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Differential distribution of β-pigment-dispersing hormone (β-PDH)-like immunoreactivity in the stomatogastric nervous system of five species of decapod crustaceans

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Summary. Pigment-dispersing hormone (PDH) acts to disperse pigments within the chromatophores of crustaceans. Using an antibody raised against β-PDH from the fiddler crab *Uca pugilator*, we characterized the distribution of β-PDH-like immunoreactivity in the stomatogastric nervous system of five decapod crustaceans: the crabs, *Cancer borealis* and *Cancer antennarius*, the lobsters, *Panulirus interruptus* and *Homarus americanus*, and the crayfish, *Procambarus clarkii*. No somata were stained in the stomatogastric ganglion (STG) or the esophageal ganglion in any of these species. Intense PDH-like staining was seen in the neuropil of the STG in *P. interruptus* only. In all 5 species, cell bodies, processes, and neuropil within the paired circumesophageal ganglia (CGs) showed PDH-like staining; the pattern of this staining was unique for each species. In each CG, the β-PDH antibody stained: 1 large cell in *C. borealis*; 3 small to large cells in *C. antennarius*; 3–8 medium cells in *P. clarkii*; 1–4 small cells in *H. americanus*; and 13–17 small cells in *P. interruptus*. The smallest cell in each CG in *C. antennarius* sends its axon, via the inferior esophageal nerves, into the opposite CG; this pair of cells, not labeled in the other species studied, may act as bilateral coordinators of sensory or motor function. These diverse staining patterns imply some degree of evolutionary diversity among these crustaceans. A β-PDH-like peptide may act as a neuromodulator of the rhythms produced by the stomatogastric nervous system of decapod crustaceans.

Key words: β-Pigment-dispersing hormone (β-PDH) – Ganglia, invertebrate – Immunohistochemistry – Antibody staining – Peptide localization – *Cancer antennarius, Procambarus clarkii, Panulirus interruptus* (Crustacea)

The stomatogastric nervous system of decapod crustaceans controls the processing and movement of food particles through the foregut. At least four different rhythmic motor patterns control the muscles that move, chew, and filter food before its entry into the hindgut (Robertson and Moulins 1981). Central pattern generators (CPGs) within this part of the central nervous system (CNS) produce these rhythmic motor patterns (see Selverston and Moulins 1987). The neurons, synaptic connections, and mechanisms for rhythm production for two of these CPGs have been well characterized (Selverston and Moulins 1987). This preparation has proven to be an excellent model system for studying the effects of neuromodulators on well-defined neural circuits (see Marder 1987; Harris-Warrick 1988). Numerous peptides have been identified as modulators of the rhythms of the stomatogastric nervous system, including proctolin (Hooper and Marder 1984, 1987; Marder et al. 1986; Heinzel and Selverston 1988; Dickinson and Marder 1989), FMRFamide-like peptides (Hooper and Marder 1984; Marder et al. 1987; Weimann and Marder 1989), and a cholecystokinin-like (CCK-like) peptide (Turrigiano and Selverston 1989, 1990).

The peptides, pigment-dispersing hormone (PDH) and red pigment-concentrating hormone (RPCH), act to disperse and concentrate pigments, respectively, within the chromatophores of crustaceans (Rao 1985). RPCH is distributed widely in the stomatogastric nervous system of the crab *Cancer borealis* (Nusbaum and Marder 1988) and the lobster *Panulirus interruptus* (Dickinson and Marder 1989). This distribution is correlated with the functional modulation of several of the rhythms produced by this part of the crustacean CNS in response to application of exogenous RPCH (Nusbaum and Marder 1988; Dickinson and Marder 1989; Dickinson et al. 1990).

Originally, PDH was called distal retinal pigment hormone (DRPH) as it was identified based on its ability to produce light-adapting movements of pigments within the crustacean retina (Kleinholz 1936). DRPH was first isolated and sequenced from the eyestalks of the shrimp *Pandalus borealis* (Fernlund 1976). Experiments on extracts from crustacean eyestalks demonstrated the existence of a melanophore-dispersing hormone (MDH) that darkened the body of several crustacean species by

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dispersing their melanophore pigments (Fingerman 1965; Kleinholz 1966; Fingerman and Fingerman 1972). MDH was shown to be identical to DRPH; they co-eluted using various chromatographic procedures and were equally capable of inducing pigment dispersion within the chromatophores of the fiddler crab *Uca pugilator* (Kleinholz 1970, 1975). Since this MDH/DRPH peptide acted to disperse pigments in melanophores, leucophores and erythrophores, the general term pigment-dispersing hormone (PDH) has been adopted recently (Mangerich and Keller 1988; Rao and Riehm 1988, 1989). The PDH peptide originally sequenced from *P. borealis* (Fernlund 1976) has been termed α-PDH (Asn-Ser-*Gly*-*Met*-Ile-Asn-Ser-Ile-Leu-Gly-*Ile*-Pro-*Arg*-Val-Met-*Thr*-*Glu*-Ala-NH_2); the peptide sequenced from *U. pugilator* (Rao et al. 1985) has been termed β-PDH (Asn-Ser-*Glu*-*Leu*-Ile-Asn-Ser-Ile-Leu-Gly-*Leu*-Pro-*Lys*-Val-Met-*Asn*-*Asp*-Ala-NH_2). The amino acid sequences of these two peptides, α- and β-PDH, are 66.7% identical; they differ at positions 3, 4, 11, 13, 16, and 17 (italicized above; see Kleinholz et al. 1986). More recently, several new PDH peptides have been sequenced from a number of different crustaceans (McCallum et al. 1988; Phillips et al. 1988; Rao et al. 1989) and these results show that crustacean PDH peptides can be classified as members of either the α- or β-PDH peptide family (Rao and Riehm 1989). Pigment-dispersing factors, PDH-like peptides in insects, also have been isolated and sequenced from the lubber grasshopper *Romalea microptera* (Rao et al. 1987) and the domestic cricket *Acheta domesticus* (Rao and Riehm 1988). The amino acid sequences of these insect pigment-dispersing factors are, respectively, 77.8% and 83.3% identical to β-PDH (Rao and Riehm 1989).

PDH-immunoreactive neurons and fibers were first described in the optic ganglia of the crab *Carcinus maenas* and the crayfish *Orconectes limosus,* indicating that PDH may function as a neurotransmitter or neuromodulator within the CNS (Dircksen et al. 1987; Mangerich et al. 1987). More recently, neurons and processes showing PDH-like immunoreactivity have been found throughout most of the CNS of *C. maenas* and *O. limosus* (Mangerich and Keller 1988). We wish to know if PDH acts as a neuromodulator within the stomatogastric nervous system. As a first step, we used an antibody raised against synthetic *U. pugilator* β-PDH to examine the distribution of PDH-like immunoreactivity in the stomatogastric nervous system of five decapod crustaceans. The overall pattern of PDH-like immunoreactivity for each species was unique, with some similar-looking features in more closely related species. Some of this work was presented in an abstract (Mortin and Marder 1989).

Materials and methods

Animals

Five species of decapod crustaceans were used in this study: the Pacific lobster *Panulirus interruptus* ($n=25$), the Atlantic lobster *Homarus americanus* ($n=8$), the Pacific crab *Cancer antennarius* ($n=9$), the Atlantic crab *Cancer borealis* ($n=13$), and the freshwater crayfish *Procambarus clarkii* ($n=11$). The Atlantic lobsters and crabs were obtained from local fish markets in Boston, Mass. The Pacific lobsters and crabs were purchased from Marinus Biologicals of Long Beach, Calif. and Pacific Biomarine of Santa Monica, Calif. The crayfish were purchased from Carolina Biological Supply Company of Burlington, NC. The marine animals were kept in artifical sea water tanks at 12°–15° C until used. Crayfish were maintained in freshwater aquaria at room temperature until used. Male and female animals were used weighing between 40 and 600 g.

The stomatogastric nervous system

Before fixation and staining, the foregut was removed from each animal and the entire stomatogastric nervous system was dissected away from the stomach and muscles, as detailed previously (see Selverston and Moulins 1987). The stomatogastric nervous system of decapod crustaceans includes the stomatogastric ganglion (STG), the esophageal ganglion (OG), the paired circumesophageal ganglia (CGs), and the network of nerves connecting these four ganglia to each other and to the muscles of the stomach (Fig. 1). The stomatogastric nerve (stn) is the only source of input to the STG from other parts of the CNS. The stn connects to a pair of superior esophageal nerves (sons), which originate from each CG, and a single esophageal nerve (on), which connects to the OG. The paired inferior esophageal nerves (ions) provide a second linkage from each CG to the OG (see Fig. 1). There are about 30 neurons in the STG. The stn contains many fibers: 50–70 in *C. borealis* (B.J. Claiborne, unpublished results), 120 in *P. interruptus* (King 1976), and 240 in *H. americanus* (Maynard 1971). There are 14–18 neurons in the OG (B.J. Claiborne, unpublished observations) and several hundred neurons in each CG (Maynard and Dando 1974). The medial and lateral ventricular nerves (mvn and lvn, respectively) contain axons from STG motor neurons that innervate stomach muscles. The circumesophageal connectives (ccs)

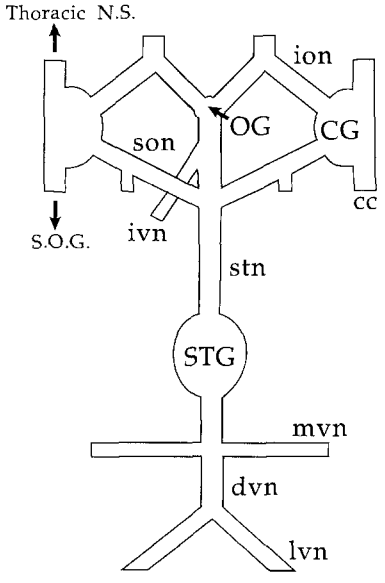

Fig. 1. Schematic diagram of the stomatogastric nervous system of decapod crustaceans. Ganglia: *CG* circumesophageal ganglion; *OG* esophageal ganglion; *STG* stomatogastric ganglion. Nerves: *ion* inferior esophageal nerve; *son* superior esophageal nerve; *ivn* inferior ventricular nerve; *cc* circumesophageal connective; *stn* stomatogastric nerve; *mvn* medial ventricular nerve; *dvn* dorsal ventricular nerve; *lvn* lateral ventricular nerve. The bilaterally symmetric ccs connect the supraesophageal ganglion (*S.O.G.* the "brain") to the thoracic nervous system (*Thoracic N.S.*)

connect each CG with the supraesophageal ganglion (the "brain") on one side and the thoracic portion of the ventral nerve cord on the other.

The stomatogastric nervous system was dissected in physiological saline of the following composition (in mM):

P. interruptus: 479 NaCl; 12.8 KCl; 13.7 $CaCl_2$; 3.9 Na_2SO_4; 10 $MgSO_4$; 11 Trizma Base [TRIS (hydroxymethyl) amino methane]; and 4.8 maleic acid; pH 7.5–7.6.

H. americanus: (same as *P. interruptus* or) 462 NaCl; 16 KCl; 26 $CaCl_2$; 8 $MgCl_2$; 11 glucose; 11 Trizma Base; 5.0 maleic acid; pH 7.4.

C. borealis and *C. antennarius:* 440 NaCl; 11 KCl; 26 $MgCl_2$; 13 $CaCl_2$; 11 Trizma Base; 5.0 maleic acid; pH 7.4–7.5.

P. clarkii: 195 NaCl; 5 KCl; 2.6 $MgCl_2$; 13 $CaCl_2$; 12 Trizma Base; 5 maleic acid; pH 7.5–7.6.

Antisera

Synthetic β-PDH was made using the amino acid sequence from *U. pugilator* (Rao et al. 1985). The polyclonal antibody was made by injecting rabbits with a glutaraldehyde conjugate of synthetic β-PDH and bovine thyroglobulin (Dircksen et al. 1987). The β-PDH antibody was a gift from Dr. K. Rango Rao (University of West Florida, Pensacola, Fla). The specificity of this β-PDH antibody was evaluated using an enzyme-linked immunosorbant assay (ELISA; Bonomelli et al. 1988). The antiserum recognized β-PDH with an IC_{50} of 160 fmol/well, but showed virtually no affinity for α-PDH (<0.001% relative to β-PDH; Rao and Riehm 1989). Additional tests indicated that the antibody recognizes antigens with residues similar to the C-terminus of β-PDH (Bonomelli et al. 1988; Rao and Riehm 1989).

Whole-mount immunocytochemistry

Tissue was processed by the method of Beltz and Kravitz (1983), detailed by Marder et al. (1987). The stomatogastric nervous system was fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, overnight. Tissues were then rinsed five times in cold 0.1 M phosphate buffer pH 7.3 containing 0.3% Triton X-100 and 0.1% Na azide at 1-h intervals. The primary rabbit anti-β-PDH antibody was diluted between 1:800 and 1:1600 in this phosphate buffer with 10% goat normal serum. Tissues were incubated in primary antibody for 20–24 h at 4° C. Tissues were then rinsed again in five washes of the cold phosphate buffer for 1 h each. Either fluorescein- or rhodamine-labeled goat anti-rabbit secondary antibodies were diluted 1:25 in cold phosphate buffer pH 7.3 with 10% goat normal serum. Tissues were incubated in secondary antibody for 20–24 h at 4° C. Tissues were then rinsed in five washes of cold 0.1 M phosphate buffer pH 7.3 without the Triton X-100 or the Na azide for 1 h each. The stomatogastric nervous system was then mounted on a slide in 80% glycerin with 20% 20 mM Na carbonate, pH 9.5.

Before dissection, a few animals were given an injection of colchicine (Sigma) at 15 mg/kg intramuscular to block axonal transport and enhance cellular staining. No new structures were ever revealed by colchicine pretreatment. Controls were done by preincubating the primary antibody with various peptides for 3 h at room temperature before applying the antibody to the tissues. Peptides used for preincubation included: β-PDH (gift of Dr. K. Rango Rao); proctolin, RPCH, FMRFamide, and CCK-8 (obtained from Sigma, Peninsula, or Bachem). Peptides were preincubated at a concentration of 100 μM with primary antibody at dilutions from 1:800 to 1:1600. Immunohistochemical staining was abolished only by preincubation of the primary antibody with β-PDH.

Lucifer yellow backfills

Nerves were backfilled with Lucifer yellow and then double labeled with the β-PDH antibody using a rhodamine-conjugated secondary antibody (Marder et al. 1987). A vaseline well was placed around the nerve that was to be backfilled, to isolate the solution in the well from that surrounding the rest of the nervous system. The saline within the well was replaced for 2–4 min with distilled water at room temperature. The water was then replaced with a solution of 10%–20% Lucifer yellow CH, dilithium salt (Sigma) containing 3%–5% L-α-lysophosphatidylcholine, type I from egg yolk (Sigma) in distilled water at room temperature. The nerve was then cut and the preparation was left at 4° C for 24–48 h. Occasionally, a second batch of the Lucifer yellow solution was added after 24 h. Next, the Lucifer yellow solution was replaced by cold physiological saline and the vaseline well was removed. The preparation was then fixed overnight in 4% paraformaldehyde and processed for PDH-like immunoreactivity as described above, using a rhodamine-conjugated secondary antibody.

Fluorescence imaging and photography

Preparations were viewed with a Zeiss epifluorescence microscope (IM35) with either fluorescein (excitor filter, band pass 450–490 nm; barrier filter band pass 520–560 nm) or rhodamine (excitor filter, 546–612 nm; barrier filter, long pass 590 nm) filter sets. Lucifer yellow backfills were viewed under fluorescein optics. Fluorescent images were photographed using Kodak T-Max film (ASA 400) and printed onto Ilford Multigrade III RC Rapid paper using a number 3 filter.

Results

Panulirus interruptus

The stomatogastric ganglion. A network of neuropil processes in the STG of *P. interruptus* was stained brightly by the β-PDH antibody (Fig. 2A). This stain revealed a complex, three-dimensional meshwork of interconnected processes. The cell bodies of STG neurons, located around the circumference of this neuropil ball, were not stained in this or any of the five species studied. This pattern of staining in the STG of *P. interruptus* is characteristic of other substances that appear to function as neuromodulators of the motor rhythms produced in the STG (Marder 1987). Preincubation of the primary antibody (1:1600) with 100 μM synthetic β-PDH blocked this neuropil stain in the STG.

The intense neuropil stain in *P. interruptus* comes from fibers that enter the STG via the stn (Figs. 2A, 3A). A bundle of many small stained fibers traversed the length of the stn, and this bundle usually split in two as it entered the STG. The origin of these PDH-stained stn fibers was not determined, but, as we will show later, they appear to originate from each CG. Another common feature of the PDH-like staining was the appearance of small strings of varicosities within the upper half of the stn (small arrows in Fig. 3B); these were presumed to be neuropil-like structures since they were similar to neuropil stains seen in other regions of the crustacean nervous system. A series of neuropil-like varicosities was always seen at the junction of the stn,

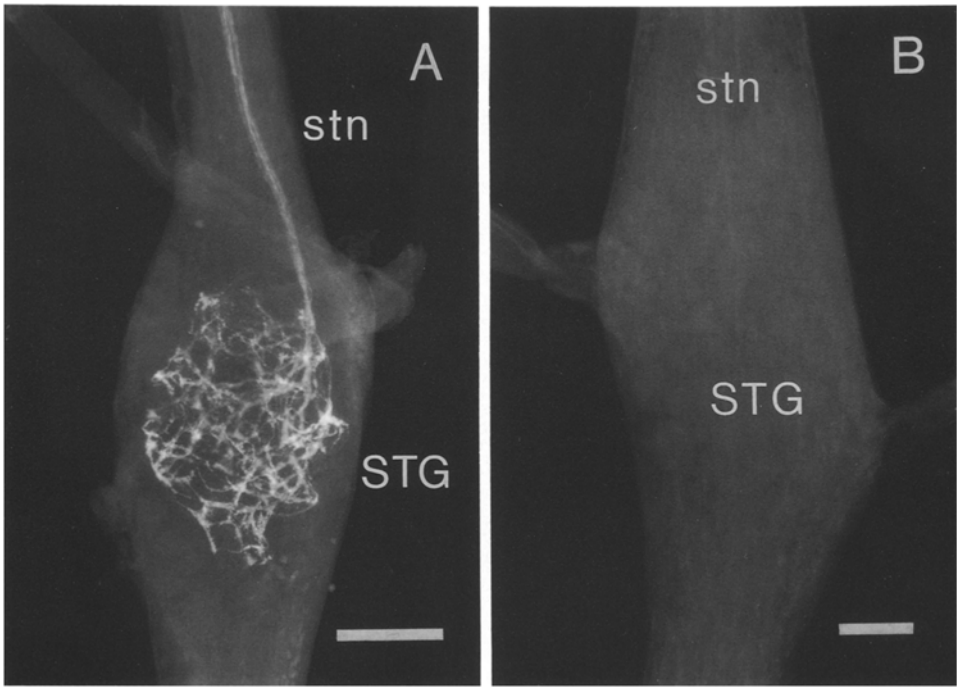

Fig. 2A, B. PDH-like staining in the stomatogastric ganglion (*STG*) of (**A**) *P. interruptus* and (**B**) *C. borealis* . Note the absence of any stain in *C. borealis*. The sole input nerve to the STG from the rest of the CNS, the *stn*, the stomatogastric nerve, is to the top. *Scale bars:* 200 μm (**A**); 100 μm (**B**)

the on, and the sons (Fig. 3C). The β-PDH antibody stained several clusters of punctate varicosities within this region (filled arrows in Fig. 3C). There were usually four or five of these neuropil clusters at this junction.

At a separate focal plane, the PDH-like immunoreactive fibers in the stn (triangles in Fig. 3C) split into two bundles, one traveling into each son (open arrows in Fig. 3C). These fibers could often be followed through each son until they entered each CG. Occasionally, a few PDH-stained somata (15–35 μm diameter) were seen in each son, near the CGs (arrows in Fig. 3D). These small, brightly stained cells had a long axon-like process that projected towards the nearest CG (Fig. 3D).

The circumesophageal ganglion (CG). The CG of *P. interruptus* showed a complex pattern of staining with this β-PDH antibody (Fig. 4). Overall, we could distinguish three distinct regions of staining (Fig. 4A). There were two different neuropil regions: the first region of neuropil-like immunolabeling was characterized by strings of punctate varicosities (solid arrows in Fig. 4A, B); the second region showed a more diffuse staining pattern that formed a tangled web (open arrows in Fig. 4A–C), and was always located closer to the son and the ion. A single through-fiber was stained in each circumesophageal connective, crossing through the first, punctate-labeled neuropil region (thin arrows in Fig. 4A, B). There also was always a cluster of between 13 and 17 small, brightly stained cells (10–30 μm) located near the son (triangles in Fig. 4A, C, D). The initial axons from this cluster of small cell bodies usually bent toward the second, more diffuse neuropil region (see Fig. 4C).

A small bundle of PDH-like stained fibers could be followed from the second, more diffuse neuropil region, passing into the son (thin arrows in Fig. 4C). This bundle of fibers would often pass nearby or sometimes through the cluster of small labeled cells in each CG. This fiber bundle could be traced through each son and into the stn, where the bundles from each son joined together into one larger fiber bundle as described above. No processes or cells were stained in either the ions or the OG of *P. interruptus*. No processes or cells were stained in the dorsal ventricular nerve (dvn) or any of the peripheral nerves below the level of the STG in this or any of the species studied (see Fig. 1).

Homarus americanus

The β-PDH-like immunoreactivity in the Maine lobster *H. americanus* was usually fainter than that seen in *P. interruptus*. In each CG of *H. americanus*, three regions of PDH immunoreactivity were identified (Fig. 5). There were two neuropil regions: one region of punctate varicosities, which appeared much like beads spread out on a tangle of strings (Fig. 5B); and a second more weakly stained region of diffuse neuropil (not shown). The former was located close to each connective, while the latter was located more medial in each CG. These two neuropil regions were very similar in location and quality to those seen in each CG of *P. interruptus*, although the punctate neuropil region usually was more extensive in *H. americanus*. A single through-fiber was also stained in each connective, intersecting the neuropil region that displayed the punctate stain (long thin arrows in Fig. 5A, B). A few small cells (10–30 μm) were also labeled within each CG of *H. americanus* (Fig. 5C, D). These faintly stained cells were usually located at the edge of each CG, near the son or the ion. The cluster of many small cells that always stained brightly with this β-PDH antibody in each CG of *P. interruptus* was not stained in the CG of *H. americanus*.

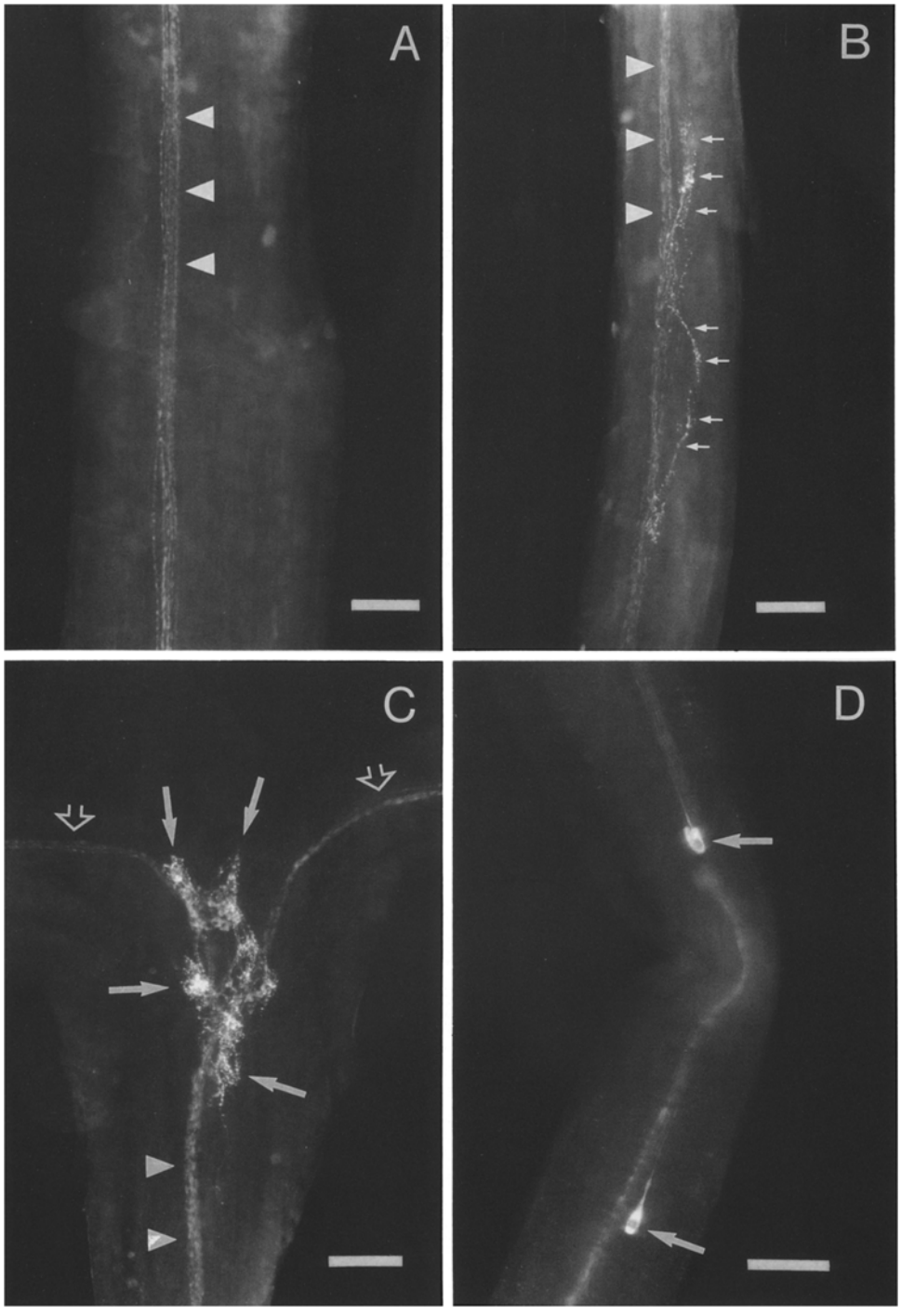

Fig. 3A–D. PDH-like staining of input fibers to the STG in *P. interruptus*. **A** PDH-stained fibers in the stn (STG towards bottom); **B** neuropil region in stn (STG towards bottom); **C** clusters of neuropil regions at the junction of the stn, the sons, and the on (stn towards bottom); **D** two PDH-stained cells in the son (nearest CG towards top). In **A–C** *triangles* point to PDH-stained nerve bundle in the stn. In **B** *small arrows* point to PDH-stained varicosities in stn. In **C** *filled arrows* point to PDH-stained neuropil clusters, and *open arrows* point to PDH-stained nerve bundle in each son. In **D** *arrows* point to PDH-stained cells in son; note that the initial process of each cell goes up towards the CG. *Scale bars:* 100 μm (**A, B, D**); 200 μm (**C**)

There was also usually a very faint PDH-like stain in the neuropil region of the STG in *H. americanus* (data not shown). PDH-like immunoreactive fibers also were sometimes seen in the sons or the stn or both. No labeling was seen in the on, the ions, or the OG of *H. americanus*.

Procambarus clarkii

In the crayfish *P. clarkii*, the β-PDH antibody stained a dense ball of neuropil structures within each CG (Fig. 6A). Many processes, varicosities, and tangles of PDH-like immunoreactivity were labeled, covering a wide area within each CG. The neuropil staining pattern in each CG was more intense and widespread in *P. clarkii* than in any of the other species studied (cf. Figs. 4A, 6A). This β-PDH antibody labeled from three to eight medium-sized cells (20–50 μm) in each CG (arrows in Fig. 6B). These PDH-immunoreactive neurons were most often nearer the son than the ion, but their localization within the neuropil often made it difficult to distinguish stained neurons from the surrounding, brightly stained processes. Therefore, three to eight β-PDH-posi-

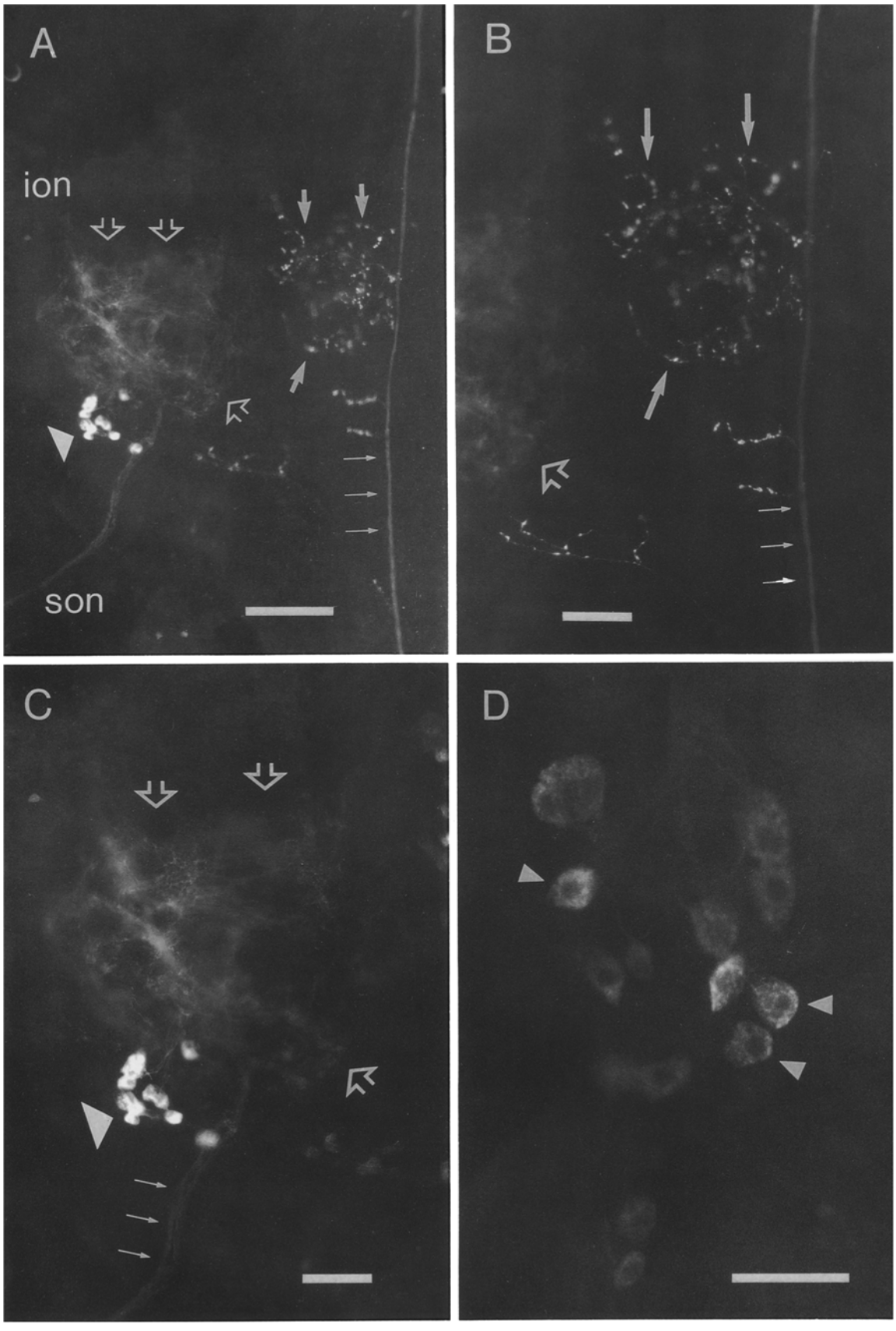

Fig. 4A–D. PDH-like staining in the circumesophageal ganglion (CG) of *P. interruptus*. **A** Overview of PDH-like immunoreactivity in the CG; **B** punctate neuropil region near the circumesophageal connective (cc); **C** diffuse neuropil region and cluster of small PDH-stained cells near the son; **D** close-up view of a PDH-stained cell cluster from another animal. In **A–C** *open arrows* point to diffuse neuropil region. In **A** and **B** *thick arrows* point to punctate neuropil region and *thin arrows* point to a through-fiber in the cc. In **A, C** and **D** *triangles* point to PDH-stained cell cluster. In **C** *thin arrows* point to PDH-stained fibers entering the son. In **A**: *ion* inferior esophageal nerve; *son* superior esophageal nerve. *Scale bars:* 200 μm (**A**); 100 μm (**B, C**); 50 μm (**D**)

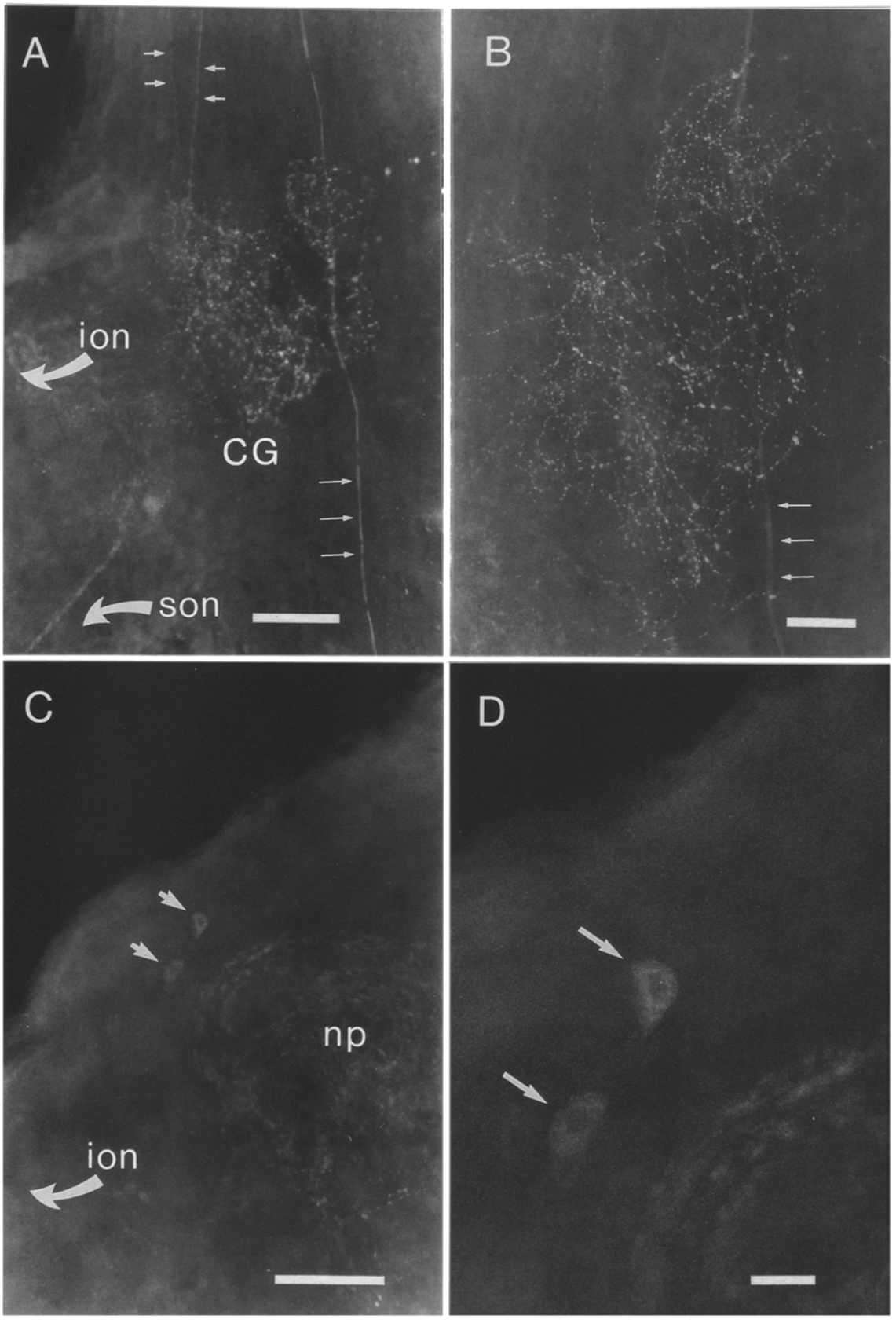

Fig. 5A–D. PDH-like staining in the CG of *H. americanus*. **A** Overview of PDH-like immunoreactivity in the *CG;* **B** punctate neuropil region near the cc; **C** two cells located near the *ion*; **D** close-up of two cells shown in **C**. In **A** *short arrows* point to two fibers entering the cc and projecting towards the thoracic nerve cord. In **A** and **B** *long arrows* point to single through-fiber in the cc. In **C** and **D** *thick arrows* point to two PDH-stained cells. In **C**: *np* neuropil of CG. *Scale bars:* 200 μm (**A**); 100 μm (**B, C**); 20 μm (**D**)

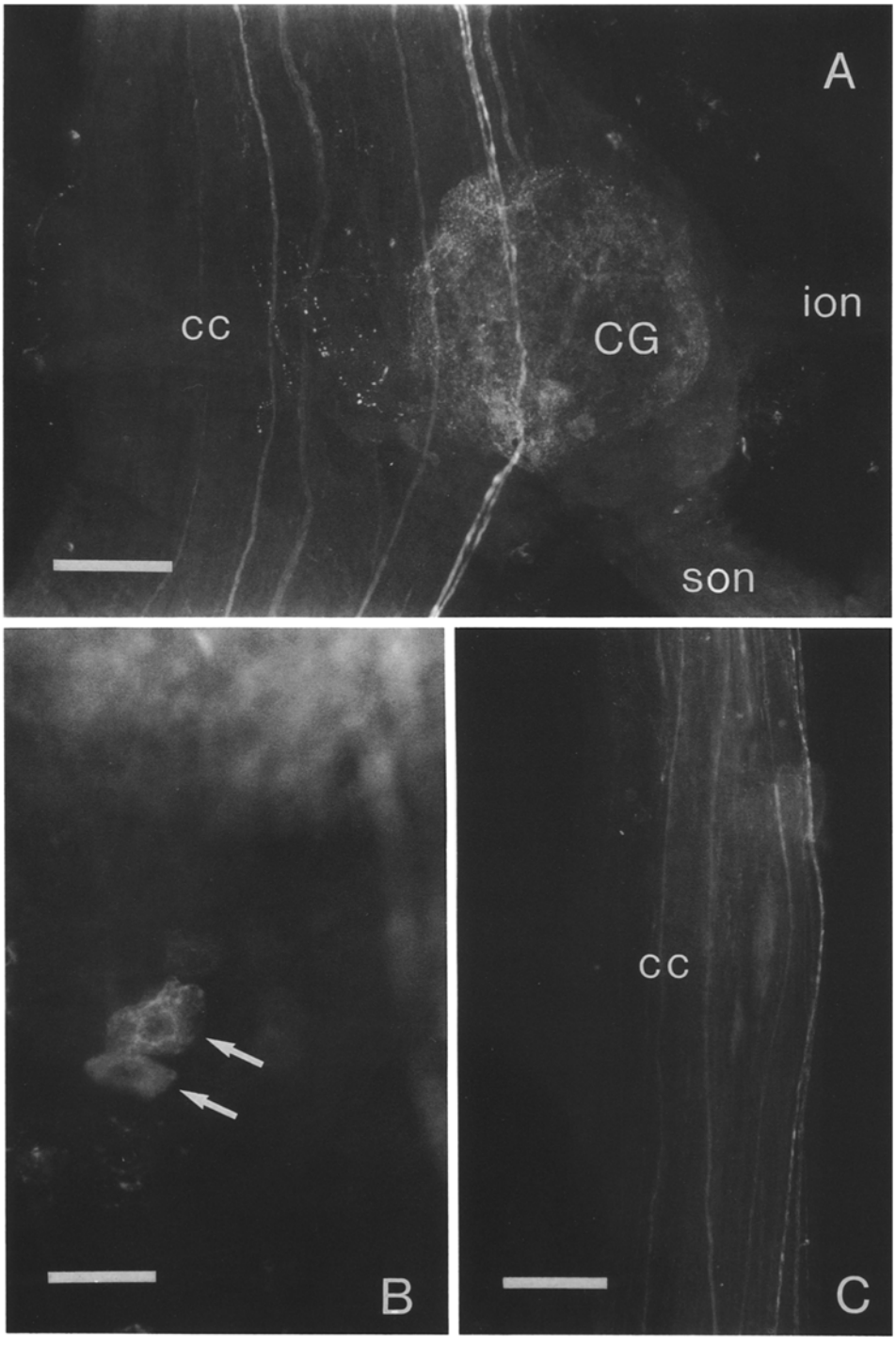

Fig. 6A–C. PDH-like staining in the CG of *P. clarkii*. **A** Overview of PDH-like immunoreactivity in the CG; **B** closeup of two cells located near the son (*arrows*); **C** PDH-stained fibers within the *cc*, the circumesophageal connective. *Scale bars:* 200 μm (**A, C**); 50 μm (**B**)

tive cells is probably a minimum for the true number of PDH-stained cells in *P. clarkii*.

Another unique feature of the PDH-like stain in *P. clarkii* is that many through-fibers were stained in each circumesophageal connective (Fig. 6A, C). At least ten PDH-immunoreactive through-processes could be seen in each connective. The intensity and quality of the staining varied greatly among these PDH-positive fibers. Some through-fibers stained brightly, while the fluorescent appearance of others was much more dim; some fibers displayed strings of stained varicosities, while other processes displayed a more even level of fluorescence throughout their length (see Fig. 6C). Possible reasons for this variability are covered later (see Discussion).

There was a very faint PDH-like stain in the neuropil region of the STG in *P. clarkii*. The staining by this β-PDH antibody in the neuropil regions of the STGs of *P. clarkii* and *H. americanus* was weaker than in any part of the stomatogastric nervous system of all the spe-

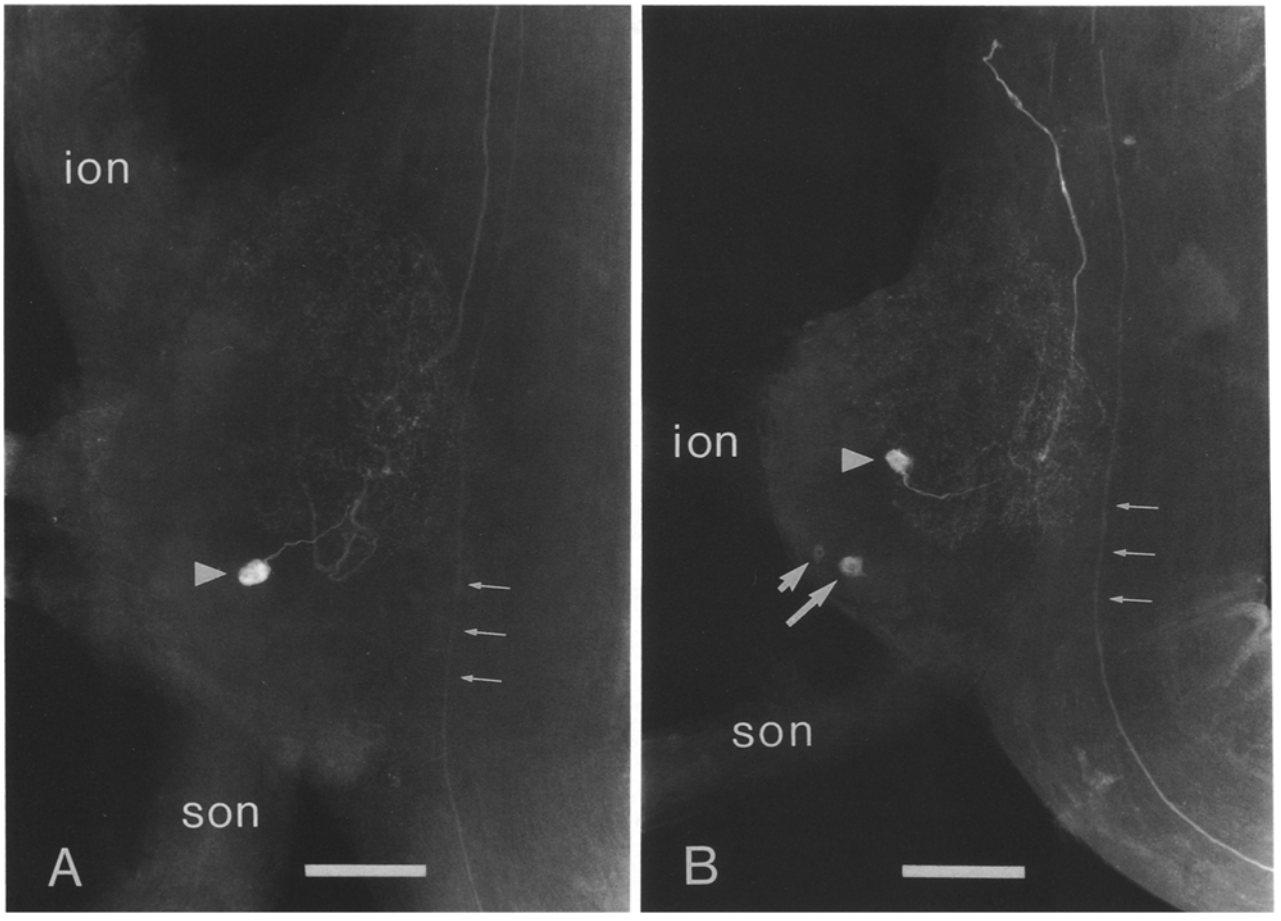

Fig. 7A, B. PDH-like staining in the CG of (**A**) *C. borealis* and (**B**) *C. antennarius*. In **A** and **B** *triangle* points to large PDH-stained neuron; *thin arrows* point to through-fiber in cc. In **B** *short* and *long arrows* point to small and medium sized cells, respectively. *Scale bars:* 200 μm

cies studied (see Discussion). No labeling was seen in the on, the ions, the OG, the sons, or the stn of *P. clarkii*.

Cancer borealis

Only one large cell (30–60 μm) was stained in each CG in the Atlantic crab *C. borealis* (Fig. 7A). A single thick initial process exited from the soma of this brightly stained cell; this process extended into the connective traveling towards the thoracic region of the crab's CNS. Before exiting the CG, the main process of this PDH-stained neuron branched extensively to contribute to a region of PDH-like immunoreactive neuropil near the connective. The axon of this cell did *not* branch into the side of the connective joining the supraesophageal ganglion (the "brain") to the stomatogastric nervous system. We could not determine if all of the PDH-stained neuropil was due to branches from this neuron or if some of the neuropil processes were from other neurons whose cell bodies were not stained in our preparations. A single through-fiber was also stained with this PDH antibody in each connective, crossing the lateral edge of the PDH-like neuropil region (arrows in Fig. 7A).

No cells or processes were stained in the STG (Fig. 2B), the OG, the stn, the sons, the on, or the ions in *C. borealis*.

Cancer antennarius

Three cells, one large (30–60 μm), one medium (25–40 μm), and one small (10–30 μm), were stained with this β-PDH antibody in each CG in the Pacific crab *C. antennarius* (Fig. 7B). The intensity of the fluorescence was roughly proportional to cell size, such that the largest of the three PDH-positive cells was the brightest. A single large process exited from the soma of the largest PDH-stained neuron in *C. antennarius* and gave rise to an axon projecting into the connective towards the thoracic nervous system. Side branches from the main process of this large PDH cell contributed to a region of neuropil within each CG. The size, position, and morphology of this large PDH-like immunoreactive neuron in *C. antennarius* suggest that it is homologous to the single large cell that showed PDH staining in *C. borealis* (cf. triangles in Fig. 7A, B).

Two small axons showed PDH-like staining in each ion of *C. antennarius* (Fig. 8A). PDH-positive fibers

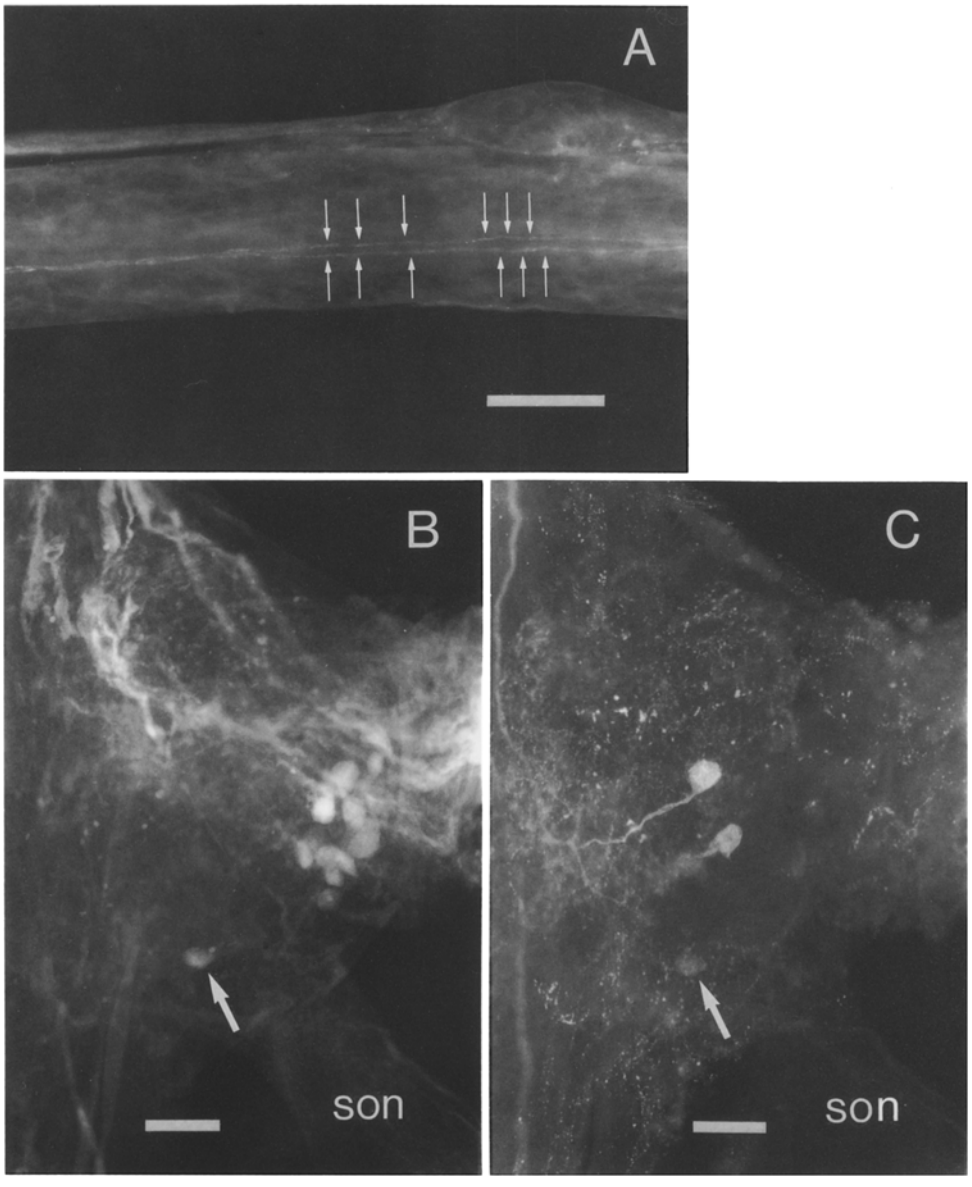

Fig. 8A–C. Lucifer yellow double-labeling of bilaterally paired crossing neurons in the CGs of *C. antennarius*. **A** Two PDH-immunoreactive fibers in the ion; **B** Lucifer yellow backfill from the ion; CG viewed under fluorescein optics (ion to upper right); **C** PDH-like immunoreactivity of the same preparation shown in part **B**; CG viewed under rhodamine optics. In **A** *thin arrows* point to the two labeled ion fibers. In **B** and **C** *arrow* points to the double-labeled neuron whose axon enters the ion. *Scale bars:* 100 μm

were not seen in the ion of the other four species studied. A pair of PDH-stained fibers could be seen exiting the CG and traveling in the ion, bending through the OG, and passing into the opposite ion and CG. We investigated the origin of these PDH-like fibers by backfilling the ion with Lucifer yellow to label cells in the CG that send their axons into the ion. These preparations were then double-labeled with the β-PDH antibody using a rhodamine-conjugated secondary antibody. An example of a Lucifer yellow backfill from one ion in *C. antennarius* is shown in Fig. 8B. Many cells and fibers near the ion in this CG showed the yellow fluorescence of Lucifer yellow. The same preparation is shown in Fig. 8C, now viewed with rhodamine filters to highlight the stain of the three PDH-immunoreactive cells. The smallest and most faintly stained PDH cell, located nearer the son in this example, was also labeled by the Lucifer yellow backfill (arrows in Fig. 8B, C). Thus, the smallest PDH-like immunoreactive cell in each CG sends an axon into the ions and through the OG to the opposite CG, yielding two PDH-stained fibers in each ion and in the OG.

No cells or processes were stained in the STG, the stn, the on, or the sons in *C. antennarius*.

Discussion

We have shown that an antibody raised against synthetic β-PDH stains a complex, three-dimensional meshwork of fibers within the neuropil region of the STG in *P. interruptus* (Fig. 2A). This bright, PDH-like stain originates from fibers that can be traced back to each CG (Fig. 9A). None of the cell bodies of the neurons within the STG were stained by this β-PDH antibody. This pattern of staining indicates that a PDH-like peptide may function as a neuromodulator of the CPGs within the STG of *P. interruptus*.

The present study adds one or more PDH-like peptides to a growing list of substances that are found in projections into the STG of various crustacean species. These include serotonin (Beltz et al. 1984), dopamine and octopamine (Barker et al. 1979), histamine (Claiborne and Selverston 1984), γ-amino butyric acid (GABA; Cazalets et al. 1987; Cournil et al. 1989, 1990), proctolin (Marder et al. 1986), FMRFamide-like peptides (Hooper and Marder 1984; Marder et al. 1987), RPCH (Nusbaum and Marder 1988; Dickinson and Marder 1989), a CCK-like peptide (Turrigiano and Selverston 1989, 1990), and a substance P-like peptide (Goldberg et al. 1988). Interestingly, robust physiological actions are produced by exogenous applications of all of these substances (see Marder and Hooper 1985), save for substance P and related peptides, although a number of tachykinins have been tested (L.I. Mortin and E. Marder, unpublished results). It remains to be seen whether a β-PDH-like peptide is physiologically active in this system.

None of the other four species of crustaceans in our study clearly displayed β-PDH-like immunoreactivity in the STG neuropil. There was no stain visible in the STG of the crabs *C. borealis* or *C. antennarius*. There was an extremely faint stain seen in the neuropil region of the STG in *H. americanus* and *P. clarkii*. The poor quality of this labeling indicates that the β-PDH antibody: (1) may not be recognizing a β-PDH-like peptide in these two species, but may be cross-reacting with another substance present in the STG neuropil (see Kvitash and Beltz 1989), or (2) that it may be demonstrating a low affinity for a different member of the PDH family of peptides (Rao and Riehm 1989). Many different forms of both the α-PDH and β-PDH peptide families have been identified in different crustacean species (McCallum et al. 1988; Phillips et al. 1988; Rao and Riehm 1988, 1989). Recently, it has been shown that a single crustacean species, the shrimp *Pandalus jordani*, can produce at least three different forms of PDH peptides, including the α- and β-PDH forms (Rao et al. 1989). In addition, PDH-like peptides have been isolated and sequenced from the grasshopper *Romalea microptera* (Rao et al. 1987) and the cricket *Acheta domesticus* (Rao and Riehm 1988), indicating that the PDHs probably constitute a family of structurally similar neuropeptides within arthropods (Rao and Riehm 1989). It will be necessary to use antibodies that are specific for different members of the PDH family (Phillips et al. 1990) and to characterize biochemically the PDH peptides in all these species, to determine whether any of the four species in our study do contain a different PDH-like peptide within the STG neuropil. Preliminary experiments using an antibody made against α-PDH (courtesy of Dr. K.R. Rao; Bonomelli et al. 1989) labeled the same neuronal structures that were stained with the β-PDH antibody, but at a lower intensity (L.I. Mortin and E. Marder, unpublished results). In competitive ELISA tests the α-PDH antiserum showed a low affinity for β-PDH and its analogs (Bonomelli et al. 1989).

We have identified a pair of neurons, one in each CG of *C. antennarius*, that send their axons from one CG to the other CG via the ions (Figs. 8, 9E). These two neurons are arranged ideally for exchanging information from one CG to the other and may function as bilateral coordinators of sensory and motor function in *C. antennarius*. To our knowledge, this is the first example of an identified neuron pair within the stomatogastric nervous system of crustaceans that display this crossed-connection morphology. Several neurons have been identified within the stomatogastric nervous system that send axonal branches to both CGs, and, therefore,

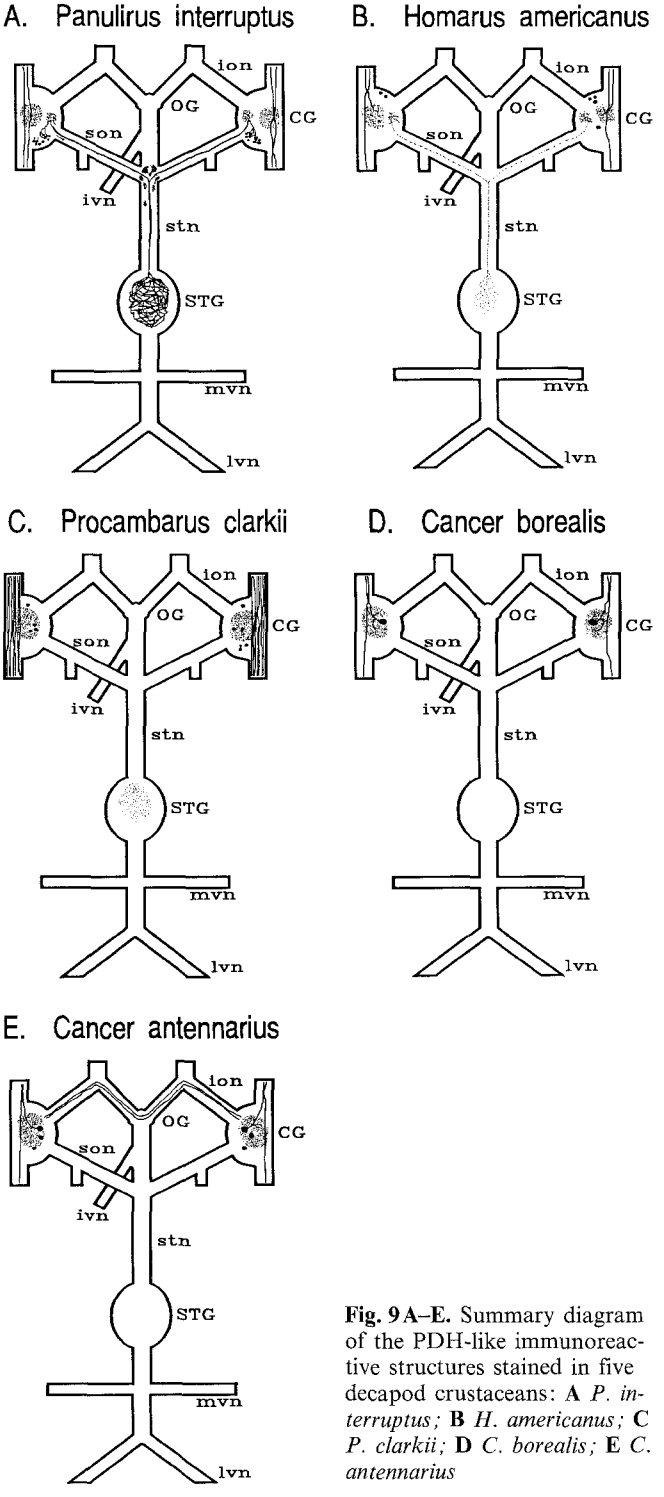

Fig. 9A–E. Summary diagram of the PDH-like immunoreactive structures stained in five decapod crustaceans: **A** *P. interruptus*; **B** *H. americanus*; **C** *P. clarkii*; **D** *C. borealis*; **E** *C. antennarius*

could send coordinated information to both sides of the stomach (these include: the anterior pyloric modulator, Moulins and Nagy 1981; Nagy and Dickinson 1983; a pair of modulatory proctolin-containing neurons, Nusbaum and Marder 1989a, b; interneuron 1, Russell 1976; two cardiac sac dilator neurons, CD1 and CD2, Vedel and Moulins 1977; Dickinson and Marder 1989; the posterior stomach receptor cells, Dando and Laverack 1969; the anterior gastric receptor neuron, Simmers and Moulins 1988a, b; and the gastropyloric receptor or GPR cells, Katz and Harris-Warrick 1989; Katz et al. 1989). None of these neurons, however, are in a position to report activity from one CG to the opposite CG directly, as would be possible with the crossing neurons that we have identified in *C. antennarius*.

Many homologies in PDH-like immunoreactivity are evident among some of the species that we studied (Fig. 9). Two qualitatively different regions of neuropil were stained in each CG in both *P. interruptus* and *H. americanus* (cf. Fig. 9A, B). The neuropil region with brightly stained, punctate varicosities was always situated near the connective. The more diffusely stained neuropil region was always situated more medial in each CG, roughly centered between the son and the ion. Similarly, the single large PDH-stained cell in each CG of *C. borealis* had the same size, shape, morphology and general pattern of arborization as the largest of the three CG cells of *C. antennarius* (cf. Figs. 7A, B; 9D, E). Every species in our study also had at least one PDH-stained through-fiber passing through the connective (Fig. 9). Whether any of these examples represent true homologies cannot be determined until more specific cell markers are established for crustaceans.

Other features of these PDH-like staining patterns were unique for individual species (Fig. 9). As mentioned above, the STG neuropil was stained brightly only in *P. interruptus* (cf. Fig. 2A, B). In addition, the cluster of 13–17 small PDH-stained neurons in each CG of *P. interruptus* was unique to that species (Figs. 4D, 9A). It is likely that these PDH-like-immunoreactive neurons are the source of the neuropil stain in the STG. The axons of many of these cells were seen projecting towards and often into the diffuse neuropil region; axonal branches or collaterals from these cells would need to reverse direction to exit the CG via the son. The PDH-like-immunoreactive fiber bundle in each son often could be followed projecting into the diffuse neuropil region. Backfills of the son with Lucifer yellow did not label any of the small PDH-immunoreactive neurons ($n=5$). This result could come about because: (1) these PDH-stained cells do not project into the son, (2) the axons of these PDH-stained neurons are too small to adequately take up and transport Lucifer yellow to their somata, or (3) the Lucifer yellow is unable to properly backfill through branch-points or collaterals in the axons of these PDH-stained neurons. The only other source of the STG neuropil stain could be from one of the through-fibers in each connective; these one or two fibers would have to branch extensively upon entering the son to make up the bundle of many small fibers traveling in the stn towards the STG. It is also possible that the titer of PDH-like peptide within the somata of the neurons projecting to the STG may be too low to detect in whole-mount preparations; an efficient transport mechanism could concentrate the peptide at the terminals.

The two smaller PDH-stained cells in each CG of *C. antennarius* distinguish its PDH-staining pattern from that in each CG of *C. borealis* (cf. Figs. 7A, B; 9D, E). The smallest of these cells in *C. antennarius* contributes to the pair of axons in each ion (Fig. 8A), as mentioned above. None of the other species studied displayed any PDH-like staining in the ions or the OG.

The PDH-like staining pattern in each CG of *P. clarkii* was unique (see Figs. 6, 9C). The brightly stained neuropil ball in this animal was very different from the CG stains in the other species. And, unlike the other animals, the PDH-immunoreactive neurons in *P. clarkii* were most often located within the large neuropil region. Finally, the circumesophageal connective of *P. clarkii* contained over ten PDH-stained through-fibers, whereas at most two through-fibers were stained in any of the other species. These through-fibers in *P. clarkii* varied greatly in the quality and quantity of their PDH-like stain. Two brightly stained through-fibers always transected the lateral edge of the CG neuropil region (Fig. 6A). Other less intensely stained fibers remained physically separate from CG structures and most likely represent through-signal lines between the supraesophageal ganglion (the "brain") and more caudal CNS centers (see Fig. 6C). Preliminary examination of whole-mounts of the brain in *P. clarkii* showed PDH-like immunoreactivity in at least four cells and in numerous processes and neuropil-like structures.

The intensity and quality differences in the labeling of the PDH-stained through-fibers in *P. clarkii,* and in cells and processes in all of our preparations, could be due to several factors. First, individual axons may contain different quantities of PDH-like peptide, or the peptide may be packaged differently in different neurons. In particular, some neurons may utilize a form of non-synaptic release such as local neurosecretion (Jan and Jan 1982). Second, the depth of each process within the three-dimensional lattice of the tissue could account for some of these differences. Since our staining was done in whole-mount preparations, the level of penetration of primary and secondary antibodies into different regions of the tissue could vary greatly. Third, the level of peptide concentration within neurons and their processes may show circadian or state-dependent variability. Pigment migration within the retina and chromatophores of crustaceans has been known to exhibit a circadian rhythmicity (Welsh 1930; Webb 1950; Thurman 1988). Fourth, there could be more than one form of PDH within a species (Rao et al. 1989) and among species (McCallum et al. 1988; Phillips et al. 1988). If different neurons contain different PDH peptides, then the affinity of the antibody against *U. pugilator* β-PDH for these other PDH moieties would vary (see Rao and Riehm 1989). Biochemical extraction and isolation of the various PDH peptides within each species will be necessary to resolve this last issue.

There are many similarities between the pattern of PDH-like staining described here and the immunohistochemical staining of CCK-like peptides (Turrigiano and Selverston 1989, 1990). For example, antibodies against CCK stained an intense three-dimensional neuropil structure in the STG of *P. interruptus* that looks remarkably similar to that seen with our β-PDH antibody. Using an antibody against CCK (Turrigiano and Selverston 1989), we stained a single large cell in the CG of *C. borealis* that resembles in position and morphology the PDH-like immunoreactive neuron in this animal. CCK-8 (100 μM) did not block PDH-like staining (1:800) and β-PDH (100 μM) did not block CCK-like staining (1:150). Since both antibodies were made in rabbits, we were unable to do double-labeling experiments in whole-mount preparations. However, only one large cell was stained in the CG of *C. borealis* when we used both the CCK and β-PDH antibodies together ($n=2$); this result suggests the colocalization of CCK- and β-PDH-like peptides (L.I. Mortin, G.G. Turrigiano, and E. Marder, unpublished results). Many other neurons may colocalize both a CCK-like peptide and a PDH-like peptide. Rigorous double-labeling experiments utilizing sectioned preparations will be necessary to substantiate these results (but see Kvitash and Beltz 1989).

Previous immunohistochemical studies have demonstrated that the distribution of other neuroactive substances within the stomatogastric nervous system, including peptides, can vary greatly among crustacean species. Antibodies to the amine, serotonin (5-HT), stained the neuropil of the STG, stn fibers, and dorsal ventricular nerve fibers in *H. americanus, C. borealis,* and *C. irroratus,* but showed no 5-HT in the STG of *P. interruptus* (Beltz et al. 1984; Katz et al. 1989). Antibodies to the pentapeptide, proctolin, stained three neurons in the OG of *C. borealis,* but no proctolin-like immunoreactive cells were stained in the OG of *P. interruptus* or *H. americanus* (Marder et al. 1986; Siwicki and Bishop 1986). In contrast, the largest neuron in the CG of *C. borealis* and *H. americanus* were stained with proctolin antibodies whereas the homologous neuron was not stained in *P. interruptus* or *P. clarkii* (Marder et al. 1986; Siwicki and Bishop 1986). Antibodies raised against the peptide FMRFamide stained four neurons in the OG of *C. borealis,* whereas only two of these neurons were stained in *P. interruptus* (Marder et al. 1987). Again the largest CG neuron showed FMRFamide-like immunoreactivity in *C. borealis,* but not in *P. interruptus* (Marder et al. 1987). Antibodies to RPCH stained two OG neurons that project into the inferior ventricular nerve in both *P. interruptus* and *C. borealis,* but a third OG neuron was stained in *P. interruptus* only (Nusbaum and Marder 1988; Dickinson and Marder 1989). Antibodies to the mammalian peptide, substance P, stained STG neuropil, one OG neuron, and a specialized neuropil structure and many neurons in the CG of *C. borealis, P. interruptus,* and *H. americanus* (Goldberg et al. 1988). Differences were seen in substance P-like immunoreactivity: OG neuropil was labeled in *P. interruptus* only, and there were significant differences in the intensity and shape of the CG neuropil structure (Goldberg et al. 1988). Recently, an antibody against crustacean cardioactive peptide (CCAP) has been shown to stain a few cells and neuropil in the stomatogastric nervous system of *P. interruptus,* but not in *C. borealis* (Mortin and Marder 1990). This may reflect a difference in whether CCAP is used hormonally or more as a neurotransmitter in one species versus the other (see Beltz et al. 1984).

Identified neurons within the stomatogastric nervous system also display variability regarding their immunoreactive content. In *C. borealis* the GPR cells contain 5-HT and acetylcholine (Beltz et al. 1984; Katz et al. 1989), whereas in *H. americanus* they contain 5-HT, and FMRFamide- and CCK-like peptides (Katz and Harris-Warrick 1990). Cells homologous to GPR in *P. interruptus* do not stain for 5-HT (Beltz et al. 1984). The pair of modulatory proctolin-containing neurons in *C. borealis* show proctolin- and GABA-like immunoreactivity (Nusbaum et al. 1989); the homologous neurons in *H. americanus* show GABA-like immunoreactivity but do not stain for proctolin (Cournil et al. 1989, 1990). These studies demonstrate that there is a great deal of phylogenetic variability regarding the transmitter content of neurons within the stomatogastric nervous system. The functional significance of this variability is not known.

The five species of decapod crustaceans used in our study come from the large suborder Reptantia. These Reptantia are grouped into three different infraorders: the crabs *C. borealis* and *C. antennarius* are Brachyurans, whereas *P. interruptus* is a Palinuran, and *H. americanus* and *P. clarkii* are Astacurans (Kaestner 1970) or Astacideans (Schram 1982). The two crabs displayed very similar staining patterns with this β-PDH antibody. But even these closely related species showed divergence relative to their PDH-like immunoreactivities. *U. pugilator,* the original source of β-PDH, is a Brachyuran crab. Extracts from the eyestalks of two other crabs, *Cancer magister* (Kleinholz et al. 1986) and *Callinectes sapidus* (Mohrherr et al. 1990), indicate that the amino acid sequence of β-PDH may be highly conserved among Brachyurans. *P. interruptus* shares some PDH-staining characteristics with *H. americanus,* and both of these lobsters are most distinct from *P. clarkii.* Preliminary ELISA analysis of extracts from the nervous system of *P. interruptus* indicates the presence of a β-PDH-like peptide but not an α-PDH-like peptide (S.L. Bonomelli, L.I. Mortin, E. Marder and K.R. Rao, unpublished results). A β-PDH analog has been isolated from *P. clarkii* and differs from *U. pugilator* β-PDH by only one amino acid (glu-17 in place of Asp-17; McCallum et al. 1988). We must exercise caution in drawing any general evolutionary conclusions from examining the staining pattern or peptide content for only one in a large family of peptides. As yet, we do not know if these PDH-staining patterns shed any additional light on the evolutionary relationships among decapod crustaceans. It will be necessary to compare numerous homologous neurons from these different infraorders as to their transmitter/peptide content in order to draw such insights.

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References

Barker DL, Kushner PD, Hooper NK (1979) Synthesis of dopamine and octopamine in the crustacean stomatogastric nervous system. Brain Res 161:99–113

Beltz BS, Kravitz EA (1983) Mapping of serotonin-like immunoreactivity in the lobster nervous system. J Neurosci 3:585–602

Beltz B, Eisen JS, Flamm R, Harris-Warrick RM, Hooper SL, Marder E (1984) Serotonergic innervation and modulation of the stomatogastric ganglion of three decapod crustaceans (*Panulirus interruptus, Homarus americanus* and *Cancer irroratus*). J Exp Biol 109:35–54

Bonomelli SL, Rao KR, Riehm JP (1988) Development and application of an ELISA for crustacean β-PDH. Am Zool 28:117A

Bonomelli SL, Rao KR, Riehm JP (1989) Preparation and evaluation of an antiserum for crustacean α-PDH. Am Zool 29:49A

Cazalets J-R, Cournil I, Geffard M, Moulins M (1987) Suppression of oscillatory activity in crustacean pyloric neurons: implication of GABAergic inputs. J Neurosci 7:2884–2893

Claiborne BJ, Selverston AI (1984) Histamine as a neurotransmitter in the stomatogastric nervous system of the spiny lobster. J Neurosci 4:708–721

Cournil I, Meyrand P, Moulins M (1989) Identification of all GABA immunoreactive projections to the lobster stomatogastric ganglion. Soc Neurosci Abstr 15:366

Cournil I, Meyrand P, Moulins M (1990) Lobster stomatogastric GABA system. In: Wiese K, Krenz WD, Tautz J, Reichert H, Mulloney B (eds) Frontiers in crustacean neurobiology. BCR Birkhäuser, Basel, pp 448–454

Dando MR, Laverack MS (1969) The anatomy and physiology of the posterior stomach nerve (p.s.n.) in some decapod crustacea. Proc R Soc Lond (Biol) 171:465–482

Dickinson PS, Marder E (1989) Peptidergic modulation of a multioscillator system in the lobster. I. Activation of the cardiac sac motor pattern by the neuropeptides proctolin and red pigment-concentrating hormone. J Neurophysiol 61:833–844

Dickinson PS, Mecsas C, Marder E (1990) Neuropeptide fusion of two motor pattern generator circuits. Nature 344:155–158

Dircksen H, Zahnow CA, Gaus G, Keller R, Rao KR, Riehm JP (1987) The ultrastructure of nerve endings containing pigment-dispersing hormone (PDH) in crustacean sinus glands: identification by an antiserum against a synthetic PDH. Cell Tissue Res 250:377–387

Fernlund P (1976) Structure of a light-adapting hormone from the shrimp, *Pandalus borealis*. Biochem Biophys Acta 439:17–25

Fingerman M (1965) Chromatophores. Physiol Rev 45:296–339

Fingerman M, Fingerman SW (1972) Evidence for a substance in the eyestalks of brachyurans that darkens the shrimp *Crangon septemspinosa*. Comp Biochem Physiol 43A:37–46

Goldberg D, Nusbaum MP, Marder E (1988) Substance P-like immunoreactivity in the stomatogastric nervous system of the crab *Cancer borealis* and the lobsters *Panulirus interruptus* and *Homarus americanus*. Cell Tissue Res 252:515–522

Harris-Warrick RM (1988) Chemical modulation of central pattern generators. In: Cohen AV, Rossignol S, Grillner S (eds) Neural control of rhythmic movements in vertebrates. Wiley, New York, pp 285–331

Heinzel H-G, Selverston AI (1988) Gastric mill activity in the lobster. II. Proctolin and octopamine initiate and modulate chewing. J Neurophysiol 59:551–565

Hooper SL, Marder E (1984) Modulation of a central pattern generator by two neuropeptides, proctolin and FMRFamide. Brain Res 305:186–191

Hooper SL, Marder E (1987) Modulation of the lobster pyloric rhythm by the peptide, proctolin. J Neurosci 7:2097–2112

Jan JY, Jan YN (1982) Peptidergic transmission in sympathetic ganglia of the frog. J Physiol (London) 327:219–246

Kaestner A (1970) Invertebrate zoology, Crustacea, vol. III, 2nd edn. Wiley, New York

Katz PS, Harris-Warrick RM (1989) Serotonergic/cholinergic muscle receptor cells in the crab stomatogastric nervous system. II. Rapid nicotinic and prolonged modulatory effects on neurons in the stomatogastric ganglion. J Neurophysiol 62:571–581

Katz PS, Harris-Warrick RM (1990) Actions of identified neuromodulatory neurons in a simple motor system. Trends Neurosci 13:367–373

Katz PS, Eigg MH, Harris-Warrick RM (1989) Serotonergic/cholinergic muscle receptor cells in the crab stomatogastric nervous system. I. Identification and characterization of the gastropyloric receptor cells. J Neurophysiol 62:558–570

King DG (1976) Organization of crustacean neuropil. I. Patterns of synaptic connections in lobster stomatogastric ganglion. J Neurocytol 5:207–237

Kleinholz LH (1936) Crustacean eye-stalk hormone and retinal pigment migration. Biol Bull 70:159–184

Kleinholz LH (1966) Hormonal regulation of retinal pigment migration in crustaceans. In: Bernard CG (ed) The functional organization of the compound eye. Pergamon, Oxford, pp 89–101

Kleinholz LH (1970) A progress report on the separation and purification of crustacean neurosecretory pigmentary-effector hormones. Gen Comp Endocrinol 14:578–588

Kleinholz LH (1975) Purified hormones from the crustacean eyestalk and their physiological specificity. Nature 258:256–257

Kleinholz LH, Rao KR, Riehm JP, Tarr GE, Johnson L, Norton S (1986) Isolation and sequence analysis of a pigment-dispersing hormone from eyestalks of the crab, *Cancer magister*. Biol Bull 170:135–143

Kvitash Z, Beltz BS (1989) SCP_B and FMRFamide immunoreactivities in *Homarus americanus* neurons: colocalization of two peptides – or – colabeling of a single peptide? Soc Neurosci Abstr 15:366

Mangerich S, Keller R (1988) Localization of pigment-dispersing hormone (PDH) immunoreactivity in the central nervous system of *Carcinus maenas* and *Orconectes limosus* (Crustacea), with reference to FMRFamide immunoreactivity in *O. limosus*. Cell Tissue Res 254:199–208

Mangerich S, Keller R, Dircksen H, Rao KR, Riehm JP (1987) Immunocytochemical localization of pigment-dispersing hormone (PDH) and its coexistence with FMRFamide-immunoreactive material in the eyestalks of the decapod crustaceans *Carcinus maenas* and *Orconectes limosus*. Cell Tissue Res 250:365–375

Marder E (1987) Neurotransmitters and neuromodulators. In: Selverston AI, Moulins M (eds) The crustacean stomatogastric nervous system. Springer, Berlin Heidelberg New York, pp 263–300

Marder E, Hooper SL (1985) Neurotransmitter modulation of the stomatogastric ganglion of decapod crustaceans. In: Selverston AI (ed) Model neural networks and behavior. Plenum Press, New York, pp 319–337

Marder E, Hooper SL, Siwicki KK (1986) Modulatory action and distribution of the neuropeptide proctolin in the crustacean stomatogastric nervous system. J Comp Neurol 243:454–467

Marder E, Calabrese RL, Nusbaum MP, Trimmer B (1987) Distribution and partial characterization of FMRFamide-like peptides in the stomatogastric nervous systems of the rock crab, *Cancer borealis*, and the spiny lobster, *Panulirus interruptus*. J Comp Neurol 259:150–163

Maynard DM, Dando MR (1974) The structure of the stomatogastric neuromuscular system in *Callinectes sapidus, Homarus*

americanus and *Panulirus argus* (Decapoda Crustacea). Philos Trans R Soc Lond (Biol) 268:161–220

Maynard EA (1971) Electron microscopy of stomatogastric ganglion in the lobster, *Homarus americanus*. Tissue Cell 3:137–160

McCallum ML, Rao KR, Riehm JP, Mohrherr CJ, Morgan WT (1988) Isolation of a β-PDH analog from the crayfish, *Procambarus clarkii*. Am Zool 28:117A

Mohrherr CJ, Rao KR, Riehm JP, Morgan WT (1990) Isolation of β-PDH from sinus glands of the blue crab, *Callinectes sapidus*. Am Zool 30:28A

Mortin LI, Marder E (1989) Localization of pigment-dispersing hormone (PDH)-like immunoreactivity in the crustacean stomatogastric nervous system. Soc Neurosci Abstr 15:366

Mortin LI, Marder E (1990) Crustacean cardioactive peptide (CCAP): a hormone neuromodulator of the stomatogastric nervous system. Soc Neurosci Abstr 16:1131

Moulins M, Nagy F (1981) Participation of an unpaired motor neurone in the bilaterally organized oesophageal rhythm in the lobsters *Jasus lalandii* and *Palinurus vulgaris*. J Exp Biol 90:205–230

Nagy F, Dickinson PS (1983) Control of a central pattern generator by an identified modulatory interneurone in Crustacea. I. Modulation of the pyloric motor output. J Exp Biol 105:33–58

Nusbaum MP, Marder E (1988) A neuronal role for a peptide similar to crustacean red pigment concentrating hormone: neuromodulation of the pyloric rhythm in the crab, *Cancer borealis*. J Exp Biol 135:165–181

Nusbaum MP, Marder E (1989a) A modulatory proctolin-containing neuron (MPN). I. Identification and characterization. J Neurosci 9:1591–1599

Nusbaum MP, Marder E (1989b) A modulatory proctolin-containing neuron (MPN). II. State-dependent modulation of rhythmic motor activity. J Neurosci 9:1600–1607

Nusbaum MP, Cournil I, Golowasch J, Marder E (1989) Modulating rhythmic motor activity with a proctolin- and GABA-containing neuron. Soc Neurosci Abstr 15:366

Phillips JM, Rao KR, Riehm JP, Morgan WT (1988) Isolation and characterization of a pigment dispersing hormone from the shrimp *Penaeus aztecus*. Soc Neurosci Abstr 14:534

Phillips JM, Fox DL, Bonomelli S, Rao KR, Riehm JP (1990) Immunocytochemical studies of the distribution of α- and β-PDH in *Callinectes* and *Pandalus*. Am Zool 30:28A

Rao KR (1985) Pigmentary effectors. In: Bliss DE, Mantel LH (eds) The biology of crustacea, vol 9. Integument, pigments, and hormonal processes. Academic Press, Orlando, pp 395–462

Rao KR, Riehm JP (1988) Pigment-dispersing hormones: a novel family of neuropeptides from arthropods. Peptides 9 [Suppl 1]: 153–159

Rao KR, Riehm JP (1989) The pigment-dispersing hormone family: chemistry, structure-activity relations, and distribution. Biol Bull 177:225–229

Rao KR, Riehm JP, Zahnow CA, Kleinholz LH, Tarr GE, Johnson L, Norton S, Landau M, Semmes OJ, Sattelberg RM, Jorenby WH, Hintz MF (1985) Characterization of a pigment-dispersing hormone in eyestalks of the fiddler crab *Uca pugilator*. Proc Natl Acad Sci USA 82:5319–5322

Rao KR, Mohrherr CJ, Riehm JP, Zahnow CA, Norton S, Johnson L, Tarr GE (1987) Primary structure of an analog of crustacean pigment-dispersing hormone from the lubber grasshopper *Romalea microptera*. J Biol Chem 262:2672–2675

Rao KR, Kleinholz LH, Riehm JP (1989) Characterization of three forms of pigment-dispersing hormone from the shrimp *Pandalus jordani*. Soc Neurosci Abstr 15:367

Robertson RM, Moulins M (1981) Control of rhythmic behaviour by a hierarchy of linked oscillators in Crustacea. Neurosci Lett 21:111–116

Russell DF (1976) Rhythmic excitatory inputs to the lobster stomatogastric ganglion. Brain Res 101:582–588

Schram FR (1982) The fossil record and evolution of Crustacea. In: Abele LG (ed) The biology of crustacea, vol 1. Systematics, the fossil record, and biogeography. Academic Press, New York, pp 93–147

Selverston AI, Moulins M (1987) The crustacean stomatogastric nervous system. Springer, Berlin Heidelberg New York

Simmers J, Moulins M (1988a) A disynaptic sensorimotor pathway in the lobster stomatogastric system. J Neurophysiol 59:740–756

Simmers J, Moulins M (1988b) Nonlinear interneuronal properties underlie integrative flexibility in a lobster disynaptic sensorimotor pathway. J Neurophysiol 59:757–777

Siwicki KK, Bishop CA (1986) Mapping of proctolin-like immunoreactivity in the nervous systems of lobster and crayfish. J Comp Neurol 243:435–453

Thurman CL (1988) Rhythmic physiological color change in Crustacea: a review. Comp Biochem Physiol 91:171–185

Turrigiano GG, Selverston AI (1989) Cholecystokinin-like peptide is a modulator of a crustacean central pattern generator. J Neurosci 9:2486–2501

Turrigiano GG, Selverston AI (1990) A cholecystokinin-like hormone activates a feeding-related neural circuit in lobster. Nature 344:866–868

Vedel J-P, Moulins M (1977) Functional properties of interganglionic motor neurons in the stomatogastric nervous system of the rock lobster. J Comp Physiol 118:307–325

Webb HM (1950) Diurnal variations of response to light in the fiddler crab, *Uca*. Physiol Zool 23:316–337

Weimann JM, Marder E (1989) Activation of the gastric rhythm of the crab stomatogastric ganglion by SDRNFLRFamide. Soc Neurosci Abstr 15:1047

Welsh JH (1930) Diurnal rhythm of the distal pigment cells in the eyes of certain crustaceans. Proc Natl Acad Sci USA 16:386–395