Neurons of the copepod, *Calanus finmarchicus* exhibiting immunoreactivity to serotonin and crustacean hyperglycemic hormone

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The monoamine serotonin (5HT) and the peptide, crustacean hyperglycemic hormone (CHH) are important neurotransmitters/neurohormones in crustacean nervous systems (reviews: Beltz and Kravitz[2]; Cooke and Sullivan[3]). In decapods, they appear in consistent patterns in different parts of the nervous system, where they take part in a variety of integrative, modulatory and regulatory functions. The consistency in the locations of CHH and 5HT- containing neurons aids identifying homologies of form and function in nervous systems of related taxa. This is the first report of the distribution of putative CHH-containing neurons in the nervous system of the most abundant and successful, albeit little-studied, crustacean class, the Copepoda.

Calanus finmarchicus obtained from net hauls in the Gulf of Maine were maintained at 4°C in the lab until use. Animals were fixed in 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS) overnight, rinsed in 0.1M phosphate buffer with Triton X (PBTX) (15 min; 6 changes), and incubated 12 h. in a mixture of polyclonal rabbit antiserotonin antibody (1:1000) and guinea pig anti-CHH antibody (1:200). Material was rinsed again (15 min x 6) in PBTX, incubated 12 h. in goat anti-rabbit and anti-guinea pig IgG secondary antibodies conjugated respectively to Alexa 594 and Alexa 488 fluorophors (Invitrogen). After a final rinse (15 m x 6) animals were mounted in GelMount (Biomeda) and imaged with epifluorescence or laser scanning confocal microscopy.

Reidentifiable groups of 5HT-immunoreactive (5HT-IR) somata of the nervous system included ~3 pairs of anteriorly-situated neurons with fine processes ramifying in more posterior neuropil and one pair located in mid-commissure. The situation for 5HT-IR somata in the ventral nerve cord (VNC) was less clear, but 1-2 pairs on average occurred in each segment. Several 5HT-IR axons were present in the VNC. At the commissural level, 3 paired fibers (1 large; 2 small) occurred in mid-cord and 1 pair was located very close to the midline. Reidentifiable groups of CHH-IR somata in the *Calanus finmarchicus* nervous system included 2 pairs of anterior neurons close to the 5HT cells and projecting to the same region, an additional pair projecting anteriorly to an unknown destination, and a pair of neurons located at about the oesophageal level. Two pairs of CHH-IR fibers run in the thoracic VNC, one arising from the oesophageal somata and one possibly from one of the pairs of anterior somata. They show varicosities and short ramifications in the VNC suggestive of release sites, and then ramify posteriorly in the ventral musculature of the mid-thoracic segments.

Distributions of 5HT-IR somata, especially in the VNC, are similar to those reported in other arthropods.[1,2] The anterior CHH-IR somata match the location reported for an X-organ homolog in an isopod[4], but no clear homolog of the sinus gland release complex of decapods was identified.

Supported by NSF grant 0451376 UH BRIN (NIH), the MDIBL High School Research Fellowship program and the Betterment Fund, and a New Investigators Award from the Salisbury Cove Fund and the Maren Foundation.

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