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Cerebrospinal fluid-contacting neurons of the central canal and terminal ventricle in various vertebrates*

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Summary. Cerebrospinal fluid (CSF)-contacting neurons were studied by means of electron microscopy in the spinal cord and/or terminal ventricle of the ray, *Raja clavata* (Elasmobranchii), the opossum, *Didelphis virginiana* (Marsupialia), the mouse, *Mus musculus,* and the guinea pig, *Cavia cobaya* (Rodentia).

Dendrites of the CSF-contacting neurons in the spinal cord of the ray penetrate the ependyma of the central canal and form terminals bearing stereocilia. Axons apparently belonging to these neuronal perikarya terminate on the basal lamina of the spinal cord near the fila of the radix ventralis. In the opossum, a representative of metatherian mammals, the dendritic terminals of the CSF-contacting neurons resemble those of the phylogenetically ancient lower vertebrates and are endowed with many stereocilia. In such eutherian mammals as the mouse and the guinea pig, the corresponding stereocilia are usually less developed. There are numerous CSF-contacting neurons in the wall of the terminal ventricle of the mouse.

Since the dendritic terminals of the spinal CSF-contacting neurons resemble those of known sensory cells and the axon terminals on the basal lamina resemble ultrastructurally neurosecretory endings, we suppose that the former are receptive to stimuli exerted by the internal (ventricular) CSF and capable of translating them into a neurosecretory output directed toward the external (subarachnoid) CSF. With their periradicular terminations the axons of the CSF-contacting neurons establish an extended, special site for neurosecretory release along the ventrolateral sulcus of the ray spinal cord.

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As representatives of the caudal component of the CSF-contacting neuronal system the medullospinal CSF-contacting neurons are specialized intra- and subependymal nerve cells located around the central canal of the medulla oblongata, spinal cord and terminal filum (for references, see Vigh and Vigh-Teichmann 1973; Vigh-Teichmann and Vigh 1979). Displaying morphologically similar features from cyclostomes to mammals, each of these nerve cells emits a dendritic process into the central canal; this dendritic terminal is endowed with stereocilia and in most cases also with a single $9 \times 2 + 2$ cilium (cf. Vigh et al. 1979, 1980). Only few stereocilia are found on the CSF-contacting dendrites of the spinal neurons of the lancelet, *Branchiostoma lanceolatum* (Vigh and Vigh-Teichmann 1981). It should be noted that solitary sensory cilia occur on a certain type of bipolar neuron in the radial nerve cord and hyponeuronal nerve of echinoderms, e.g. the starfish, *Pisaster ochraceus, Asterina gibbosa,* and the sea cucumber, *Holothuria forskali* (Vigh and Vigh-Teichmann 1982); the above-mentioned neural structures are devoid of a central cavity. These ciliated central neurons of echinoderms and other invertebrates may represent phylogenetic precursor of the CSF-contacting neurons (cf. Lentz 1968).

From a phylogenetic point of view elasmobranch fishes and primitive mammals, such as the marsupials, are of special interest. To date, in these animals, electron-microscopic data on the structural organization of the CSF-contacting neurons are lacking. Therefore, in the present study emphasis was placed on the spinal CSF-contacting neurons of the ray and the opossum. The ultrastructure of these neurons was compared to that of spinal CSF-contacting neurons in eutherian mammals, i.e., the mouse and the guinea pig.

Materials and methods

Eight rays (*Raja clavata*), 8 opossums (*Didelphis virginiana*), 6 guinea pigs (*Cavia cobaya*), and 5 mice (*Mus musculus*) were examined. These animals were anesthetized with a 5% solution of sodium hexobarbital injected intraperitoneally, and fixed by transcardiac perfusion with 2–5% glutaraldehyde in phosphate buffer (pH 7.3) or in cacodylate buffer of varying osmolarity. The spinal cord of the ray was fixed by immersion. Slices of various segments of the spinal cord and the terminal conus of all species were postfixed in 1% buffered OsO_4, and embedded in Araldite. Ultrathin sections stained with uranyl acetate and lead citrate were examined in a JEM 6C electron microscope. For orientation sections were stained with toluidine blue-azure II.

Results and discussion

The apical dendrite of the spinal CSF-contacting neurons of the ray penetrates into the central canal and forms a terminal endowed with stereocilia. Similar dendritic terminals were previously found in the teleosts *Anguilla*

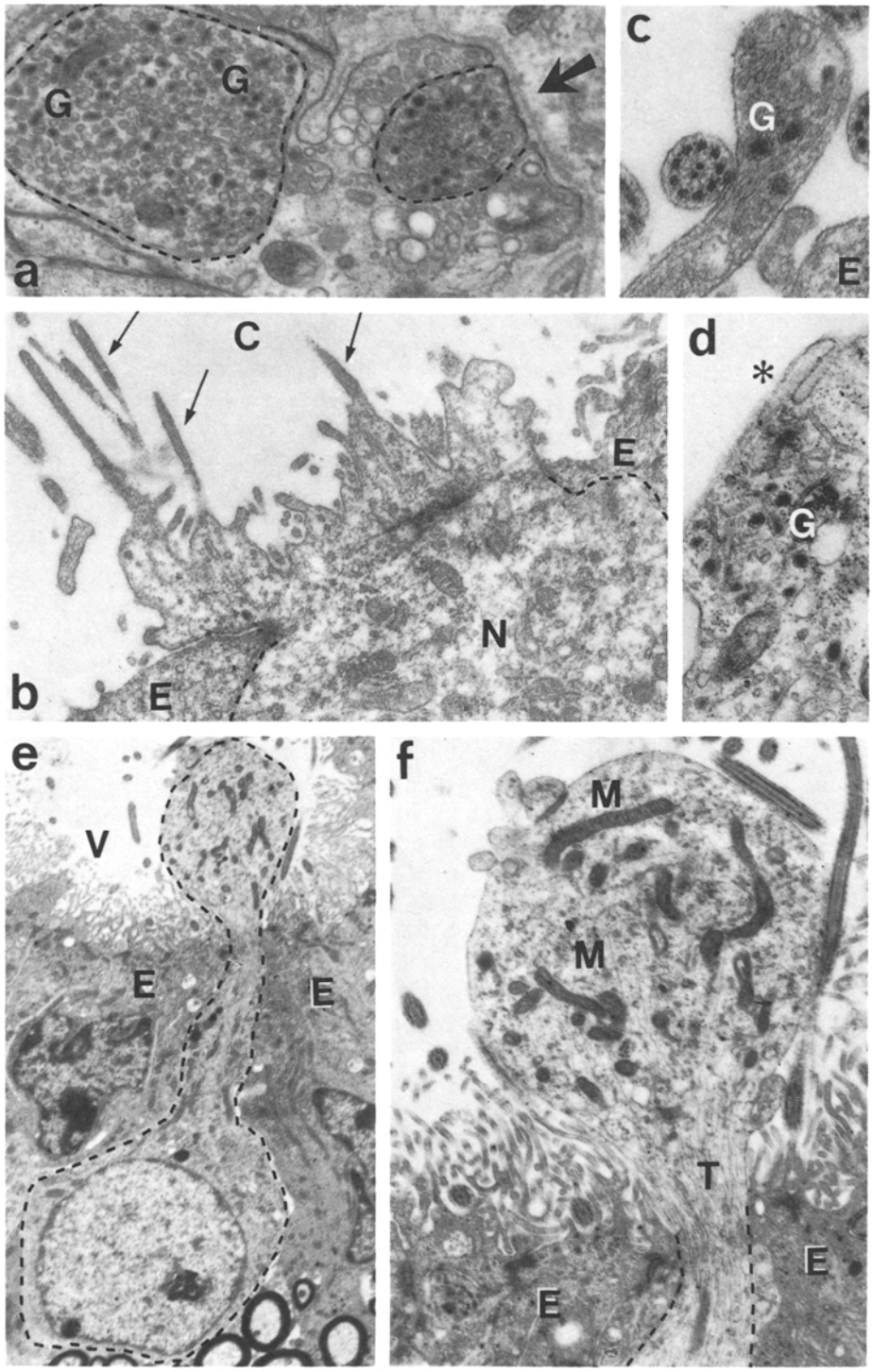
anguilla, Cyprinus carpio, Phoxinus phoxinus, Cichlasoma nigrofasciatum, and *Misgurnus fossilis* (cf. Vigh et al. 1977), and in the lamprey (*Lampetra fluviatilis:* Vigh et al. 1980). Based on the morphological similarity of the stereocilia-bearing terminals of CSF-contacting dendrites to sensory structures of known mechanoreceptors, we suggested that they might respond to changes in the flow (or pressure?) of the CSF, although a chemoreceptive function should also be taken into consideration (Vigh and Vigh-Teichmann 1973; Vigh et al. 1977, 1979).

In the present study of the ray, we observed axon terminals apparently belonging to the perikarya of CSF-contacting neurons at the external surface of the spinal cord. Near the surface, these axons branch and terminate with enlargements on the basal lamina of the neural tissue in a manner known for neurosecretory terminals (Fig. 1a). Similar axon endings were described in the spinal cord and/or terminal filum of the lamprey, several teleosts, amphibians, reptiles, and a number of avian species (cf. Vigh et al. 1977, 1980). Furthermore, we could trace the silver-impregnated axons of the CSF-contacting neurons to the surface of the spinal cord (Vigh et al. 1977).

In the axon terminals of the ray, granular vesicles 90 nm in diameter and synaptic vesicles were found. Such axon endings are most numerous in the ventrolateral sulcus of the spinal cord, but they also occur at the lateral and medial aspect, near the origin of the fila of the radix ventralis. The ventrolateral sulcus appears to represent a release area for these neurosecretory axon terminals; in bilateral arrangement this area extends along the spinal cord.

To date, different bioactive agents (e.g., monoamines, neurohormones, amino acids) have been demonstrated to occur in the CSF (cf. Rodríguez 1976). These might be discharged by neurosecretory axon terminals into the subarachnoid space at the external circumference of the spinal cord. The monoamine content of some of the CSF-contacting neurons (cf. Vigh and Vigh-Teichmann 1971) speaks in favor of this hypothesis. In some species, the neurosecretory axons terminate in juxtaposition with or near the blood vessels facing the basal lamina of the spinal cord (*Pleurodeles waltlii, Xenopus laevis:* Vigh et al. 1977). The neurosecretory material located in these terminals was already suggested to be involved in the control of the vascular circulation of the spinal cord (Vigh et al. 1977; Vigh-Teichmann and Vigh 1979). However, in this respect experimental evidence is still lacking.

In the opossum, a marsupial, considerable numbers of CSF-contacting neurons occur in the wall of the central canal of the spinal cord. In a study on Reissner's fiber in the sacral spinal cord and terminal filum of the Australian possum, *Trichosurus vulpecula,* Tulsi (1982) mentioned the light-microscopic occurrence of CSF-contacting nerve cells. In the Virginian opossum they are larger than ependymal cells and are located mainly intraependymally at the lateral and ventral aspects of the central canal; these neurons contain a relatively large number of granular vesicles 80–120 nm in diameter. The dendrites of these CSF-contacting neurons are short and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form a conspicuous terminal in the CSF. These dendritic endings bear numerous stereocilia resembling those observed in lower vertebrates. The stereocilia (Fig. 1b) are filled with microfilaments and extend radially into the lumen of the central canal. In the latter, nerve fibers were found containing granular vesicles approximately 95 nm in diameter, in addition to synaptic vesicles. Such axons were numerous in the central canal of the medulla oblongata (Fig. 1c). Some of them establish synaptic contacts on the free apical surface of the ependyma, sometimes also on its microvilli, as has also been demonstrated in the third ventricle of the opossum brain (Vigh-Teichmann et al. 1981); this general finding suggests a neural regulatory influence on the functional activity of the ependyma (cf. Vigh-Teichmann and Vigh 1979).

In the guinea pig, the stereocilia on the rather large CSF-contacting dendritic clubs are thin, and relatively less numerous compared to the opossum. Granular vesicles approximately 130 nm in diameter are found in the dendritic CSF-contacting endings and the corresponding perikarya (Fig. 1d). These CSF-contacting neurons exhibit axo-somatic synapses formed by axons containing synaptic and granular vesicles; the latter measure 65 nm in diameter. In analogy to our findings in the opossum and to those of Leonhardt (1976) in the rabbit, supraependymal axons can be demonstrated in the central canal of the spinal cord of the guinea pig. They were also observed at the level of the medullary conus (conus terminalis).

The terminal ventricle of the mouse is a dorso-ventrally elongated cavity at the caudal portion of the medullary conus. Especially in the ventral wall of its lumen numerous CSF-contacting neurons can be found (Fig. 1e). Their CSF-contacting dendrite terminals are round, rather large and exhibit only a few stereocilia. Further, they display numerous mitochondria, a feature also described for similar neuronal elements of the central canal of the rabbit (Leonhardt 1976, 1980). The slender dendrites connecting the CSF-contacting terminals with the corresponding subependymal perikarya exhibit numerous microtubules (Fig. 1f). In accord with the ultrastructural pattern in the opossum and guinea pig, the cell bodies contain granular vesicles approximately 130 nm in diameter and are supplied with axo-so-

Fig. 1a–f. Ultrastructural details of CSF-contacting neurons in the spinal cord and terminal conus of the ray (**a**) and several mammals (**b–f**). **a** Axons (axolemma *dotted*) containing synaptic and granular (dense-core) vesicles (*G*) terminate on the basal lamina (*arrow*) at the surface of the spinal cord of the ray. × 28500. **b** Intraependymal CSF-contacting neuron (*N* plasmalemma *dotted*) and its CSF-contacting dendrite terminal bearing stereocilia (*arrows*) in the spinal cord of the opossum, *Didelphis virginiana*. *E* ependymal cells, *C* lumen of the central canal. × 15700. **c** Axon in the central canal of the medulla oblongata of the opossum. *E* ependyma, *G* granular and synaptic vesicles. × 38400. **d** Portion of a CSF-contacting dendrite terminal in the medulla oblongata of the guinea pig. *Asterisk* stereocilium in the central canal. *G* granular vesicles. × 25900. **e** CSF-contacting neuron (plasmalemma *dotted*) in the terminal ventricle (*V*) of the mouse. *E* ependymal cells. × 5000. **f** Intraependymal dendrite (plasmalemma *dotted*) and its large CSF-contacting dendrite terminal in the terminal ventricle of the mouse. *M* mitochondria, *T* microtubules. × 12200

matic synapses, the presynaptic axons of which are characterized by synaptic and granular vesicles (diameter approximately 65 nm). The synapses on the CSF-contacting neurons may represent modulatory afferents aiding the receptive and/or secretory functions of the neurons. Since the granular vesicles found in the CSF-contacting neuronal perikarya differ from those in the presynaptic axon endings, these afferents appear to originate from an unknown neuronal source.

As already mentioned, the morphological organization and especially the polarization of the spinal CSF-contacting neurons suggest that they might perceive stimuli from the internal (ventricular) CSF in the central canal by means of their stereocilia-bearing CSF-contacting dendritic terminals. In the phylogenetically ancient lancelet the stereocilia are poorly developed (Vigh and Vigh-Teichmann 1981); on the other hand, terminals bearing few stereocilia can be found even in eutherian mammals (guinea pig, mouse). Leonhardt (1976, 1980) described stereocilia-bearing and stereocilia-free terminals in the central canal of one and the same individual (rabbit).

Interestingly, some spinal CSF-contacting neurons contain monoamines, while others are acetylcholinesterase-positive, a pattern apparently speaking in favor of at least two different types of neuronal elements (cf. Vigh and Vigh-Teichmann 1971). It is, however, open to discussion whether the stereocilia-rich or stereocilia-poor dendritic terminals are characteristic of either the monoamine-containing or the acetylcholinesterase-positive CSF-contacting neurons of the spinal cord.

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