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Introduction. A neurosecretory tissue of the crustacean eyestalk is known as the X-organ-sinus gland system (XOSG). This is the major crustacean neuroendocrine center for diverse physiological functions. Ionic bases of intracellular action potentials observed in somata (X-organ), axons, and terminals (sinus gland) were described elsewhere. Light- and electron-microscopic studies showed that the terminals have several morphological types. In this paper, I describe morphological and electrophysiological features of XOSG neurons fully identified by the technique of Lucifer yellow injection.

Materials and methods. The eyestalk of crabs (Portunus trituberculatus) or crayfish (Procambarus clarkii) was used. The optic ganglion with optic tract was isolated from the eyestalk by removing the exoskeleton and overlying muscles. Under a dissecting microscope, the sinus gland was visible as an iridescent tissue on the surface of the optic ganglion and the X-organ was discernible as a cluster of cells on the opposite surface of the ganglion to the sinus gland. They were dissected together with connecting axons which usually passed through the optic neuropil. When the X-organ was not visible it was isolated together with the surrounding connective tissue.

The XOSG preparation was pinned to the bottom of a chamber made of the Sylgard. The chamber was continuously perfused with a fresh saline. The saline used was natural seawater in crab experiments or a solution containing 205 mM NaCl, 5.4 mM KCl, 13.5 mM CaCl₂, 2.6 mM MgCl₂, and 1–3 mM NaHCO₃ (pH 7.4) in crayfish experiments. A microelectrode filled with a 1 M LiCl solution containing 3% Lucifer yellow was inserted into a soma or into an axon. After recording the resting and action potentials the dye was iontophoretically injected by applying hyperpolarizing pulses of current (1 nA, 1 sec duration) for about 30 min at a frequency of 0.1 Hz. Then, the XOSG was fixed overnight with 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) in the dark. The preparation was rinsed with the phosphate buffer, immersed in glycerol and observed under an epi-fluorescence microscope (Labophot, Nikon).

Results and discussion. Experiments were performed on 14 crabs and 11 crayfish. Intracellular penetration was successfully done on somata, axons or terminals in both species. In some preparations, however, the dye did not spread well, especially into the terminals, but in 11 crab and 10 crayfish preparations the spread was considered successful and photographed. The XOSG's of two species of crustacea showed some differences. Axons in crabs were thin but individual terminals were clearly visible. Some axons in crayfish were giants but their terminals were small and unclear.

In three preparations of crabs, the spread of Lucifer yellow looked complete
and the soma, axon and terminals were all visible in the same neuron (Fig. 1). In other eight preparations, the dye spread was limited to the soma, to the axon, to the terminal, or to two of them. When the dye was injected into the soma, the dendrites and thin branches from the axon were shown out as well as a long axon and terminals but the fine arborizations of the terminals were not well discernible. On the other hand, when the dye was injected into an axon near the terminals, a great number of thin arborizations were traced in the sinus gland side. The diameter of the soma was usually more than 50 µm, whereas the dilatations of the terminal were less than 15 µm in diameter. Axons close to the soma had even thinner diameters with many thin branches but no varicosity.

The electrical responses of XOSG neurons could be studied with the same electrode as used for the Lucifer yellow injection, although sometimes the membrane potential was not stable enough because of electrode plugging. The resting potentials of XOSG neurons were usually about −60 mV. Some evoked and spontaneous action potentials were overshot. The spontaneous action potentials were so labile that they were not observed after the Lucifer yellow injection. The inset of Fig. 1B shows action potentials of a soma evoked by intracellular stimulation with a current pulse. These were recorded from the soma of the same neuron as shown in the figure. The inset of Fig. 1C shows action potentials obtained from the axon that was injected with the dye. When the soma was stimulated with a long pulse (1 sec duration), the amplitude of the repetitive action potentials usually declined (Fig. 1B). However, this was not the case in the axon (Fig. 1C) or in the terminal (not shown).
In contrast to crab preparations, the XOSG of crayfish showed a variety in shape from one preparation to another. Of 10 preparations of crayfish, three were traced with Lucifer yellow by more than 2 mm in length, while in the others tracing was possible only for 1 mm. Fig. 2 shows several examples of XOSG of crayfish. The diameter of the soma in crayfish was usually smaller than those of crabs. The axon showed a wide variety in its diameter, length and collaterals. The terminals had less frequent arborizations and their dilatations were rather small in comparison to those of crabs.

In both species examined, some of the soma looked very small and lacked long axons, and did not show any trace of connection to the terminal even after a 20 min injection of the dye, although it sometimes had a small network of dendrites in the X-organ. From these neurons the spontaneous or evoked action potential was rarely observed, even though their resting potentials (about -70 mV) were stable. Electrophysiologically, it is known that some somata in the X-organ show no action potential. It is therefore surmised that these non-excitible neurons do not send the axon to the terminal, but only to the limited area within the X-organ. The role of these somata is unclear at the moment. Andrew et al. made an observation with iontophoretic cobalt-backfilling and concluded that small non-neurosecretory cells in the X-organ of the crayfish do not send the axon into the sinus gland. The electrically quiescent cells described here may correspond to those cells.

In one experiment one injection of the dye into an axon visualized two somata as shown in Fig. 2B. One of the somata (near the top of the figure) showed a much weaker fluorescence than the other, although the colors of fluorescence of both somata were the same. This finding indicates that there is a dye-coupling between the two neurons. Stewart or Adanina et al. suggested that some dye-coupled cells are also electrically coupled to each other. It is likely that some somata in the X-organ have electrical interactions between them to process the neuronal information and to modify the secretory function.
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References