WAYS OF TRANSPORT OF THE SECRETION FROM THE HYPOTHALAMIC CENTERS INTO THE NEUROHYPOPHYSIS

A. A. Voitkevich

Department of Histology and Embryology (Director, Corresponding Member, Academy of Medical Sciences USSR, Prof. A. A. Voitkevich) Voronezh Medical School
(Presented by Active Member, Academy of Medical Sciences, USSR N. A. Kraevskii
(Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 99-103, July, 1961
Original article submitted October 12, 1960

The conception according to which a neurosecretory product is transported through the supraoptico-hypophyseal tract in mammals and through its analogue in lower vertebrates—the preoptico-hypophyseal tract from the hypothalamus to the pituitary gland—is based on the fact that along the nerve fibers a Gomori-positive substance can be found, and on the results following the transsection of these fibers [7,10,11,12,16,18]. The neurosecretory substance, which is transported over numerous nerve fibers, is condensed in the region of the median eminence of the infundibulum or in the posterior lobe of the pituitary gland, round the endothelial walls of the numerous blood vessels. These vessels, taken as a whole, represent two portal systems which secure an independent supply of the modified secretion to the anterior and intermediate lobe of the pituitary gland. [4,6,8,9,18]. Part of the nerve fibers from the posterior lobe enter the intermediate lobe of the pituitary gland; in a similar manner nerve fibers from the median eminence and the infundibulum enter, together with the blood vessels, the tuberal part [5,6,19]. According to recent findings in mammals a great number of nerve fibers connect the wall of the infundibulum with the meninges embracing the pituitary gland and its tuberal lobe [15]. Such nerve fibers are absent from the anterior lobe of the pituitary gland.

In recent years an increasing number of authors reported that the hypothalamo-hypophyseal tract does not represent the only way in which the neurosecretory substance can be transported. It could be established that the product of neurosecretion is conveyed toward the oral cavity [5,15]; others reported its transfer into the cerebrospinal fluid of the IIIrd ventricle [8,12,13]. The rich vascularization of the hypothalamic nuclei, the close contact between individual neurons and the endothelium and the numerous capillaries emphasize the possibility of a direct outflow of the neurosecretory substance with the blood. The discrepancy between the quantity of secretion found in the posterior lobe of the pituitary gland in tadpoles and its low concentration in various parts of the preoptico-hypophyseal tract suggests that the neurosecretory substance may be transported in other ways as well [1].

In the present paper we quote new findings concerning various ways of transport of the neurosecretory substance and in particular we discuss its possible transport by wandering cells.

METHOD

Anurans of various age, white rats and puppies served as objects of investigation. Amphibia are interesting in view of the fact that from a phylogenetical point of view their hypophyseal system represents the earliest stage of the formation of the organization so characteristic for all higher vertebrates which is developed to perfection in mammals. The material was fixed in Bouin's liquid, fixative of Shabadash, Zenker-formol and 10% neutral formalin; the material was embedded in paraffin; sagittal and transversal sections were stained with Gomori's hematoxylin with phloxin, aldehyde-fuchsin, asano (according to Heidenhain), and by the method of Halmy as modified by Dyban, and the P.A.S. reaction for polysaccharides was used. Also, the sections were treated by the method of Brachet to demonstrate nucleic acids.

RESULTS

Study of the preoptic region in Rana esculenta and Rana temporaria, the spadefoot toad (Pelobates fucus) and the green toad (Bufo viridis) revealed that the structure of the neurosecretory substance is different [2,3] in various species with regard to the process of secretion and formation. In the toad granules, and in the frog droplets, of varying diameter predominate; the path of outflow, however, seems to be identical (see Figs. a,b). The preoptic nuclei, which are large and extend over a considerable area, are arranged symmetrically in the walls of the preoptic fossa and can be divided into two parts: the lower elongated ventromedial and the upper, more compact, dorsolateral part. In the lower part of the nucleus the neurosecretory substance, which gives a positive staining reaction, extends along the axons or the ependymal processes, in the shape of granules or spheroid formations of varying diameter, mainly toward the site opposite the ependyma. The latter is here formed by densely arranged cuboidal cells between which small fragments or droplets of neurosecretory substance can only rarely be seen.

The more dorsally situated ependyma which covers the compact part of the preoptic nuclei has a quite different appearance (see Fig. c): here the cells are very tall, of typical bipolar character (see Figs. d, e). Along the processes of the ependymal cells, between the cell bodies and on the apical surface, fragments and droplets as well as dilated processes of the neurons containing a granular secretion can be seen (see Figs. d, e). In this region the neurosecretory cells are of larger size (see Fig. c). Part of the secretion which is formed by these cells enters the surrounding nervous tissue. The bulk of the neurosecretory substance, however, moves here toward the side of the ependyma (see Figs. b, d). On sections cut in different planes and stained with the aid of various methods a great number of droplets can be seen in the immediate vicinity of the ependyma. In this region we found small cells with a spheroid nucleus and a dense zone of compact cytoplasm which gave a brilliant color when stained with aldehyde-fuchsin (see Fig. e, arrow). The tinctorial properties of the cytoplasm in these cells are similar to those of the neurosecretory substance. These cells in the ependymal region do not yet display the dense deep color which is typical for the cells found in the more caudal parts of the brain. This fact suggests that the neurosecretory substance accumulates gradually in the cells. Similar cells can be found in various parts of the brain but they are not found anterior to the preoptic region. Their character resembles that of the reticulopituicytes described by Romeis [17]. They are freely wandering and can be found on the tall-celled ependyma in the region of the preoptic nuclei, on the ependyma of the IIIrd ventricle, in the infundibular region, in the capillary lumen of the brain stem and in the walls of the infundibulum between the ependymal cells of the median eminence and on its base as well as between the cells of the intermediate lobe of the pituitary gland (see Figs. f,g,h,i,k,l,m). Such cells are never found in the anterior lobe of the hypophysis; these wandering cells are not "mastcells": they are of different size and lack the characteristic granulation.

We extirpated the preoptic part of the hypothalamus in tadpoles and young yearlings of R. esculenta and the spadefoot toad Pelobates fucus. This operation was followed by a rapid reduction of the neurohypophysis. The region of the lesion and also the more caudally situated parts of the brain were investigated histologically. We did not in all operations achieve the complete resection of the preoptic fossa down to the optic chiasma or produce in all cases necrosis of the hypothalamic nuclei. Under these conditions the number of wandering cells increased considerably in the immediate vicinity of the lesion as well as in the cavity of the infundibulum; the cytoplasm of these cells contained large quantities of neurosecretory substance which gave a positive P.A.S. reaction.

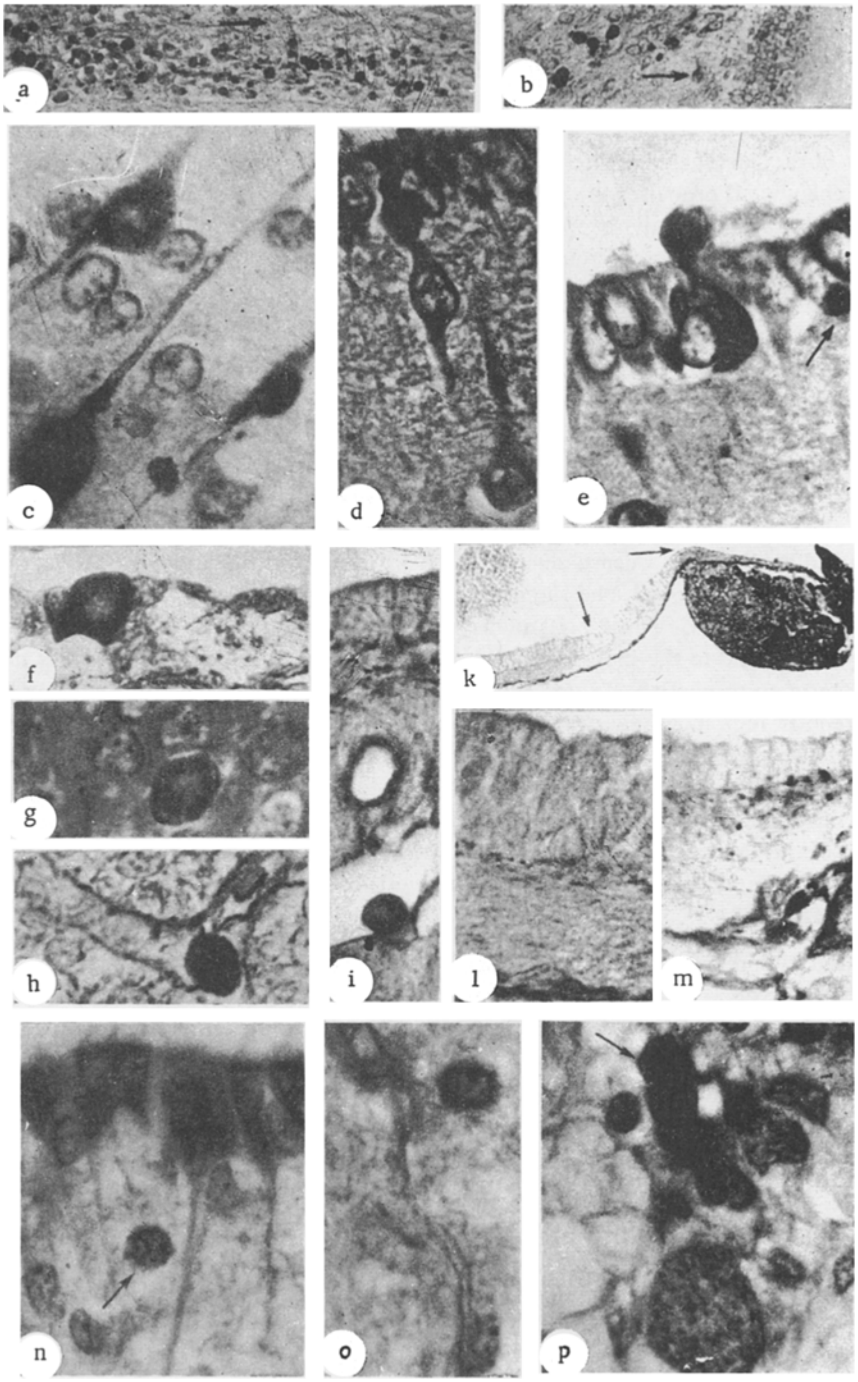
The transport of the neurosecretory substance by wandering cells does not represent a phenomenon restricted only to lower vertebrates, as it occurs also in mammals. Figures n, o and p show intensively staining wandering cells in the region of the median eminence, part of which migrate toward the underlying tuberal lobe of the pituitary gland. As we said above with regard to amphibia these cells acquire a dendritic shape when entering the nervous tissue; when they are situated on the ependyma surface or within a blood vessel or in the cerebrospinal fluid, however, they are of round shape. Such cells occur in great numbers also in the proximal part of the neurohypophysis of young puppies (see Figs. o,p) and frequently swell up to take on the shape of large spheroid formations filled with fine granules.

The data discussed above show that along with nerve fibers wandering cells take part in the transport of the neurosecretory substance. It is characteristic that the latter mainly occur in the immediate vicinity of the source of neurosecretion as well as in the region where this secretion is being used up—namely the neurohypophysis. The staining reaction of these cells to polysaccharide stains corresponds to their high content of neurosecretory substance.

Other data suggest that the neurosecretory substance can be transported through the cerebrospinal fluid [8,12,13]. Collin and Barry [8] observed cells situated on the ependyma surface discharging granules of secretory sustance into the cerebrospinal fluid of the IIIrd ventricle. Discharge of the secretion on the ependyma surface and into the lumen of the preoptic cleft, not only by means of wandering cells but also in the shape of small granules or larger droplets (see Figs. d,e), could repeatedly be observed in our material. These findings as well as the difference found in the state of the ependymal lining in some parts of the cerebral cavities induced us to accept the conclusion of Löfgren [14] according to which the neurosecretory substance is transported to the recess of the infundibulum with the flow of

A

B



Various means of formation and transportation of the neurosecretory substance. A) in amphibia; B) in mammals; a, b) parasagittal section through the preoptic region of R. esculenta, at a magnification of ×240; the arrow shows the ventrodorsal direction; a) abundance of large droplets of neurosecretory substance; b) movement of the droplets of neurosecretory substance toward the ependyma of the IIIrd ventricle (staining according to Halmy-Dyban); c) neurons containing a multitude of neurosecretory granules in the dorsal part of the nucleus in a toad at a magnification of ×1200 (aldehyde-fuchsin); d, e,) tall ependymal cells in the dorsal part of the nucleus of R. temporaria (aldehyde-fuchsin); d) progression of the secretion between the ependymal cells; e) secretion reaching the surface of the ependyma, at a magnification of ×1200; f, g, h, i) wandering cells at a magnification of ×1800 (Halmy-Dyban stain); f) between the ependymal cells of the median eminence of R. esculenta; g) in the intermediate lobe of the pituitary gland of the spadefoot toad (Pelobates fucus); h) in a capillary lumen within the brain tissue of a yearling of the spadefoot toad Pelobates fucus; i) in the same animal in the vicinity of a capillary in the median eminence; k, l, m) the bottom of cerebrospinal fluid. Here the neurosecretory substance is precipitated and enters through the loose layer of ependymal cells lining the bottom of the infundibulum the numerous capillaries of the pituitary portal system or directly the tuberal lobe; this transport is mediated by the glia cells.

And indeed in the region of the bottom of the infundibulum the glia cells are, in the animals investigated by us (amphibia and mammals), very tall and arranged loosely in two-three rows. The ependymal lining seems highest in those small areas of the deepening in the IIIrd ventricle where the outflow of neurosecretory substance into the cerebrospinal fluid is most marked of all. A similar picture of a stratified ependyma was found in amphibia also at the bottom of the infundibulum during the metamorphosis of the frog. This organization develops already in the tadpole stage. At this time the intermediate and distal lobes of the pituitary gland are formed under the influence of the earlier differentiated neurohypophysis and begin to function. Figures k, l, m show that the bottom of the infundibulum consists of a mass of longitudinally arranged non-myelinated nerve fibers which are covered by a layer of tall ependymal cells with a loose cuticular surface which shows fine processes; although the nerve fibers are evenly distributed within the bulk of the infundibular wall the granules of the neurosecretory substance are nevertheless mainly situated near the base of the ependymal cells both in the area anterior to the pituitary gland and in the region of the median eminence.

The local changes of the ependyma in two topographically separated areas deserve attention inasmuch as the glia cells represent mediators and apparently also transformers of the neurosecretory substance on its way into the blood stream. A concentration of granules and fragments of the neurosecretory substance round the endothelium of the capillaries in the median eminence of the neurohypophysis is a common finding in histological sections. The morphological organization of the pituitary gland in the larvae of amphibia deserves attention: the infundibular deepening represents the deepest point in the path of the cerebrospinal fluid; the spoon-shaped base of the infundibulum is bent in the caudal direction. It is to be expected that in such an elongated bag-shaped formation the liquid will stagnate and exert a higher pressure upon the wall of the base than in other parts of the brain. In other words it is here that the most favorable conditions for accumulation and diffusion of the neurosecretory substance into the wall of the infundibulum exist. It is in this region that considerable concentration of granules and fragments of secretion giving a positive stain reaction can be observed immediately under the ependyma. In whatever way the neurosecretory substance is transported it is subjected to changes, changes which become manifest in its tinctorial properties. On its path the neurosecretory substance passes through several (glial and endothelial) membranes.

The results of transection of the hypothalamo-hypophyseal tract quoted above seem to constitute a serious argument against the existence of additional ways of transportation of the secretion as discussed above [11]. After this operation the neurosecretory substance disappears from the neurohypophysis and accumulates at a site located proximally of the point of transection. These experiments showed with great force of conviction that an outflow takes place along the nerve fibers of the hypothalamo-hypophyseal tract. The transection leads, however, as is well known, to degenerative changes in the hypothalamic nuclei themselves, a fact which cannot but impair the other ways of transportation of the neurosecretory substance as well. Far from denying the flow of the neurosecretory substance along the axons we thus report data concerning other ways of transportation of this substance to the pituitary gland. The existence of such additional ways is further supported by the repeatedly observed discrepancy between the quantity of neurosecretory substance found in various parts of the hypothalamo-hypophyseal tract on the one hand and the two main parts of the neurohypophysis on the other hand.

SUMMARY

Neurosecretory nuclei of the hypothalamus and the area of infundibulum were subjected to histological investigation in tadpoles and frogs of various age. Different methods were used. Both intact animals and those subjected to diencephalon excision were studied. Apart from this, albino rats and puppies were also taken as objects for this

---

the infundibulum of a spadefoot toad tadpole—the stage before the onset of metamorphosis (Halmy-Dyban stain); k) magnification × 144; l, m) magnification × 1200; at the base of the tall ependymal cells numerous neurosecretory granules can be seen; n) tall ependymal cells in the region of the median eminence of the neurohypophysis of a puppy; intensively stained wandering cell at a magnification of × 1800 (Gomori-hematoxylin); o,p) proximal part of the neurohypophysis of a puppy at a magnification of × 1800 (aldehyde-fuchsin); o) two cells in the vicinity of a capillary; p) a wandering cell and two spheroid accumulations of neurosecretory granules of different size.

experimental work.

The presence of neurosecretion was established not only in the bodies and the processes of the neurons and the surrounding tissues, but also in the wandering cells accumulating in their cytoplasm the positive aldehyde-fuchsinophilic substance (and in this connection staining intensively). The data discussed concerns the transmission of neurosecretion through the cerebrospinal fluid; the active state of the cells in the ependymal lining of the neurohypophysis (its proximal part) was especially emphasized.

LITERATURE CITED

1. A. A. Voitkevich, Dokl. AN SSSR, 135, 1 (1960) p. 221.
2. A. A. Voitkevich, E. I. Zubkova, and I. I. Grigor'ev, Dokl. AN SSSR, 130, 4 (1960) p. 940.
3. L. B. Levinson, Proceedings of the 5th All-Union Congress of Anatomists, Histologists and Embryologists [in Russian] (Leningrad, 1951) p. 410.
4. A. L. Polenov, Uchen. Zapiski Leningradsk. Med. Inst., 3 (1959) p. 176.
5. J. Barry, C. R. Soc. Biol., 152 (1958) p. 809.
6. H. Bertha, Wien. Z. Nervenheilk. 9 (1954) p. 146.
7. H. Brettschneiderr, Morphol. Jb., 95, Pt. 1 (1955) p. 265.
8. R. Collin and J. Barry, C. R. Soc. Biol., 148 (1954) p. 1457.
9. A. R. Cruz, Acta Anat., 36 (Basel, 1959) p. 153.
10. B. Hanström, Acta Neuroveget. 8 (Wien, 1954) p. 269.
11. G. W. Harris, Neurol. Control of the Pituitary Gland (E. Arnold, London, 1955).
12. E. Kivalo and S. Talanti, Acta Endocr., 26 (Kbh) (1957) p. 128.
13. J. Kolaczkowski and M. Wender, Folia Morph., 6 (Warszawa, 1955) p. 75.
14. F. Löfgren, Lunds Univ. Årsskr., 54, 11 (1958) p. 15.
15. J. Metuzals, Experientia, 15 (1959) p. 36.
16. R. Rabl, Arch. Path. Anat., 326 (1955) p. 444.
17. B. Romeis, in: W. Moellendorff. Handbuch der mikroskopischen Anatomie des Menschen, 6, 3 (Berlin, 1940).
18. F. Stutinsky, Année Biol., 29, 3 (1955) p. 487.
19. L. D. Wilson, J. A. Weinberg, and H. A. Bern, J. Comp. Neurol., 107 (1957) p. 253.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.