Secretion into the Cerebrospinal Fluid by Caudal Neurosecretory Neurons

Abstract. *Neurons of the regenerating caudal neurosecretory system of* Tilapia mossambica *and of the normal system of* Albula vulpes *send processes into the central canal of the spinal cord. Evidence for appreciable release of secretion into the cerebrospinal fluid from these processes has been obtained with both light and electron microscopes.*

Presumed dendritic processes of diencephalic neurosecretory cells containing large amounts of stainable material extend into the third ventricle in teleosts (*1*) and amphibians (*2*, *3*). Discharge of secretion into the ventricle has been claimed by several workers, but Dierickx (*4*) has suggested that the dendrites in amphibians may function as osmoreceptors. Caudal neurosecretory cells in the isospondylous teleost *Albula vulpes* also send blunt processes into the central canal, possibly indicating an apocrine type of release of secretion into the cerebrospinal fluid (*5*).

During the course of a study of regeneration of the caudal neurosecretory system in the cichlid teleost *Tilapia mossambica* (*6*), we encountered unquestionable evidence for the occurrence of neurosecretory processes extending into the central canal and strong evidence for the discharge of neurosecretion from these processes. These observations form the basis for the present report.

The entire caudal neurosecretory (urophysial) system including all recognizable neurosecretory (Dahlgren) cells was removed from mature *Tilapia* by ligature and section of the caudal peduncle (*7*).

About 5 months after the operation a new neurosecretory system had regenerated (*6*), which possessed neurohemal areas within the spinal cord (Fig. 1*A*). A conspicuous feature of the regenerated system is the enormous pile-up of acid violet–stained (*8*) neurosecretory material in processes extending toward the central canal; these processes resemble Herring bodies. Processes also project into the lumen of the central canal where large masses of secretion seem to be released in an apocrine manner (Fig. 1*A*). (Reissner's fiber does not stain with acid violet and therefore is not to be mistaken for neurosecretory material.)

Under the electron microscope, processes from subependymal neurosecretory cells were seen projecting between ependymal cells into the central canal (Fig. 1*B*). These processes showed variable morphology: some were filled with elementary neurosecretory granules typical of a neurohemal area (Fig. 1*C*), whereas others contained fewer granules and more granular endoplasmic reticulum (Fig. 1*B*) typical of the circumnuclear region of a Dahlgren cell. Mitochondria and Golgi lamellae were also noted. Of particular interest was the large number of elementary neurosecretory granules (800 to 2000 Å in diameter) free in the cerebrospinal fluid among apparent cellular debris and between apical cytoplasmic blebs of ependymal cells. The granules ranged in form from membrane-limited, electron-dense structures to electron-lucent, apparently disintegrating structures without a limiting membrane (Fig. 1, *B–D*). After apocrine release, the granules appear to undergo dissolution in the cerebrospinal fluid.

Although some processes are similar to those described by Smoller (*3*) in *Hyla regilla*, not all processes project deeply into the lumen of the central canal. Some cells possess desmosomes, terminal bars, cilium, accessory centriole, and striated rootlet (Fig. 1*D*), in addition to elementary neurosecretory granules. Extrusion of secretory

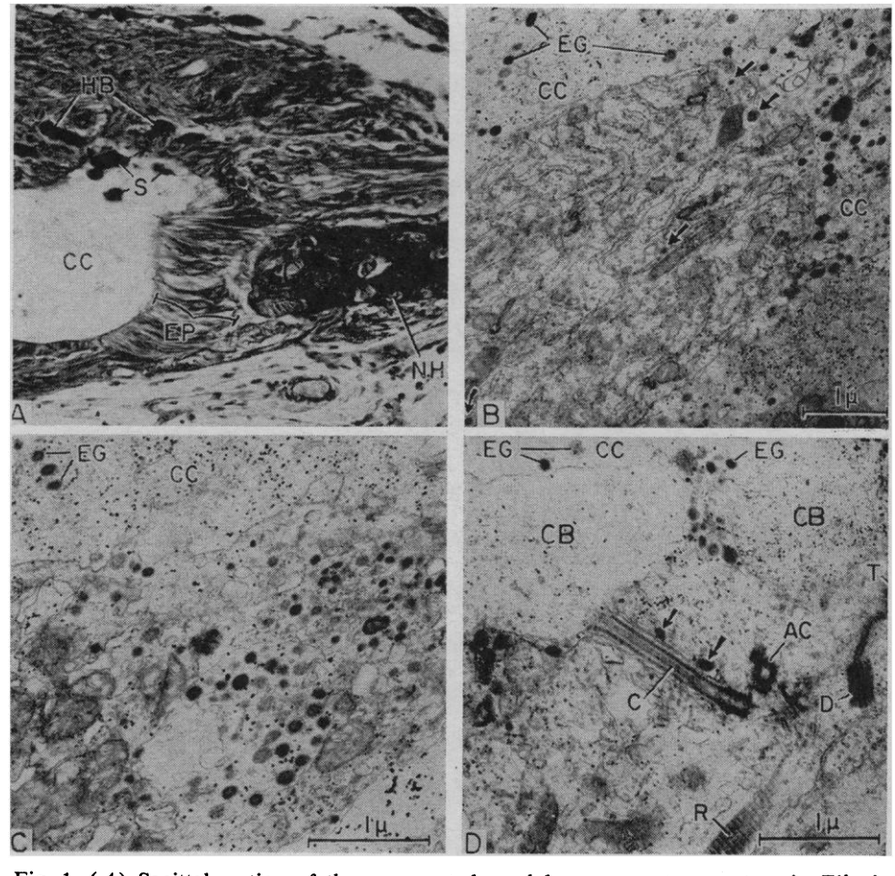

Fig. 1. (*A*) Sagittal section of the regenerated caudal neurosecretory system in *Tilapia mossambica*, 5 months after removal of the caudal peduncle. Secretion (*S*) stained with acid violet is free in the central canal (*CC*). Pile-up of neurosecretory material is evident as Herring bodies (*HB*) associated with processes extending toward the central canal. The regenerated neurohemal area (*NH*) is seen within the spinal cord. *EP*, ependyma (× 230). (*B*) Tip of neurosecretory process extending into the central canal (*CC*) which contains much cellular debris. Arrows indicate elementary neurosecretory granules in the process, which shows well-developed granular endoplasmic reticulum. Elementary granules (*EG*) of variable electron density are free in the cerebrospinal fluid (× 10,800). (*C*) Neurosecretory process with numerous elementary granules bordering the central canal (*CC*). Free elementary granules (*EG*) lie in the cerebrospinal fluid (× 15,800). (*D*) Portions of several cells adjacent to the central canal (*CC*). One cell shows terminal bar (*T*), desmosome (*D*), cilium (*C*), accessory centriole (*AC*), and striated rootlet (*R*), in addition to elementary granules (arrows). Two other (ependymal) cells are represented by almost structureless cytoplasmic blebs (*CB*). *EG*, Elementary granules in lumen (× 15,800).

granules seems to occur without evident disruption of the ciliary apparatus.

Electron micrographs of the central canal area adjacent to the neurosecretory cells in the caudal system of normal *Albula vulpes*, a more "primitive" teleost, also reveal elementary granules free in the cerebrospinal fluid, paralleling the picture seen in regenerating *Tilapia*. Occasionally elementary granules have been found in the central canal of normal *Tilapia*. The finding of elementary neurosecretory granules in extracellular locations in the insect corpus cardiacum has been reported recently by Normann (*9*); here granule extrusion occurs from the axonal processes. A somewhat different mode of discharge of electron-dense material from caudal neurosecretory axon terminals has also been encountered (*10*).

The abundance of release of neurosecretion into the central canal in the regenerating system may represent a physiologically important pathway for neurohormone release, correlated with the reduced extent of the neurohemal area, which in the regenerated system never reaches the same size as in normal fish. It is also possible that secretion into the central canal is a consequence of the failure of some axonal processes (normally projecting to the urophysis) to establish operative physiological contact with capillaries, resulting in a kind of temporary "pile-up" of secretion at the cellular level.

The significance of this alternative pathway in normal fish is difficult to estimate; however, absorption of neurohormone from the cerebrospinal fluid by the choroid plexuses is conceivable, with eventual vascular transport to the target organ(s) at the periphery. Similarly, neurohormonal effects upon other nervous centers can also be visualized.

GUNNAR FRIDBERG
RICHARD S. NISHIOKA
Department of Zoology and
Cancer Research Genetics Laboratory, University of California, Berkeley

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Predominance of Hemoglobin Gower 1 in Early Human Embryonic Development

Abstract. *Hemoglobin Gower 1, the structure of which is thought to be* ϵ_4, *is the predominant hemoglobin in early human embryonic life. This finding suggests that the production of* ϵ*-chains initially exceeds that of other known* (α, β, γ, *and* δ) *chains.*

Starch-gel electrophoresis of hemoglobins (Hb) from small human embryos has shown two embryonic hemoglobins: Hb Gower 1 and Hb Gower 2 (*1–4*).

Hemoglobin Gower 2 consists of four polypeptide chains, two of which on hybridization and fingerprinting are consistent with α-chains. The other two chains of Hb Gower 2 differ from the β-, γ-, and δ-chains by more than one amino acid substitution in their tryptic-digest pattern and have been termed ϵ-chains (*3, 4*). Hybridization experiments with Hb Gower 1 suggest that it may consist entirely of ϵ-chains (*3, 4*).

In previous studies (*1, 2, 4*) Hb Gower 1 and Hb Gower 2 were detected in all embryos less than 85 mm in crown-rump (CR) length (presumably less than 3 months in gestation). The proportions of the embryonic hemoglobins were greater, the smaller, and presumably the younger, the embryo (Table 1). In all the embryos, fetal hemoglobin (Hb F) was the predominant hemoglobin. The smallest embryo examined was 25 mm in CR length.

Table 1. Proportions of various hemoglobins in human embryos.

Crown-rump of embryo (mm)	Hemoglobin			
	Gower 1 (%)	Gower 2 (%)	F (%)	A (%)
*Previously studied embryos**				
63	1.0	0.5	PH†	
50	3.4	1.3	PH	
46	5	1.7	PH	
34	17	16	PH	
25	24	13	PH	
Our embryos‡				
21.3	24	27	36	14
18.2	42	17	25	16
16.3	42	24	21	13

* Ref. (*2*). † Predominant hemoglobin present. ‡ Proportions of various hemoglobins were estimated by transmission densitometry of photographic negatives of the stained starch gels by use of a Spinco model RB Analytrol.

We here report hemoglobin studies in several embryos with CR lengths of 21.3, 18.2, and 16.3 mm. The 21.3- and 18.2-mm specimens were the products of spontaneous abortions. Their respective gestational ages were estimated as 41 days and 39 days (*5*), and their development was judged by the criteria of Streeter (*5*) to be at horizons XX and XIX. Both specimens were in excellent condition and appeared externally normal. Chromosomal studies of fibroblasts could only be done on the 21.3-mm specimen. These studies disclosed a normal female chromosomal complement. The 16.3-mm specimen was obtained from a tubal pregnancy. Its estimated gestational age was 37 ± 1 days (*5*), and developmentally it was at horizon XVIII. This embryo was likewise in excellent condition and appeared externally normal. Serial sections were made of this embryo (but not of the other two embryos) and appeared normal.

Blood (less than 0.1 ml) was obtained by cannulation of the umbilical vessel from each embryo. The erythrocytes were washed with isotonic saline and disrupted with distilled water, and the hemolyzate was examined promptly by starch-gel electrophoresis in a tris-ethylenediamine tetraacetate–borate (*6*) or tris-citrate-borate (*7*) buffer system at *p*H 8.4 These buffer systems allowed clear separation of both embryonic hemoglobins and separation of Hb F from Hb A (adult hemoglobin). The starch gels were stained with benzidene and photo-