CRF-LIKE IMMUNOREACTIVITY IN THE DAHLGREN CELLS OF THE CAUDAL NEUROSECRETORY SYSTEM OF THE LITTLE SKATE, RAJA ERINACEA

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The caudal neurosecretory system of fishes (CNSF) consists of variably sized neurosecretory cells that are located in the distal tail (Friedberg and Bern, Biol Rev 43:175-199, 1968). This system is diffuse in elasmobranchs and primarily contains large secretory cells, the Dahlgren cells, that distribute in the ventrolateral central grey of the caudal spinal cord. In contrast, several species of teleosts possess a specialized organ, the urophysis, in which collections of neurosecretory processes, rich in hormone peptides, are concentrated (Chan and Bern, Cell Tiss Res 174:339-357, 1976). Identification of the types of hormone peptides present in the CNSF will provide information necessary for the appreciation of the functional significance of this system which is still poorly understood. Urotensin I (UT) is one of the peptides that was isolated from the urophysis, and its mammalian neuropeptide homolog is corticotropin releasing factor (CRF). Recently, marked similarities were found between CRF and UT (Lederis et al, Science 218:162- 164, 1982). Subsequently, localization of CRF was accomplished in several teleosts (Onstott and Elde, Soc. Neurosci. Abstr 9:1016, 1983). The present study has demonstrated immunocytochemical localization of CRF-like peptides in the caudal spinal cord of the skate.

Anesthetized skates of both sexes were perfused transcardially with 4% paraformaldehyde dissolved in phosphate buffered elasmobranch ringer (pH 7.5). The distal two thirds of the tail spinal cord were studied in sections cut in longitudinal and transverse planes at light microscopic and electron microscopic levels. The peroxidase anti-peroxidase procedure of Sternberger (Immunocytochemistry, 2nd Edn, John Wiley and Sons, New York, 1979) was used to localize CRF-like immunoreactivity. Antisera to synthetic mammalian ovine CRF were obtained commercially (Immunonuclear).

Figure 1 illustrates two longitudinally cut Dahlgren cells (D) that are surrounded by small glia cell nuclei (arrow),and stained with cresyl violet. The Dahlgren cells were distributed in small intermittent clusters of a few cells or of solitary cells positioned lateroventrally to the central canal. Such cells were intensely immunoreactive with antisera to CRF. Localization of CRF imunoreactive Dahlgren cells is shown in longitudinal (Fig. 2) and transverse (Fig. 3) planes of the most caudal spinal cord regions (last 10 to 15 vertebrae). Antisera to serotonin (5HT), tyrosine hydroxylase (TH), or cholecystokinin(CCK) did not label the Dahlgren cells, although some immunoreactive afferent fiber inputs, labeled by either 5HT or TH, were evident in the ventral cord. Dahlgren cells containing CRF-like immunoreactivity had processes of different calibers as shown in Figure 4. Such processes distributed in the adjacent neuropile and they projected predominantly to the ventromedial surface (arrows in Figs. 2, 3).

Ultrastructural studies of cells of the CNSF in Raja erinacea confirmed some previous findings in other species (Afzelius and Friedberg, Z Zellforsch 59:289-308, 1963;O'Brien and Kriebel, Brain Res Bull 10:89-95, 1983).

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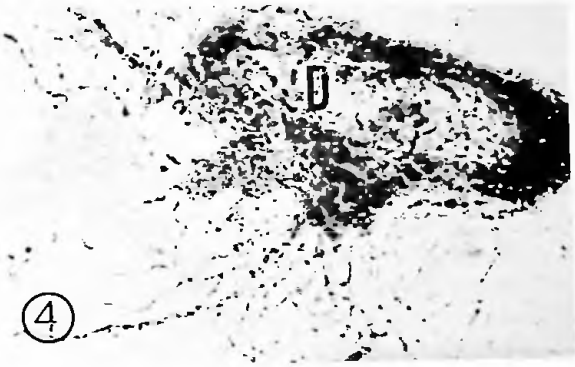

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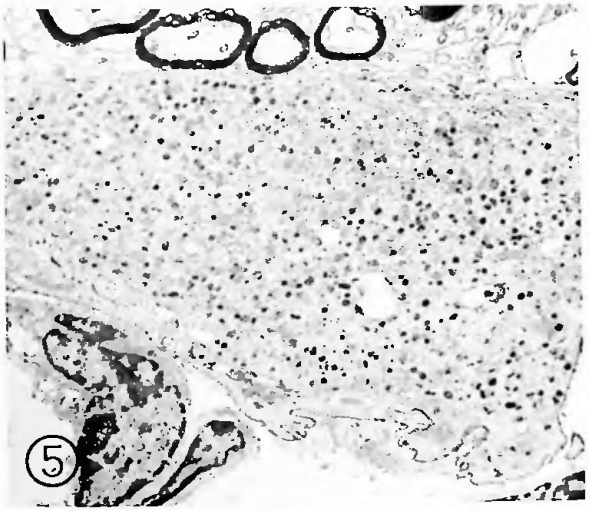

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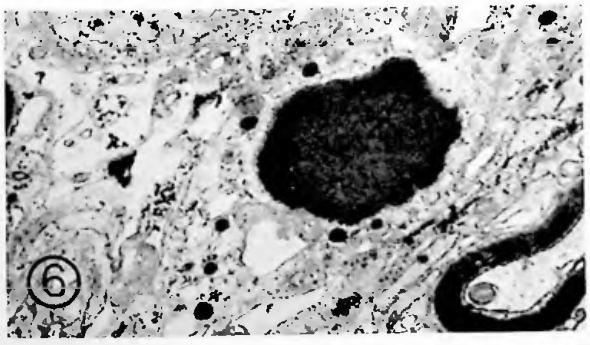

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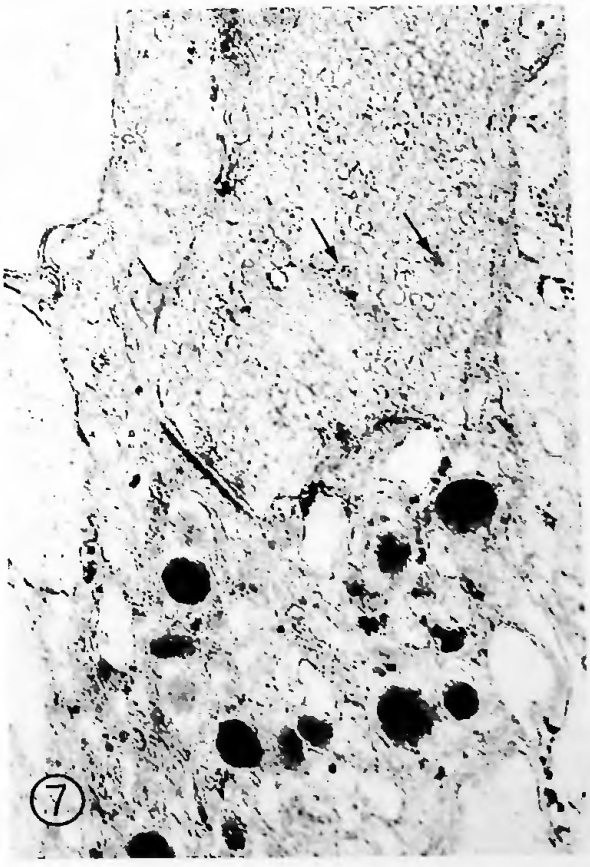

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Variously sized electron dense secretory vesicles distributed in somata and processes of the Dahlgren cells (Figs. 5,7). In addition accumulations of large electron dense droplets (Fig. 6) occurred in some processes of the skate Dahlgren cells. Large bulbous processes of Dahlgren cells contributed to the ventromedial surface of the spinal cord (Fig. 5). These processes were covered by thin cytoplasmic extensions derived from glia and by typical glia endfeet which abbutted a continuous sheath of basal lamina. A few of the Dahlgren cell processes projected between the ependymal cells of the central canal into which some of the large secretory droplets appeared to be released. Somata and proximal processes of Dahlgren cells were postsynaptic to numerous axon terminals. A typical example of an asymmetric synapse is illustrated in Figure 7. The contacting axon terminals contained clear vesicles and a few small dense core vesicles (arrows).

This study demonstrated the presence of CRF-like immunoreactivity in Dahlgren cells of the skate. Work is in progress to identify other hormone peptides secreted by Dahlgren cells, and to further determine the origin and transmitter types of the afferent fibers that impose synaptic control on the caudal neurosecretory system in the skate.

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