Frontiers of Hormone Research

Cerebrospinal Fluid (CSF) and Peptide Hormones

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1st International Symposium on Cerebrospinal Fluid and
Peptide Hormones, Valdivia, November 24–25, 1980

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111 figures and 11 tables, 1982

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Frontiers of Hormone Research

Vol. 8: ACTH and LPH in Health and Disease. Tj. B. van Wimersma Greidanus, Utrecht and L. H. Rees, London (eds.)
X + 210 p., 25 fig., 14 tab., 1981. ISBN 3–8055–1977-X

National Library of Medicine, Cataloging in Publication
Cerebrospinal fluid (CSF) and peptide hormones
Volume editors, E. M. Rodríguez, Tj. B. van Wimersma Greidanus. – Basel, New York, Karger, 1982.
(Frontiers of hormone research; v. 9)
Proceedings of an international symposium held at the Universidad Austral de Chile in November 1980
1. Cerebrospinal Fluid – physiology – congresses 2. Endocrinology – congresses 3. Neurosecretion – congresses 4. Peptides – physiology – congresses I. Rodríguez, E. M. (Esteban M.) II. Wimersma Greidanus, Tj. B. van III. Series
Wl FR946F v.9/WL 203 C413 1980
ISBN 3-8055-2823-X

Printed in Switzerland by F. Reinhardt AG, Basel
ISBN 3-8055-2823-X

Frontiers of Hormone Research

Vol. 9

Series Editor
Tj. B. van Wimersma Greidanus, Utrecht

S. Karger · Basel · München · Paris · London · New York · Sydney

Front. Horm. Res., vol. 9, pp. VII–VIII (Karger, Basel 1982)

Introduction

The story of the relationship between the cerebrospinal fluid (CSF) and the neural control of endocrine function started early in this century. Thus, during the first three decades Spanish and German histologists published several papers describing intraventricular nerve fibers. Simultaneously, British and French authors postulated that the hormones of the neural lobe and pars intermedia of the hypophysis were principally released into the CSF. A second epoch, between 1920 and 1940, is clearly characterized by numerous controversial publications concerned with the presence or absence in the CSF of neurohypophysial principles. Perhaps the best defined and profitable epoch started in 1949 when *Bargmann* published his classical paper describing the hypothalamic neurosecretory system. In this very first paper *Bargmann* reported the existence of neurosecretory fibers reaching the ventricular cavity. This and many other publications that followed encouraged pharmacologists to reinvestigate the probable existence in the CSF of neurohypophysial hormones. During the 1970s not only neurohypophysial hormones have been shown to be present in the CSF but also all the other pituitary hormones and those of the peripheral glands. Brain substances such as the neurotransmitters and peptides are also present in the CSF. Many authors, some of whom are here today, dedicated their efforts to study the regional specializations of the ventricular walls trying to clarify the functional relationship between the CSF and neuroendocrine mechanisms.

The recent invasion of the central nervous system by neuroendocrinologists pushed by the driven force of immunocytochemistry, the findings of behavioural effects of pituitary hormones, the fascinating possibility that there may be neurons capable of secreting adenohypophysial

hormones, such as ACTH or prolactin, the probable involvement of neuropeptides in neurotransmission might all be taken as signs that a new period of research in neurobiology is starting. It is hoped that this epoch would be characterized by a more integrative view. In this context the CSF and the related structures appear as a fruitful meeting point for endocrinologists, neurochemists, behavioural scientists and clinical investigators.

The 25th Anniversary of this University provided a good reason for an attempt to gather together leading scientists concerned with different aspects of the CSF, who usually work in parallel but are not always cognizant of likely relationships between divergent functional aspects.

I have found great pleasure and satisfaction in organizing this international symposium. It has allowed me to reinforce the relations with old friends, and I do hope it will result in new and friendly relationships not only for myself but for all the participants. I wish that all of you coming from abroad will have the same experience I have had during the 4 years I have spent in this remote and beautiful spot of the world, that is to enjoy the warm Chilean hospitality.

This meeting would not have been possible without the dedicated cooperation of many colleagues and the generous financial support of some institutions and people of good will. The Universidad Austral de Chile not only provided the main financial support, but also the appropriate environment for this symposium. Further financial support was obtained from the Chilean Biological Society, Sandoz (Chile), and the following people of Valdivia: Mr. *Victor Kunstmann,* Mr. *Guillermo Michaelis,* Mr. *Esteban Fried* and Mr. *Adolfo Kaehni.* I also want to acknowledge the personal financial efforts of all our invited participants who have had to defray on their own an important part of their travelling expenses.

It is not possible to acknowledge the help of all those who gave so generously of their time to assist me to organize the symposium. Three, however, must be mentioned, for I regard them as the fluid currents of the Valdivian ventricular system: the two secretaries of the Institute of Histology and Pathology, Miss *Rosario Andrade* and Mrs. *Elizabeth Santibáñez,* and Prof. *Italo Caorsi,* Head of the Institute who not only encouraged me constantly but also took care of the finances. Last, but not least, my sincere thanks to *Tjeerd van Wimersma Greidanus,* who promptly and gladly accepted my invitation to organize this meeting together.

E.M. Rodríguez

Contents

Front. Horm. Res., vol. 9, pp. 1–3 (Karger, Basel 1982)

Opening Remarks

It gives me great pleasure to provide the opening remarks for this symposium and add to the welcome expressed by Dr. *Pessot* and *Rodríguez*. This meeting has been organized by Drs. *Rodriguez* and *van Wimersma Greidanus* for the purpose of having a group of eminent neurobiologists discuss their views on the subject of peptide hormones in the cerebrospinal fluid and examine the hypothesis that they are exercising some physiological role in this medium of the brain.

The possibility that the cerebrospinal fluid may be involved in some central neuroendocrine processes has been suggested by numerous investigators during the past 20 years but the supporting evidence has to date not been so convincing as to incorporate this idea into our general concepts of neuroendocrinology. The original basic tenants of neural control of the pituitary included specific rules on how hypothalamic hormones are delivered to the pituitary gland and these did not entertain other possibilities such as involvement of the cerebrospinal fluid. These initial rules governing the interactions of the hypothalamo-pituitary axis were sound and offered a framework on which a great deal of our neuroendocrine knowledge has been constructed. However, new information in recent years may require an enlargement of our views of these brain hormones with respect to where they are delivered, how they are delivered and what functions they perform.

The list of neuroendocrine-related central and peripheral neuropeptide systems grow progressively longer and their organization more complex. Many of the hypothalamic peptides thought to be exclusively associated with median eminence and pituitary function are now known to be delivered to other areas of the brain. Almost all of these peptides have been

detected in the cerebrospinal fluid. Other factors such as substance P, the enkephalins and the opiocortin peptides, initially studied in relation to descending pain control mechanisms, are now recognized to have widespread distribution in the brain and are clearly related to neuroendocrine functions. Many of these peptides are present in cerebrospinal fluid also. In addition hormones of peripheral origin are present in brain and cerebrospinal fluid as well.

The functional significance of peptides in the cerebrospinal fluid must be considered in the light of the basic anatomy of the cerebrospinal fluid system. Nature saw fit to provide tissue of the central nervous system with a highly specialized environment that is sheltered almost completely from the blood and maintained at very precise chemical composition. The cerebrospinal fluid system consists of two compartments: (a) the interstitial space of brain parenchyma, and (b) the macroscopic cavities. These internal and external spaces are in direct anatomical continuity. Of fundamental importance to understanding cerebrospinal fluid physiology and central peptide endocrinology will be the development of final proof that cerebrospinal fluid may in part be formed by bulk flow of extracellular fluid into the cerebrospinal fluid cavities. In order to maintain chemical composition of the extracellular fluid it is obvious that products of neuronal and glial activity must be continuously removed. Cerebrospinal fluid is not a mere cushion for the brain. It is, in fact, a dynamic medium in constant circulation in continuous formation and absorption. It is, as Harvey Cushing recognized 50 years ago, a modified lymphatic system of the brain.

The presence of neuropeptides in cerebrospinal fluid presents two fundamental questions, namely how do they enter this compartment and what are they doing there? Neuropeptides such as vasopressin or sleep factor, whose origins are in the brain, may be delivered to the ventricular cavities for the purpose of being carried by cerebrospinal flow and acting at specific distance sites or by acting in a neuromodulatory role in broad regions of the brain. In this situation hormone concentration in the cerebrospinal fluid might be expected to be highest at the site of its release. Alternately, peptides may be released from nerve terminals in the brain parenchyma and after exercising their effector roles, be washed away in the extracellular fluid to the ventricles. The concentration in cerebrospinal fluid would be highest in the region where the peptide has acted and thus be a reflector of neuronal activity of that particular site in the brain. These neuropeptides may be enzymatically inactivated or they may remain bio-

logically active. Implicit in these considerations is the probability that the concentration of neuropeptides will vary regionally in the cerebrospinal fluid cavities. It follows from this that the site from which a sample of cerebrospinal fluid is obtained will influence considerably its value in analyzing the events transpiring at the sites of release or action of any given peptide.

The presence in cerebrospinal fluid of pituitary, gastrointestinal and other peptides of peripheral origin opens vistas of considerable interest. In addition to the question of their functions in the brain, their mode of entry into this protected environment must also be described. Circumventricular organs such as the median eminence, the organum vasculosum and the subfornical organ have been recognized as specialized windows of the brain, where there may be free exchange between blood and cerebrospinal fluid. Are these the check points of entry? Are these windows selective and specialized?

These and other questions are the subject of this symposium. I am sure that this morning there is no single statement which unifies all our views and all the facts. By the time we leave this lovely city of Valdivia, however, I believe that the participants of this meeting may agree upon the fact that many peptides and a variety of other factors are indeed present in cerebrospinal fluid and that in some cases their presence here is not an irrelevant consequence of brain metabolism.

Karl M. Knigge

Front. Horm. Res., vol. 9, pp. 4–14 (Karger, Basel 1982)

Secretion and Circulation of the Cerebrospinal Fluid[1]

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Introduction

The cerebrospinal fluid (CSF) occupies the ventricles, canals and spaces surrounding the central nervous system (CNS). Although the CSF provides physical support and protection for the CNS, it has become widely appreciated that the CSF also provides a controlled chemical environment for the neurones and glia of the CNS. The CSF is in relatively free communication with the extracellular fluids of the brain parenchyma across the ependymal and pial membranes covering the external and internal surfaces of the brain. While the CSF resembles an ultrafiltrate of plasma, the CSF composition remains fairly constant despite wide fluctuations in plasma, e.g. the CSF K remains between 2 and 4 mEq/l even when plasma K varies between 1 and 12 mEq/l. Disturbances in CSF composition have profound influences on normal brain function and this is illustrated by the 4-fold increase in respiratory ventilation rates when the CSF pH is lowered by 0.05 pH units. Finally, in recent years it has also become evident that the CSF plays an important role in neuroendocrinology [14]: First, the CSF may be involved in the distribution of hormones within the brain, e.g. in the putative feedback mechanisms for neurohypophysical hormones; second, the CSF may provide the pathway for melatonin transport between the pineal gland and the hypothalamus; and third, the CSF may provide the route for the clearance of hormones and their metabolic products from the brain to the blood. Thus, it is appropriate in a symposium on peptide hormones in the CSF to review some

[1] This work was supported by grants from the USPHS (NS 09666).

aspects of CSF physiology. I will review the secretion and circulation of the CSF in one model system – the frog brain. Readers are referred to the monographs by *Davson* [3], *Milhorat* [7], *Rapoport* [13] and *Bradbury* [2] for general discussion of the fluids of the brain, and to the reviews by *Wright* [24, 25] for specific consideration of CSF transport processes.

CSF Secretion and Circulation

In man the total volume of the CSF is about 140 ml and about 20% of the volume is contained within the lateral, third and fourth ventricles. CSF is produced at a rate of 0.35 ml/min, and it has been estimated that about 65% of the fluid is secreted in the ventricles by the choroid plexuses. Extrachoroidal sources of CSF may include the metabolic production of water from glucose, fluid movement across the blood-brain barrier (cerebral capillaries) and the secretion of fluid by the arachnoid membrane. Fluid produced in the ventricles causes bulk flow of CSF out of the fourth ventricle (through the foramina of Luschke and Magendie) into the cisterna and over the external surface of the brain in the subarachnoid spaces. Eventually, the CSF drains into the blood in the dural sinuses across the arachnoid villi. Circulation of CSF is poorly understood but, apart from flow in response to fluid secretion, pressure changes associated with variations in cardiac and respiratory pulses, postural movements, coughing and sneezing are thought to promote mixing and bulk flow. Within the ventricles and canals the ciliated ependymal cells cause CSF to flow over the internal surface of the brain, but it is unlikely that the cilia contribute to bulk flow in large animals.

Table I shows the rates of CSF secretion by the choroid plexuses in various species. The rates are comparable in cold blooded animals, sharks and frogs, and, given the differences in temperature and a reasonable activation energy ($\sim$20 kcal/mol) for the transport processes involved, these rates are comparable to those recorded in mammals. In an adult bullfrog (500 g) the choroid plexuses weigh 5 mg, and so the total CSF secretion amounts to 0.25 µl/min.

Analysis of freshly secreted CSF collected from the surface of the frog plexus [23] shows that the fluid is hypertonic to plasma (236 vs 205 mosm/l) and this is largely due to an increase in sodium concentration (125 vs 110 mEq/l in plasma). Ouabain (1×10^{-4} *M*) in the ventricle blocks CSF production and eliminates the concentration differences between

Table I. Rates of CSF Secretion

	$\mu l\ min^{-1}\ mg^{-1}$
Man	0.25
Sheep	0.13
Cat	0.4
Shark	0.05
Frog	0.07

Rates of secretion are expressed per mg of wet weight of choroid plexus. The experiments with shark and frog were carried out at room temperature, while the mammalian experiments were performed at 37 °C. Data from *Davson* [3] and *Wright* et al. [23].

CSF and plasma. These studies suggest that active sodium transport is directly responsible for CSF secretion in the frog.

Sodium is actively transported across the frog choroid plexus from blood to CSF in two steps (fig. 1). In the first, sodium enters the choroidal ependyma from plasma across the basolateral membrane down its electrochemical potential gradient. The intracellular sodium concentration is about 10 mEq/l and the cell interior is –45 mV with respect to the extracellular fluids, i.e. the electrochemical driving force for sodium entry into the cell is greater than 150 mV. There is some evidence that Na enters the cell by Na/H exchange, and it is also possible that NaCl cotransport contributes as in some epithelia (gallbladder and intestine). In the case of Na/H exchange the intracellular protons are supplied by the dissociation of H_2CO_3 which can enter the cell by non-ionic diffusion. Carbonic acid is also formed by the hydration of CO_2 mediated by the enzyme carbonic anhydrase. This explains why carbonic anhydrase inhibitors, e.g. Diamox, reduce the rate of CSF secretion in mammals.

The second stage of Na transport across the choroidal ependyma is the uphill movement of Na out of the cell across the brush border membrane into the ventricle. Uphill Na transport is mediated by Na/K pumps. Evidence for the location of Na/K pumps in the brush border includes: (1) ^{3}H-ouabain binding and autoradiography [12]; (2) the active accumulation of K within the plexus across the brush border membrane by a sodium-dependent, ouabain-sensitive process [24]; and (3) the abrupt depolarization of the brush border membrane potential by ouabain [28].

The mechanism of active sodium and potassium transport across the brush border membrane has been studied in considerable detail using both

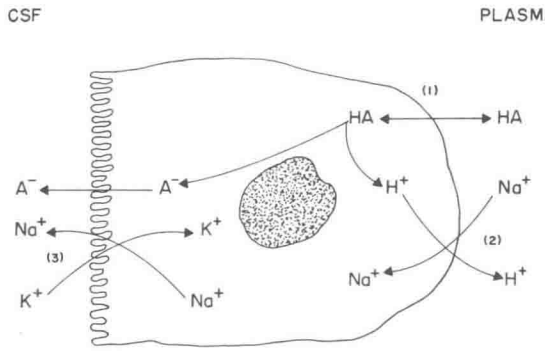

Fig. 1. A simple model for sodium secretion across the frog choroid plexus. In this cheme sodium is transported across the tissue in two steps: the first is where Na enters the choroidal ependymal cell from plasma via a sodium/proton exchange on the basolateral plasma membrane (2); the second is where sodium is transported out of the cell into the CSF by a Na/K exchange pump in the brush-border membrane (3). The intracellular supply of protons for Na/H exchange (2) is generated by the dissociation of buffers (HA) that enter the cell by non-ionic diffusion (1). The most important buffer is H_2CO_3 which can also enter the cell via diffusion of CO_2 and the subsequent hydration of CO_2 in the cell mediated by carbonic anhydrase. The buffer anions (A^- or HCO_3) then accompany Na^+ as it is pumped out of the cell across the brush border (3). The Na/K pump is electrogenic owing to the exchange of 3 Na^+ ions for 2 K^+ ions during each cycle of the pump. Most of the K pumped into the cell returns to the CSF across the brush-border membrane, but a small fraction escapes to the blood across the basolateral membrane to account for a small net transport of K from CSF to blood [from ref. 22].

tracer and electrophysiological techniques [16, 24, 27, 28]. Both types of experiment are consistent with an electrogenic pump which transports 2 K ions into the cell for every three sodium ions pumped out. There are 10×10^6 pumps per cell, as estimated from ^{3}H-ouabain-binding studies, and each pump turns over approximately 10 times/s. In the steady state the electrical potential difference across the brush border membrane is –45 mV, cell interior negative: the electrogenic pump contributes 10 mV to the potential difference. The potassium transported by the pump leaks back into the CSF owing to the high permeability of the brush border membrane, except for a small percentage which leaks out across the basolateral membrane to produce a small net flux of K from CSF to blood.

Analysis of the unidirectional ion fluxes across the plexus [18] and the composition of the fluid secreted by the frog plexus [23] suggest that a mixture of NaCl and $NaHCO_3$ is transported across the tissue, i.e. Cl and

HCO_3 ions accompany the active transport of sodium across the choroidal epithelium. The nature of the coupling between anion and sodium transport across the brush border membrane is still not clear, but it could be simply related to the anion electrochemical potential gradients and the permeability of the brush border membrane. This possibility is under investigation.

Little is known about the regulation of CSF secretion by the choroid plexus. However, there is histochemical and ultrastructural evidence that the choroidal ependyma is innervated by adrenergic, cholinergic and peptidergic (VIP) fibers [6]. Sympathetic denervation of the rabbit choroid plexus increased the rate of CSF production 33%, whereas bilateral electrical stimulation of the superior cervical ganglia decreased CSF production more than 30%. Additional experiments also showed that the intraventricular infusion of norepinephrine caused a 40% reduction in secretion. The adrenergic innervation of the plexus has been further studied by monitoring the properties of adenylate cyclase in the tissue [8, 15].

These experiments lead to the conclusion that the plexus contains β_2-adrenergic receptors and that these may be involved in the secretory activity of the epithelium. Inconsistencies arise because cholera toxin, which also stimulates adenylate cyclase in the plexus, has been shown to increase CSF production by a factor of five [4]. Further work is needed to clarify this important aspect of CSF physiology.

Circulation of the CSF

In our group we have been interested in the role of cilia in the circulation of CSF within the ventricles and canals of the brain. Examination of the ependymal surfaces of the brain by scanning electron microscopy has established the distribution and density of cilia most clearly. In the frog we have reported [10] that about 20–40 cilia, some 20 μm long, project from each cell of the plexus and ependyma of the fourth ventricle. Although the distribution of cilia in the plexuses is quite variable from species to species, a high density of ependymal cilia is found in all species examined. Cilia are missing only in specialized regions of the ependyma, e.g. the periventricular organs and median eminence.

Cilia in the frog beat at a frequency of 5–20 c/s and it appears that the frequency is related to the activity of adenylate cyclase; cyclic AMP and theophylline both accelerate the ciliary beat up to a maximum of 30 c/s.