Yellow CH (Sigma) tip fill with 1mol⁻¹ lithium chloride in the barrel; 5% Neurobiotin (Chemicon) tip fill with 3mol⁻¹ KCl in the barrel; 5% carboxyfluorescein (Sigma) tip fill with either 3mol⁻¹ KCl or 2mol⁻¹ potassium acetate in the barrel. Dye solutions were pressure-injected with a Picospritzer II pressure injection system. Cells injected with Lucifer Yellow or carboxyfluorescein were observed live in the recording dish using a Nikon mercury-vapor epifluorescence microscope. For Neurobiotin injections, the tissue was fixed as for immunohistochemical preparations, briefly exposed to Triton X-100 (1h) or cold methanol (20min), treated with the ABC reagent of the Vectorstain kit, reacted with diaminobenzidine with H₂O₂, post-fixed in 1% osmium tetroxide in 2.5% bicarbonate buffer, dehydrated in ethanol and propylene oxide, and embedded in an Epon substitute (EMBED 812: Electron Microscopy Sciences). Cold methanol treatment allowed adequate penetration of the immunocoehemicals while giving the best ultrastructural preservation. Sections 1–2μm thick were cut with glass knives and mounted on slides, unstained, for light microscoical examination. The Lucifer
Yellow immunohistochemical preparations were similarly post-fixed, embedded and examined.

The following survey of 5-HT-immunoreactive cells and processes is based on 29 separate immunohistochemistry experimental series prepared over a period of 6 years and including animals collected in the months of January, February, March, May, June and July. Included in this study were animals that possessed immature reproductive structures, mature reproductive structures and post-coital characteristics, such as expanded ovotestes containing large, apparently mature, oocytes and sperm receptacles filled with sperm. Some animals of this latter stage were observed to lay large clusters of fertilized eggs in our holding containers. Serotonin-immunoreactive staining was qualitatively consistent throughout this period and in adult animals of different sexual stages.

Results

The nervous system of *Clione limacina* was first described and elegantly illustrated by Wagner (1885). The central ganglia include cerebral, pedal, pleural, intestinal and buccal pairs (Fig. 1). The buccal ganglia are attached to the posterior end of the buccal mass and are connected to the cerebral ganglia by a long pair of cerebro-buccal connectives. The other ganglia form a circumesophageal ring, with all but the cerebral ganglia being placed inferior to the esophagus. The cerebral ganglia are connected to one another by a very short commissure, to the pedal ganglia by cerebro-pedal connectives and to the pleural ganglia by cerebro-pleural connectives. The pleural ganglia are connected to the pedal ganglia by very short pleuro-pedal connectives and to the intestinal ganglia by pleuro-intestinal connectives. The pedal ganglia communicate with one another via a stout pedal commissure and a very thin parapedal connective (not shown in Fig. 1). The pair of intestinal ganglia are separate, but directly connected to one another.

Immunoreactive neuron somata were found in the cerebral and pedal ganglia only (Figs 1, 2). The buccal, pleural and intestinal ganglia did not contain immunoreactive somata, although all contained neuropilar processes that showed dense immunoreactive staining. In the buccal ganglia, the processes originated from immunoreactive axons in the cerebro-buccal connectives rather than from the buccal mass. Stained axons were also found in the other cerebral nerves, which innervate the body wall of the head and neck, and the head structures including the buccal cones (prey-capture appensages).

10–14 immunoreactive neuron pairs were found consistently in the cerebral ganglia of *Clione limacina* (Figs 1, 2A,B, 3A). One pair of cells had large (up to 90 μm) dorsal somata occurring in a position similar to that of the giant cerebral neurons (metacebral neurons) of other molluscs (Figs 2B, 3A). Although the axons were difficult to trace, it appears that these cells were the source of the axons running to the buccal ganglia via the cerebro-buccal connectives and to the labial nerve. On the dorso-medial side of the ganglia was a paired cluster of 7–8 immunoreactive somata, found on the anterior margin of the ganglia in a medial position near the cerebral commissure (anterior cluster; Figs 2A, 3A). These cells were small (15–20 μm in diameter) and showed intense staining. Another paired cluster of 3–4 somata was found on the dorsal side of the ganglia, on the posterior margin, near the commissure (posterior cluster; Figs 2A, 3A). The diameters of these somata were in the range 15–30 μm. Both anterior and posterior cluster cells contributed axons that formed a tract descending to the pedal ganglia via the cerebro-pedal connectives.

On the ventral side of the cerebral ganglia, only one pair of immunoreactive cells was found, posterior to the commissure (Fig. 3A). The left-hand cell was fairly large (40 μm), while the contralateral homologue had a smaller soma (25–30 μm). Both cells sent axon branches to both ipsilateral and contralateral cerebro-pleural connectives. These cells were not the source of the axons running to the buccal mass and are connected to the cerebral ganglia by a long pair of cerebro-buccal connectives. The other ganglia form a circumesophageal ring, with all but the cerebral ganglia being placed inferior to the esophagus. The cerebral ganglia are connected to one another by a very short commissure, to the pedal ganglia by cerebro-pedal connectives and to the pleural ganglia by cerebro-pleural connectives. The pleural ganglia are connected to the pedal ganglia by very short pleuro-pedal connectives and to the intestinal ganglia by pleuro-intestinal connectives. The pedal ganglia communicate with one another via a stout pedal commissure and a very thin parapedal connective (not shown in Fig. 1). The pair of intestinal ganglia are separate, but directly connected to one another.

Immunoreactive neuron somata were found in the cerebral and pedal ganglia only (Figs 1, 2). The buccal, pleural and intestinal ganglia did not contain immunoreactive somata, although all contained neuropilar processes that showed dense immunoreactive staining. In the buccal ganglia, the processes originated from immunoreactive axons in the cerebro-buccal connectives rather than from the buccal mass. Stained axons were also found in the other cerebral nerves, which innervate the body wall of the head and neck, and the head structures including the buccal cones (prey-capture appensages).

10–14 immunoreactive neuron pairs were found consistently in the cerebral ganglia of *Clione limacina* (Figs 1, 2A,B, 3A). One pair of cells had large (up to 90 μm) dorsal somata occurring in a position similar to that of the giant cerebral neurons (metacebral neurons) of other molluscs (Figs 2B, 3A). Although the axons were difficult to trace, it appears that these cells were the source of the axons running to the buccal ganglia via the cerebro-buccal connectives and to the labial nerve. On the dorso-medial side of the ganglia was a paired cluster of 7–8 immunoreactive somata, found on the anterior
dorsal and dorso-lateral region of each pedal ganglion in an anterio-medial position, immediately adjacent to the pedal commissure (Figs 2C, 3B). Up to nine somata have been found in this cluster. These cells sent axons to the ipsilateral wing.
nerve (Fig. 3B) and to two additional pedal nerves. One of these nerves ran to the body wall of the head, neck and the anterior portion of the body. The other nerve ran posteriorly to unknown destinations. In addition to the dorsal cluster of immunoreactive somata, a single asymmetric soma was found in a central position on the dorsal surface of the left pedal ganglion (Fig. 2C). This cell was in a position similar to that of the heart excitor neuron described by Arshavsky et al. (1990). A small (15 μm in diameter) soma was found on the dorsal, posterior surface of each ganglion near the emergence of the wing nerve. Ventral immunoreactive cells included up to six large (up to 90 μm in diameter) scattered somata found in the medial half of the ganglia and a cluster of two or three very small cells (10 μm in diameter) found in the posterior region near the emergence of the wing nerve (Fig. 2D). Axons of the ventral cells could not be followed. A dense mesh of fine immunoreactive processes filled the medial neuropil of each pedal ganglion and extended to the anterio-lateral (near the...
cerebro-pedal connective) and posterio-lateral (near the origin of the wing nerve) neuropilar regions (see Fig. 3B). Each pedal ganglion innervated the ipsilateral parapodium (wing) via a single wing nerve. Either four or five immunoreactive axons were found in each wing nerve (Fig. 4A). The close spacing of the axons made it difficult to determine whether this was true variability or whether the fifth axon was simply not visible in some preparations. After entering the wings, the axons followed the four primary branches of the wing nerve and divided repeatedly to supply the entire dorsal and ventral surfaces of the wing (see Satterlie, 1991a, for a description of wing nerve branches). Immunoreactive processes were found in the same focal plane as the layers of swim musculature and tended to run parallel to the long axis of the muscle bundles (Fig. 4A). The finest processes contained numerous swellings along their lengths, again in the same focal plane as swim musculature.

Since our primary interest is in the control of locomotion, the dorsal cluster of immunoreactive neurons of each pedal ganglion was initially singled out for further investigation. The main reason for their selection was that the axons of at least some of these cells ran to the ipsilateral wing and sent branches to all parts of the wings. Furthermore, immunoreactive processes were found in the same focal plane as the swim musculature, suggesting a role in the regulation of swimming activity.

Lucifer Yellow and carboxyfluorescein fills of these pedal neurons confirmed that at least five of the cells sent axons to the ipsilateral wing, where they branched repeatedly and appeared to innervate the entire surface of the wing (either dorsal or ventral muscles). Three of these cells sent processes into three of the four major branches of the wing nerve (but not into the parapodial connective, which runs to the contralateral wing; Fig. 4B). The other two sent axon branches to all four of the wing nerve branches. Other neurons of the pedal cluster sent axons to the head and body wall of the anterior part of the body and to the posterior regions of the body. Several experiments in which all of the cells in the cluster were filled with dye confirmed that up to five axons run to the ipsilateral wing via the wing nerve. These multiple fills also showed that the pedal cells contribute processes to the neuropil adjacent to the cell bodies and to the anterio-lateral region near the emergence of the cerebro-pedal connective. Axons of these cells did not run to other ganglia.

To verify that the recorded, filled neurons were the same as those showing immunoreactivity, the pedal 5-HT neurons were injected with carboxyfluorescein and then subjected to immunohistochemical localization of serotonin (5-HT) using the anti-serotonin antibody and a Rhodamine-labelled secondary antibody. This was carried out in three ways. First, all suspected peripheral 5-HT neurons were injected in individual preparations to verify that the extent of the cluster was properly identified. These experiments were extremely useful in showing that filled and immunoreactive axons in the wing nerves were identical in number (Fig. 5A,B). Second, a single 5-HT neuron was injected and processed for serotonin immunohistochemistry. Finally, two cells were injected, one from the 5-HT cell cluster and one known non-serotonin-immunoreactive cell whose soma was close to the cluster (Fig. 5C,D). This third experiment was necessary since the fluorescence of very bright carboxyfluorescein fills tended to ‘bleed over’ into the fluorescent image produced by Rhodamine-labelled serotonin antibody when using the proper Rhodamine excitation and barrier filters, thus potentially leading to false positives. Fortunately, the various chemical treatments of fixation and immunolocalization greatly reduced the brightness of carboxyfluorescein injections and thus did not produce false positives when non-immunoreactive neurons were injected.

Fig. 5. Double labelling of pedal neurons of the medial cluster using carboxyfluorescein injection and serotonin-immunohistochemistry with a Rhodamine secondary antibody. Paired photographs A and B show labelled axons in the wing nerve. In A, all neurons of the medial cluster were injected with carboxyfluorescein. Immunohistochemical treatment (B) shows the same distribution of labelled axons in the wing nerve as were revealed by the dye injection. Paired photographs C and D show a control for bleed-over of carboxyfluorescein. A cell outside the medial cluster of putative immunoreactive neurons (marked by the dot) was injected (C) and was found to show no fluorescence with the Rhodamine filters (D). A neuron within the medial cluster was also injected (marked by the asterisk) and showed immunoreactivity with the Rhodamine filters (D). Scale bar, 100 μm.
Neurobiotin fills, and Lucifer Yellow fills that were subsequently treated with anti-Lucifer Yellow antibody, confirmed that the cells of the pedal cluster innervate the swim musculature. In both types of fills, dense precipitates of reaction product were found in small terminals adjacent to swim muscle bundles (Fig. 6A). Immunoreactive terminals were not found adjacent to retractor muscles, which occur in the center of the wing hemocoel, or in the dorso-ventral muscles that span the width of the hemocoel. This lack of staining was not due to a penetration problem since immunoreactive axons were found in nerves in the very center of the wing hemocoel, adjacent to retractor muscles. These individual cell fills clearly indicated that each of the neurons sending axons into the wing innervated either the dorsal or the ventral swim musculature and exclusively innervated slow-twitch muscle fibers (Fig. 6). Immunohistochemical preparations using the anti-serotonin antibody, in which the second antibody was an avidin–biotin–peroxidase complex, confirmed that only slow-twitch muscles are innervated by serotonergic terminals and further showed that immunoreactive processes resemble ‘beaded strings’ reminiscent of those seen in whole mount in fluorescent preparations (Fig. 6B).

Electron microscopic examination of serotonin-immunohistochemical preparations revealed numerous examples of reactive processes within the swim muscle bundles. Vesicle-filled processes were frequently found in two configurations; directly adjacent to slow-twitch muscle cells (Fig. 7B) and adjacent to non-reactive, vesicle-filled terminals which, in turn, formed neuromuscular junctions with slow-twitch muscle cells (Fig. 7A). Reactive terminals contained vesicles with a mean diameter of 40nm (N=15 terminals). Owing to the dense precipitates of reaction product, it was not possible to tell whether the vesicles were clear or dense-cored.

**Discussion**

Serotonin immunohistochemistry was used to localize putative serotonergic neurons in the central nervous system of the pteropod mollusc *Clione limacina*. The results confirm and extend previous investigations of monoaminergic neurons of
Clione limacina using glyoxylate-induced histofluorescence (Kabotyansky and Sakharov, 1990).

The overall number of immunoreactive neurons in the central ganglia of Clione limacina is relatively low and significantly smaller than that found in Aplysia species (Goldstein et al. 1984; Ono and McCaman, 1984; Longley and Longley, 1986; McPherson and Blankenship, 1991). Nonetheless, three similarities between the two genera can be found. First, buccal and pleural ganglia do not contain immunoreactive somata, but do possess immunoreactive processes in the ganglionic neuropil. Second, both genera have a large pair of serotonin-immunoreactive neurons in the anterior region of the cerebral ganglia. Putative homologs have been found in numerous opisthobranchs and pulmonates and have been given many names, including metacerebral giant cells, giant cerebral neurons, cerebral serotonergic cells and C1 neurons (see Granzow and Rowell, 1981; Pentreath et al. 1982; Croll, 1987). These cells share very specific anatomical features, most notably they are the only source of serotonergic elements in the cerebro-buccal connectives, buccal ganglia and intrinsic musculature of the buccal mass (Croll, 1987). This appears to be consistent with the structure of the large pair of immunoreactive neurons in the cerebral ganglia of Clione limacina, which sends axons to the buccal ganglia via the cerebro-buccal connectives. These cerebral neurons were also shown to contain serotonin by the glyoxylate-induced histofluorescence technique (Kabotyansky and Sakharov, 1990). In a variety of gastropods, these metacerebral cells have been found to modulate feeding behavior, specifically the activity of the buccal mass and radula (Gillette and Davis,
1977; Granzow and Kater, 1977; Weiss et al. 1978; Gelperin, 1981; Croll et al. 1985). In Clione limacina, the activity of metacerebral cells was also shown to influence the buccal ganglion pattern generator that produces rhythmic activities of the buccal mass (Arshavsky et al. 1991).

The third similarity between Clione limacina and Aplysia species involves the distribution of serotonin-immunoreactive neurons in the pedal ganglia. In both Clione limacina and Aplysia species, clusters of immunoreactive neurons are found in the medial region of the pedal ganglia, typically adjacent to or surrounding the origin of the pedal commissure (Goldstein et al. 1984; Ono and McCaman, 1984; Longley and Longley, 1986; McPherson and Blankenship, 1991). In Aplysia brasiliana, these cells have been called ‘parapodial opener phase (POP)’ neurons. During swimming in A. brasiliana, POP neurons fire bursts in phase with those of the parapodial opener-phase motor neurons. As with the peripheral 5-HT cells of Clione limacina, POP neurons send axons to the ipsilateral parapodium and appear to innervate the parapodial musculature (McPherson and Blankenship, 1991). Furthermore, both groups of neurons enhance the contractility of parapodial muscles without directly producing a motor effect (McPherson and Blankenship, 1991; Satterlie, 1995).

The Clione limacina swim musculature is unique in comparison with other opisthobranch muscle systems in that two well-defined types of fibers, slow-twitch fatigue-resistant and fast-twitch fatigable, can be clearly identified morphologically, histochemically, ultrastructurally and physiologically (Neal, 1988; Satterlie et al. 1990; Satterlie, 1991a,b). Wing contractions during slow swimming are produced by slow-twitch fibers, while the change to fast swimming involves recruitment of fast-twitch fibers and enhanced contractions of slow-twitch fibers (Satterlie, 1991a,b, 1993). Data from both individually filled peripheral 5-HT neurons and immunohistochemical preparations indicate that peripheral 5-HT neurons only innervate the slow-twitch muscle fibers. This has been confirmed in electrophysiological tests (Satterlie, 1995). The significance of this innervation arrangement is not fully understood, although some speculation is provided in the discussion of an accompanying paper (Satterlie, 1995). Ultrastructural evidence suggests that the serotonin-immunoreactive neurons may function in two ways. The direct apposition of vesicle-filled processes and muscle cells suggests that the immunoreactive neurons may exert a direct influence on the muscle cells. The direct alignment of similar processes with non-immunoreactive terminals also suggests that the immunoreactive terminals may act presynaptically to modify muscle activity indirectly. The identity of the non-immunoreactive terminals in unknown; however, an ultrastructural investigation utilizing double-label injections and injection–immunohistochemistry combinations of motor neurons and peripheral 5-HT neurons is under way to address this problem.

All immunohistochemical preparations were consistent in terms of the number and location of immunoreactive neurons in the central nervous system of Clione limacina. The small number of immunoreactive neurons in the central ganglia allows a thorough description of the effects of immunoreactive cells on the swimming system. Our primary focus in this series of papers is on the pedal neurons that send axons to the wings and on the two medial clusters of small cerebral cells that send their axons to the cerebro-pedal connectives. The former appear to modulate the swimming musculature, while the latter are the first candidates for modification of the activity of pedal swim interneurons and motor neurons.

We thank Dr A. O. D. Willows, Director of Friday Harbor Laboratories, for providing space and generous assistance, and Lou and Alison Satterlie and Dr Claudia Mills and the rest of the Friday Harbor dock-walkers for help in collecting animals. This study was supported by NIH grant R01 NS27951.

References


Serotonin immunoreactivity in Clione limacina 903


