inhibits the secretion of pituitary STH [7], slowing of its release must be accompanied by elevation of the blood STH level in protein deficiency.

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CHOLINERGIC INNERVATION OF VASOPRESSIN-CONTAINING CELLS OF HUMAN CEREBRAL BLOOD VESSELS

A. V. Lomakin, Yu. I. Pigolkin, and P. A. Motavkin

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The role of vascular melanocytes in regulation of material mobility in the mammalian brain has been demonstrated experimentally. Stimulation of the vagus nerve by a direct current and application of acetylcholine to the pia mater cause degranulation of these cells [1]. However, the morphological substrate of the mechanism of degranulation and the vasoactive substances of melanocytes, as possible vascular effectors, are not yet known. We investigated these cells and the localization of vasopressin (VP) in them, and also their relations with cholinergic axons.

EXPERIMENTAL METHOD

The pia mater and also transverse sections through the mesencephalon and pons and blood vessels located in these regions in fetuses during the second half of intrauterine development, and in adults (aged 30-50 years 6-12 h after death) were investigated. Melanocytes were identified by presence of brown melanin granules in the cytoplasm, by Masson's reaction, and by the reaction for tyrosinase [3]. Nerve fibers were impregnaged by Campos' method and cholinergic axons were revealed by reactions for acetyl-cholinesterase (AChE) [6] and choline-acetyltransferase (ChAT) [2], and electron-microscopically by the presence of translucent synaptic vesicles in them. Material for electron-microscopic study was fixed in 1% OsO_4 and embedded in Epon-12. Sections were cut on the LKB Ultrotome and examined in the JEM-100B electron microscope. VP was detected in the MBI-15-2 luminescence microscope by an indirect immunofluorescence method [7], using monospecific hog antibodies to hog IgG, conjugated with FITC ("Sevac," Czechoslovakia). Pigmented neurons of the locus coeruleus,

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Department of Histology, Vladivostok Medical Institute.

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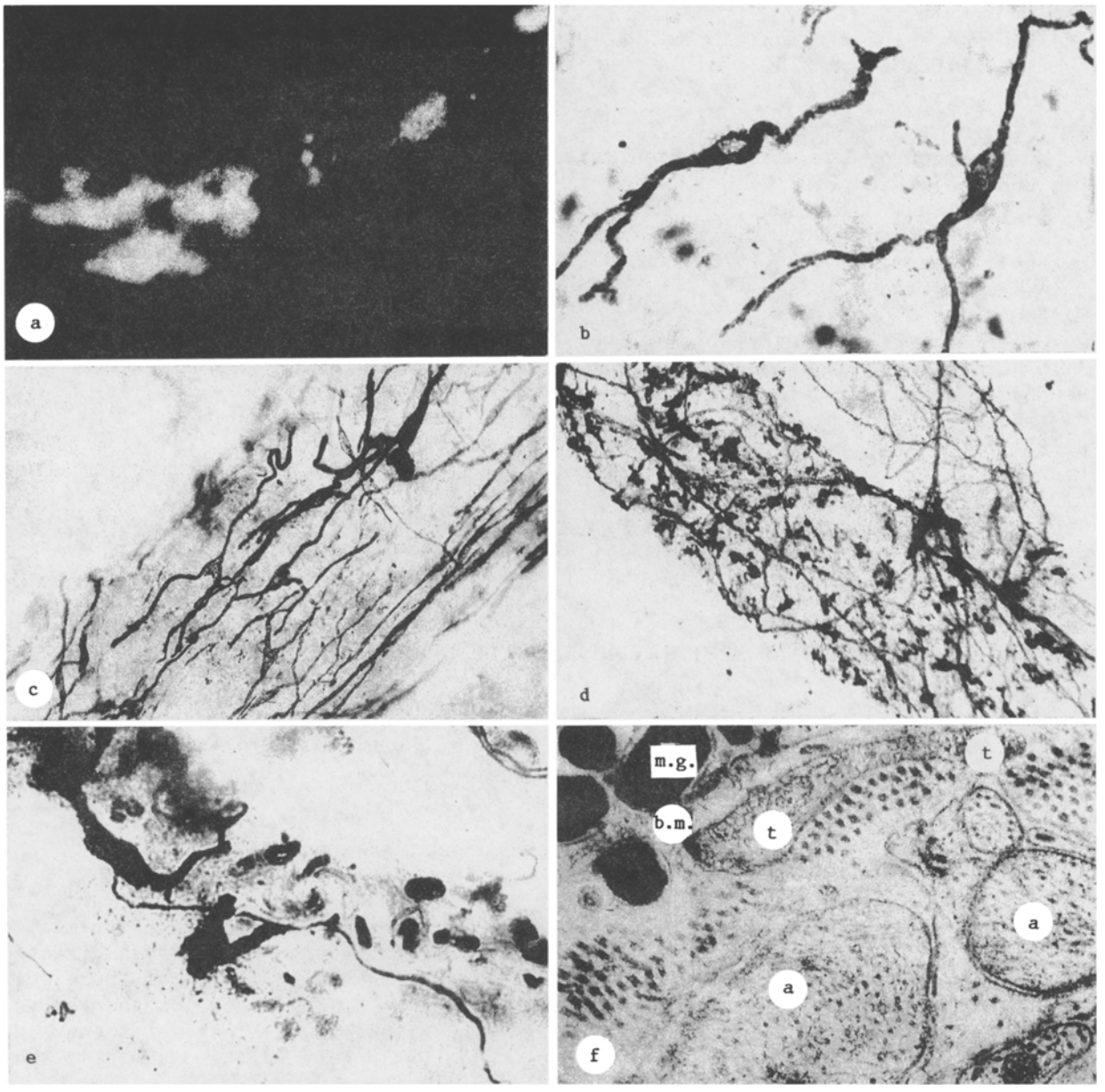

Fig. 1. Melanocytes of human cerebral arteries: a) VP-positive melanocytes of intracerebral blood vessels; man aged 32 years, indirect immunofluroescence method. 400 ×. b) Tyrosinase of melanocytes of a 28-week fetus. 280 ×. c) High concentration of melanocytes on pial artery; man aged 35 years, Masson's method. 140 ×. d) Melanocytes in cholinergic plexus on pial artery; man aged 44 years; Koelle'e method. 44.1 ×. e) Nerve fiber ending on melanocyte of an arteriole; man aged 35 years; impregnation by Campos' method. 140 ×. f) Cholinergic terminal on surface of a melanocyte: m.g.) melanin granule; b.m.) basement membrane; t) cholinergic terminal; a) axon. Electron micrograph 30,000 ×.

in which the presence of VP has been established, and of the substantia nigra, whose melanin-containing neurons have no VP, although axons converging on them do have this neuropeptide [5], and also sections not treated with monospecific serum, were studied simultaneously as the standard.

EXPERIMENTAL RESULTS

VP-containing melanocytes gave specific emerald green luminescence. The comparatively small luminescent granules filled the cytoplasm of the bodies and processes of the cells uniformly (Fig. 1a). No immune activity was found on control sections, untreated with monospecific serum. An immunopositive reaction was found in neurons of the locus coeruleus

and in vascular melanocytes. The absence of fluorescence in neurons of the substantia nigra is an additional criterion of the specificity of the method, for it enabled autoluminescence of melanin to be excluded in this case.

Melanocytes 20-40 μ in diameter were distributed in groups on the almost invisible wall of the cerebral arteries. The identity of VP-containing vascular cells as melanocytes are confirmed light-optically by the discovery of brown and black pigment granules in them or by the method of tryosinase. High activity of tyrosinase – a marker of melanin formation, was discovered in human cerebral vessels as early as in the intrauterine period of development, before the appearance of granules (Fig. 1b). Besides round or oval cells, angular cells with short processes or elongated, fusiform cells with long, infrequently branching processes, forming plexuses on the arteries, also were found (Fig. 1c). Melanocytes with processes were found more often on intracerebral vessels 100-40 μ in diameter; with a decrease in caliber of the blood vessels, there was a relative increase in the number of cells with long processes. In the central part of the cells a round or oval nucleus, poor in chromatin, could be clearly identified.

Topographically the melanocytes of the cerebral arteries were closely connected with autonomic nerves They were located in plexuses of axons giving a positive reaction for ChAT and AChE, and which, consequently, were cholinergic (Fig. 1d). Impregnation revealed nerve fibers ending on the melanocytes (Fig. 1e).

Electron microscopy showed that fibers of this kind constitute a cable system, individual axons of which contain electron-translucent vesicles and are located 100-200 nm from the surface of the melanocyte (Fig. 1f).

Previous investigations [4] showed that for a nerve terminal to be 1000 nm, or even 4000 nm, away is no obstacle to excitation of the cell. The cholinergic axon shown in Fig. 1f, which is some 10 times closer to the melanocyte, thus undoubtedly controls the function of that cell. Experiments with stimulation of parasymapthetic nerves and with application of acetylcholine to the pia mater prove that degranulation of vascular melanocytes evokes a cholinergic mechanism [1]. It is perfectly legitimate to suggest that in the melanocytes–vascular wall system, VP also is secreted during exocytosis.

The presence of VP in the melanocytes and their morphological and functional connection with cholinergic axons thus lead to the conclusion that a nervous mechanism for regulating the cerebral blood vessels exists and realizes its effect through an endocrine cell.

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