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CEREBROSPINAL FLUID CONTACTING NEURONS IN THE REDUCED BRAIN VENTRICULAR SYSTEM OF THE ATLANTIC HAGFISH, *MYXINE GLUTINOSA*

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Cerebrospinal fluid (CSF)-contacting neurons are sensory-type cells sending ciliated dendritic process into the CSF. Some of the prosencephalic CSF-contacting neurons of higher vertebrates were postulated to be chemoreceptors detecting the chemical composition of the CSF, other cells may percieve light as "deep encephalic photoreceptors". In our earlier works, CSF-contacting neurons of the mechanoreceptor-type were described around the central canal of the hagfish spinal cord. It was supposed that perceiving the flow of the CSF they are involved in vasoregulatory mechanisms of the nervous tissue. In the present work, we examined the brain ventricular system of the Atlantic hagfish with special reference to the presence and fine structure of CSF-contacting neurons.

Myxinoids have an ontogenetically reduced brain ventricular system. In the adult hagfish *(Myxine glutinosa)* the lumen of the lateral ventricle is closed, the third ventricle has a preoptic-, infundibular and subhabenular part that are not connected to each other. The choroid plexus is absent. The infundibular part of the third ventricle has a medial hypophyseal recess and, more caudally, a paired lateral recess. We found CSF-contacting neurons in the lower part of the third ventricle, in the preoptic and infundibular recess as well as in the lateral infundibular recesses.

No CSF-contacting neurons were found in the cerebral aqueduct connecting the subhabenular recess to the fourth ventricle. There is a pineal recess and a well-developed subcommissural organ at the rostral end of the aqueduct. Extending from the caudal part of the fourth ventricle in the medulla to the caudal end of the spinal cord, the central canal has a dorsal and ventral part. Dendrites of CSF-contacting neurons are protruding into the ventral lumen. Corroborating the supposed choroid plexus-like function of the wall of the dorsal central canal, segmental vessels reach a thin area on both sides of the ependymal lining.

The perikarya of the CSF-contacting neurons found in the brain ventricles are mainly bipolar and contain granular vesicles of various size. The bulb-like terminal of their ventricular dendrites bears several stereocilia and contains basal bodies as well as mitochondria. Basal bodies emit cilia of the 9 + 0-type. Cilia may arise from the basal body and accessory basal body as well. The axons run ependymofugally and enter – partially cross – the periventricular synaptic zones. No neurohemal terminals similar to those formed by spinal CSF-contacting neurons of higher vertebrates have been found in the hagfish. We suppose that CSF-contacting neurons transform CSF-mediated non-synaptic information taken up by their ventricular dendrites to synaptic one. A light-sensitive role for some (preoptic?) groups of CSF-contacting neurons cannot be excluded.

Keywords: Third ventricle – preoptic recess – Reissner's fiber – central canal – ultrastructure

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INTRODUCTION

Already present in the lancelet and representing the most ancient nerve cells ("protoneurons") of the vertebrate brain, the CSF-contacting neurons are sensory-type cells in the wall of the brain ventricles. They send a ciliated dendritic process into the CSF and show several cytologic similarities to receptor cells [22, 25, 26, 30, 31, 32].

Cyclostomes are the most simple craniate vertebrates possessing already all main brain parts known in higher vertebrates. The central nervous system of the Atlantic hagfish *(Myxine glutinosa)* was already investigated light microscopically by some pioneer researchers [1, 2, 8, 6, 9, 10, 11, 16, 18]. The immunocytochemistry and fine structure of different brain parts was also studied by several authors [3, 4, 5, 33, 34].

The central canal and brain ventricles of the cyclostome Petromyzoniformes possess a well-developed CSF-contacting neuronal system [25]. The brain of adult Myxinoids is characterised by a reduction of the cerebral ventricles [2, 12, 13, 34, 36, 38]. Despite of the reduced ventricular system, CSF-contacting neurons were found in considerable number in the ventral part of the hypothalamic ventricle and its lateral recesses of the hagfish, *Eptatretus burgeri* and *E. stouti* [13, 37]. In our earlier studies we described CSF-contacting neurons around the central canal in the Atlantic hagfish *Myxine glutinosa* [23, 24], but the various brain ventricles have not yet been studied in this respect. Therefore, in the present work, we investigated the fine structure of the brain ventricles of the Atlantic hagfish with special reference to the presence of the CSF-contacting neurons.

MATERIALS AND METHODS

Fifteen Atlantic hagfishes, *Myxine glutinosa*, measuring up to 28 cm in length, were caught at Kristineberg Zoological Station during the months of May and August. The animals were kept for one to two weeks in containers with circulating seawater until fixation. They were anesthetized with MS 222 (Sandoz, Basel) and fixed by transcardial perfusion with 1% glutaraldehyde dissolved in 0.1M phosphate buffer pH 7.2, and adjusted with sodium chloride to varying osmolarities, for 1 h at 4 °C. Due to the lack of choroid plexus in hagfish, fixatives do not penetrate well into the brain ventricular system, therefore, the appropriate conservation of CSF-contacting structures needs longer perfusion-time.

After washing the brain and parts of the spinal cord overnight the material was osmificated, then dehydrated in graded series of ethanol, followed by 1,2 propylenoxyde, and embedded in Araldite. Silver- to gold coloured ultrathin sections collected on copper grids and stained with uranyl acetate and lead citrate were used for electron-microscopy, and 1 micron thick sections stained with toluidine blue for light-microscopic examination.

RESULTS

The brain ventricular system

In the Atlantic hagfish *(Myxine glutinosa)* the lateral ventricle is absent in adult animals. The third ventricle is narrow, it has preoptic-, infundibular- and subhabenular recesses not connected to each other in adult animals (Fig. 1). The infundibular recess has a ventral neurohypophyseal part and a paired lateral evagination more caudally. The ventricles are lined by a ciliated cylidrical ependyma (Fig. 2a–b). Ependymal cilia exhibit a 9 + 2-type tubular arrangement.

There is a well-developed subcommissural organ at te beginning of the cerebral aqueduct. Formed by the secretory material of the organ, the Reissner's fiber runs through the cerebral aqueduct, fourth ventricle and central canal (Fig. 2c). It leaves the cerebroventricular system through the caudal opening of the central canal at the end of the spinal cord. A pineal-recess-like dorsal evagination of the small subhabenular recess was detected rostrally to the subcommissural organ.

The mesencephalic aqueduct connects the subhabenular recess and the fouth ventricle. The latter forms dorsal and lateral enlargements. The central canal of the oblongate medulla and spinal cord has a double lumen (Fig. 2c). The dorsal crescent-like part of the central canal contains a labyrinthic structure formed by processes of ependymal cells connected with cell junctions. On both sides of the dorsal central canal the ependymal lining is thin and the segmental spinal vessels make a close contact with them. In the rostral oblongate medulla the dorsal lumen is enlarged and the labyrinthic ependymal structure is tranformed to a squamous epithelium. In the ventral central canal runs the Reissner's fiber.

CSF-contacting neurons

CSF-contacting neurons were found around the ventral parts of the *third ventricle*, in the lower part of the preoptic and infundibular recess and in the neurohypophyseal and posterior infundibular recesses (Figs 1, 2b, 3a–c). The perikarya of CSF-contacting neuronas are bipolar, some of them are multipolar. The cytoplasm contains granular vesicles of various diameter, rough-surfaced endoplasmic reticulum, Golgi-areas and mitochondria. The axons were traced to the periventricular synaptic zone of the hypothalamus, some of the axons are crossing this zone.

The denrites of CSF-contacting neurons extend to the ventricular lumen and form bulb-like terminals above the ependyma. The terminals contain mitochondria and basal bodies. They bear a sensory cilium with 9 + 0 tubular arrangement and several stereocilia. Some dendrite terminals emit two cilia arising from the basal body and from the accessory basal body.

No CSF-contacting neurons vere detected in the subhabenular recess, mesencephalic aqueduct and in the rostral part of the fourth ventricle.

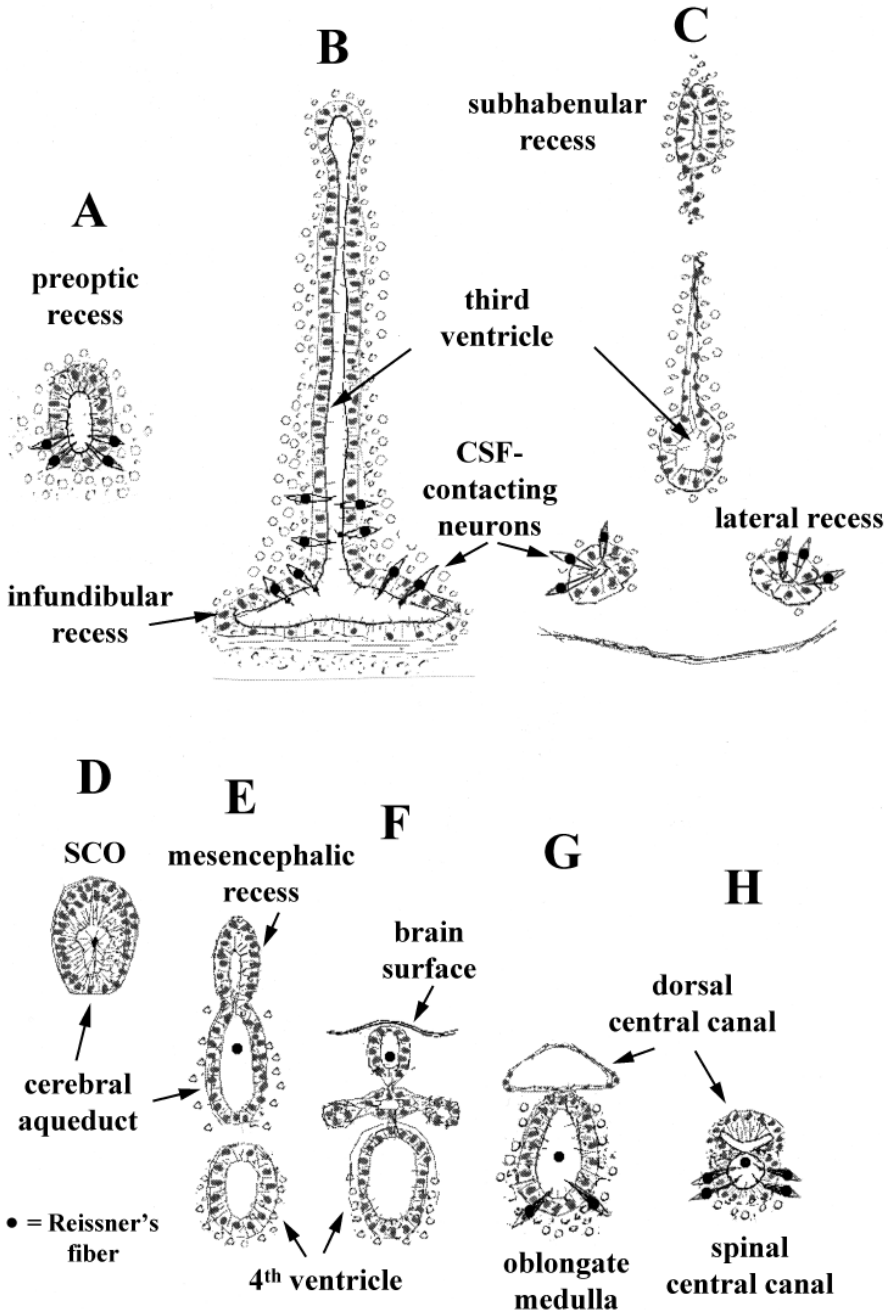

Fig. 1. Schemes of cross-sections of the ventricular system of *Myxine glutinosa*

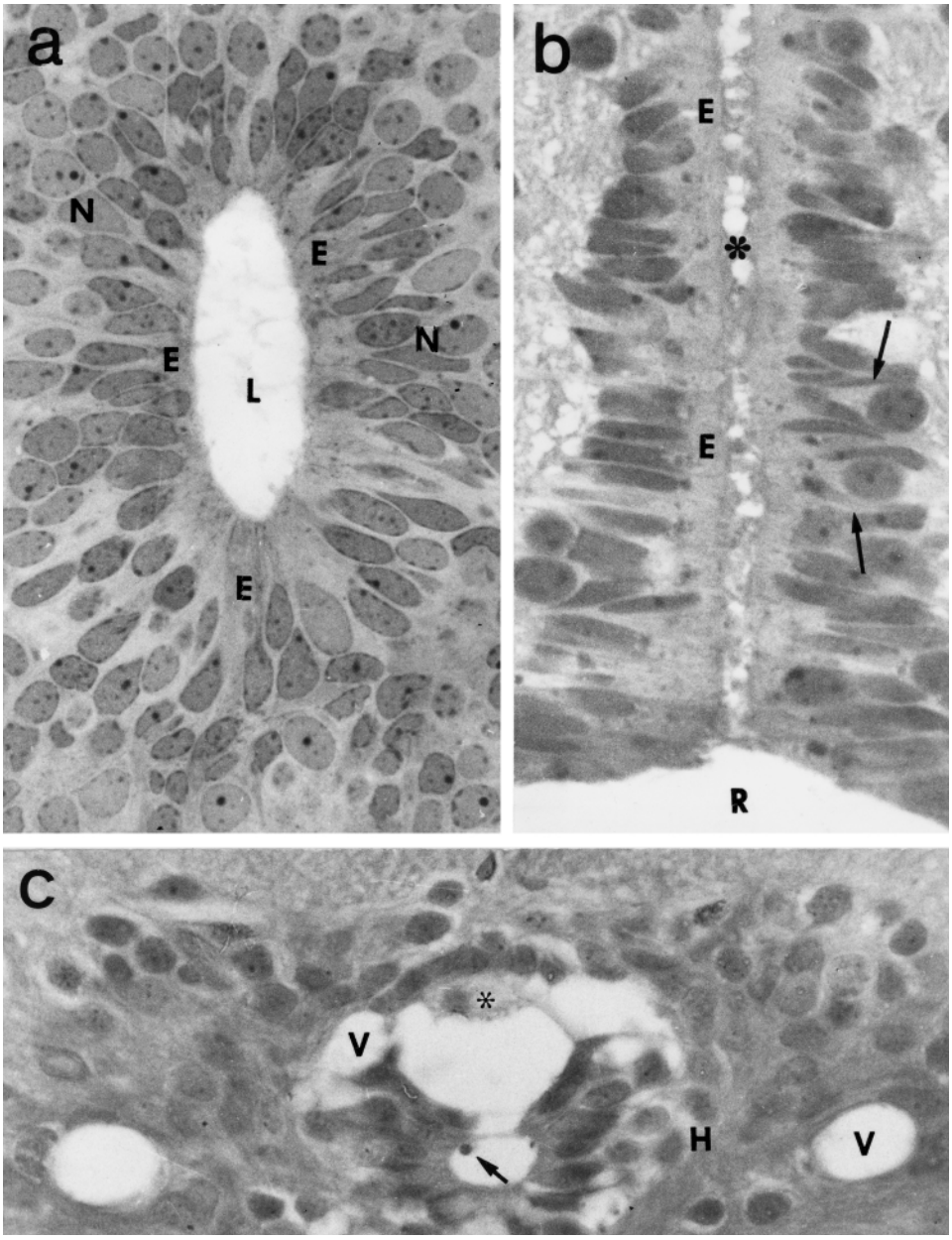

Fig. 2a–c. Parts of the brain ventricles and central canal. **a.** Preoptic recess. E: columnar ependymal cells, L: lumen of the preoptic recess, N: hypendymal neuronal perikarya, ×680. **b.** Narrow part of the third ventricle (asterisk) and the infundibular recess (R). E: ependymal cells, arrows: CSF-contacting neurons, ×830. **c.** Central canal of the transitory zone betveen the oblongate medulla and spinal cord. Arrow: Reissner's fiber in the ventral lumen of the central canal, asterisk: ependymal processes in the dorsal lumen of the central canal, H: small hypendymal neurons,V: vessels, ×450

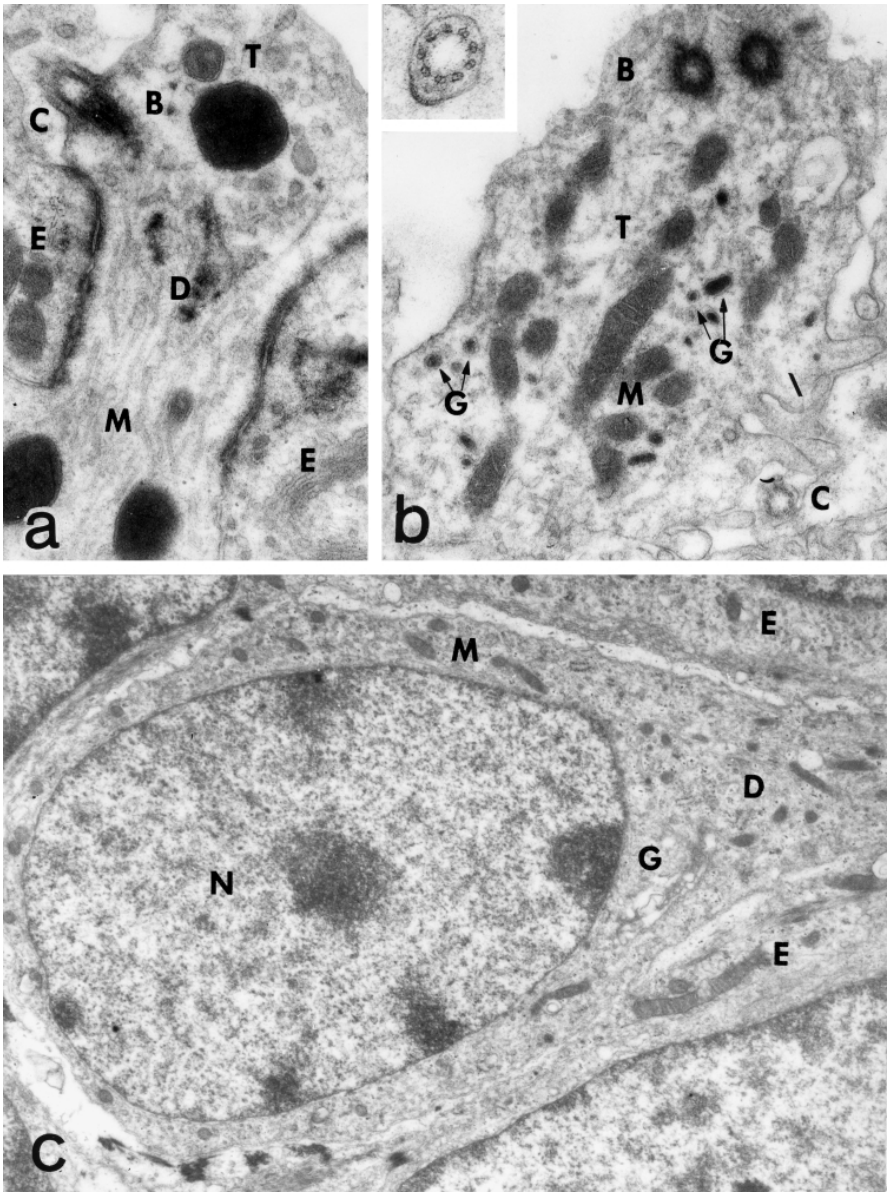

Fig. 3a–c. Fine structure of the CSF-contacting neurons of the third ventricle. **a.** Intraventricular terminal (T) and the distal part of the CSF-contacting dendrite (D). B: basal body, C: cilium, E: ependymal cells, M: microtubules, asterisks: cell junction structures between the dendrite and ependymal cells, ×23,500. **b.** Granular vesicles (G) of various size in the intraventricular dendrite terminal (T). B: basal bodies, C: cilium, M: mitochondria, ×23,500. Inset: 9 + 0 arrangement of the microtubuli in the cilium of a dendrite terminal, ×45,000. **c.** Perikaryon and proximal part of the ventricular dendrite (D) of a CSF-contacting neuron, E: ependymal cells, G: Golgi area, M: mitochondria, N: nucleus, ×9500

Spinal CSF-contacting neurons were found not only around the central canal of the spinal cord but also in the medulla. They are represented by small perikarya at both sides of the ventral part of the lumen of the central canal (Fig. 2c). Their perikarya contain granular vesicles of various size. The CSF-contacting dendrites are connected by cell-juction structures to ependymal cells, and form terminal enlargements in the lumen. The terminals bear several stereocilia and contain two basal bodies, both of them emitting a cilium of 9 + 0 tubular arrangement. The cilia can reach the Reissner's fiber.

The axons of these neurons run to the ventral surface of the nervous tissue, but we could not find any neurohormonal terminals on the basal lamina of the spinal cord as it is known in higher vertebrates. Some axons containing granular vesicles similar to those present in CSF-contacting perikarya were seen among the ventral motor fibers leaving the spinal cord.

DISCUSSION

Our results concerning the brain ventricular system of the hagfish corroborates earlier findings [1, 2] and fournishes new data about the fine structure of its ependymal lining and presence of CSF-contacting neurons. In the inshore and Pacific hagfish *Eptatretus burgeri* and *E. stouti* Kadota [13], Wicht and Northcutt, [36] described CSF-contacting neurons in the ventral part of the hypothalamic ventricle and its lateral recesses. Similar CSF-contacting neurons were found in the present study in *Myxine glutinosa* in the infundibular recess and in the neurohypophyseal and posterior infundibular recesses of the third ventricle. In addition, there is a relatively large preoptic recess in the Atlantic hagfish, where CSF-contacting neurons are equally present. The preoptic recess of the *Myxine* is isolated from the other parts of the third ventricle and from the lateral ventricle being regressed in adult animals [1, 2, 15, 36].

The ventricles are lined by a ciliated columnar ependyma. The kinocilia have 9 + 2-type tubular arrangement. Although myxinoids are considered to be devoid of pineal organ [19, 35, 36], a pineal-recess-like dorsal evagination of the subhabenular recess – the "Epiphysenaustülpung" of Edinger [6] – was found rostral to the subcommissural organ in each of the studied animals. With a similar localization, pineal tissue-like structures were found in *Eptatretus burgeri*, by Ueck and Kobayashi [20].

The secretory subcommissural ependymal organ situated at the beginning of the cerebral aqueduct is well developed in *Myxine.* The Reissner's fiber formed by the secretory material of the organ runs in the lumen of the cerebral aqueduct, fourth ventricle and central canal and finally flows out through a caudal opening of the end of the spinal cord as already described by us earlier [23]. Connecting the ventricular and subarachnoidal CSF-spaces in higher vertebrates, the medial and lateral apertures of the fourth ventricle were not found in the Atlantic hagfish. Therefore, we supposed that it is the Reissner's fiber that keeps open this caudalmost aperture of the central canal thus ensuring a continuous drainage of the CSF [24].

Myxinoids do not have choroid plexus and the CSF is thought to originate from the extracellular fluid of the nervous brain tissue. There is a double central canal in the hagfish and the ependymal cells of its crescent-like dorsal lumen form a labyrinthic structure bordering the CSF. We proposed a CSF-regulating role for this special ependyma [23]. Corroborating its supposed choroid plexus-like function, segmental vessels were found reaching a thin area both sides of the ependymal lining of the dorsal central canal.

Most of the hypothalamic CSF-contacting perikarya are bipolar, their ventricular process is dendrite-like, contains rough endoplasmic reticulum. Entering the ventricle, it is connected to neighbouring ependymal cells by cell-junction structures and terminates in the CSF by a ciliated enlargement bearing several stereocilia and one or two kinocilium. Like photoreceptor cilia, the kinocilia contain microtubuli in a 9 + 0 arrangement, but outer segment-like differentiations were not found on them.

The ependymofugal process of the CSF-contacting neurons is axon-like, contains mitochondria and microtubules. The axons were traced to the periventricular synaptic zone of the hypothalamus, some of them may cross this zone to go to farther targets. According to Kadota [13] axons of hypothalamic CSF-contacting neurons of *Eptatretus* may enter the telencephalon through the lateral part of the diencephalon or via the fasciculus basalis telencephali. We suppose that CSF-contacting neurons are transforming CSF-mediated non-synaptic information taken up by their ventricular dendrites to synaptic one, thus informing hypothalamic or telencephalic centers. The circadian pacemaker of *Eptatretus* was localized to the preoptic nucleus [16, 17]. Since deep encephalic photoreceptors of higher vertebrates are represented by CSF-contacting neurons without outer segments [7, 29] a light-sensitive role for some (preoptic?) groups of CSF-contacting neurons of the hagfish cannot be excluded.

CSF-contacting neurons similar to those present in ventral central canal were found not only in the spinal cord but also in the most caudal part of the fourth ventricle and in the oblongate medulla. They represent the so-called "medullospinal CSF-contacting neurons" of higher vertebrates [23, 25, 27, 28]. In contrast to similar dendrites of other vertebrates that have kinocilia of 9 + 2 tubular arrangement, in the hagfish these cilia are of the 9 + 0-type. In the present study, dendrite terminals bearing two cilia were also found. One of them arises from the basal body and the other from the accessory basal body. The stereocilia occasionally reach the Reissner's fiber running in the ventral lumen of the central canal. Stereocilia are known to be present on dendritic terminals of mechanopreceptors, therefore, we supposed that the movements of the Reissner's fiber influenced by the flow of the CSF may serve as a mechanoreceptor input for the CSF-contacting neurons [21, 23, 25].

Concerning the efferentation of the medullospinal CSF-contacting neurons it must be mentioned that some of them show serotonine immunoreactivity (*Myxine*: [24], *Eptatretus*: [13]). In higher vertebrates serotonin containing axons of similar neurons enter a "centro-superficial tract" running to the surface of the spinal cord and terminating on the superficial basal lamina by forming neurohormonal endings. They also protrude from the surface to meningeal vessels. Serotonin was supposed to be

released from these terminals to influence vessels in order for the regulation of the blood flow in the nervous tissue [24, 25].

In the Atlantic hagfish, neurohormonal nerve terminals were not yet found either on the surface of the spinal cord or near vessels penetrating the nervous tissue. Some fibers containing granular vesicles and being similar to those present in CSF contacting neurons were detected among motor axons leaving the spinal cord. Further studies are in progress to find the exact termination site of the axons of the hypothalamic and medullospinal CSF-contacting neurons of the hagfish.

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