ELECTRON-MICROSCOPIC INVESTIGATION OF MONOAMINERGIC FIBERS IN THE RAT POSTERIOR PITUITARY

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UDC 611.814.3

Monoaminergic fibers and their expansions containing granules with an electron-dense center 700-1000 Å in diameter were discovered electron-microscopically in the posterior lobe of the rat pituitary. Most of the expanded monoaminergic fibers were some distance away from capillaries and were surrounded by neurosecretory fibers and pituicytes. In the region of contacts between them, usually no specialized structures were found. Individual monoaminergic fibers made contact with capillaries and actually penetrated into the pericapillary space. Occasionally large expansions of these fibers were found which contained not only granules but also various polymorphic inclusions, degenerating mitochondria, and numerous small tubules.

KEY WORDS: monoaminergic structures; posterior lobe of the pituitary.

Fluorescence-microscopic studies have shown that the posterior lobe of the pituitary gland (PLP) in mammals is penetrated by a few monoaminergic fibers [4, 13, 14]. The intensity of fluorescence of the monoaminergic structures changes appreciably during activation of the functions of the hypothalamic-pituitary neurosecretory system [1, 4]. Monoaminergic fibers are observed extremely rarely in the electron microscope; these observations apply chiefly to structures located at the boundary with the pars intermedia of the pituitary [3, 14].

EXPERIMENTAL METHOD

The PLP of Wistar rats weighing 120-160 g was studied under normal conditions (15 animals) and after a high salt intake for 7, 14, 20, and 25 days (three rats at each time). Instead of drinking water, the experimental animals received a 2.5% solution of sodium chloride *ad lib.* in addition to dry food. The method of preparation of the material for electron microscopy was fully described earlier [10].

EXPERIMENTAL RESULTS AND DISCUSSION

Expansions of the nerve fibers measuring up to 5 × 9 μ, containing elementary granules 700-1000 Å in diameter, synaptic vesicles, and small round mitochondria with a matrix of average or increased density, were found in the PLP of the rats, together with expansions of neurosecretory fibers, on the 7th, 14th, 20th, and 25th days of salt loading. Besides elementary granules, granules of the same types as in neurosecretory fibers were found in the expansions of these fibers [5]. By the use of 5-hydroxydopamine to label the monoaminergic granules, and of 6-hydroxydopamine to cause degeneration of monoaminergic fibers, fibers of this type could be identified with monoaminergic fibers in the PLP of the rat [13]. The membranes of some granules had tubular evaginations measuring about 1300 × 360 Å, with only slightly osmiophilic contents, connected by intermediate forms with pale tubules of the

Laboratory of Neuroendocrinology, Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 87-90, December, 1975. Original article submitted November 5, 1974.

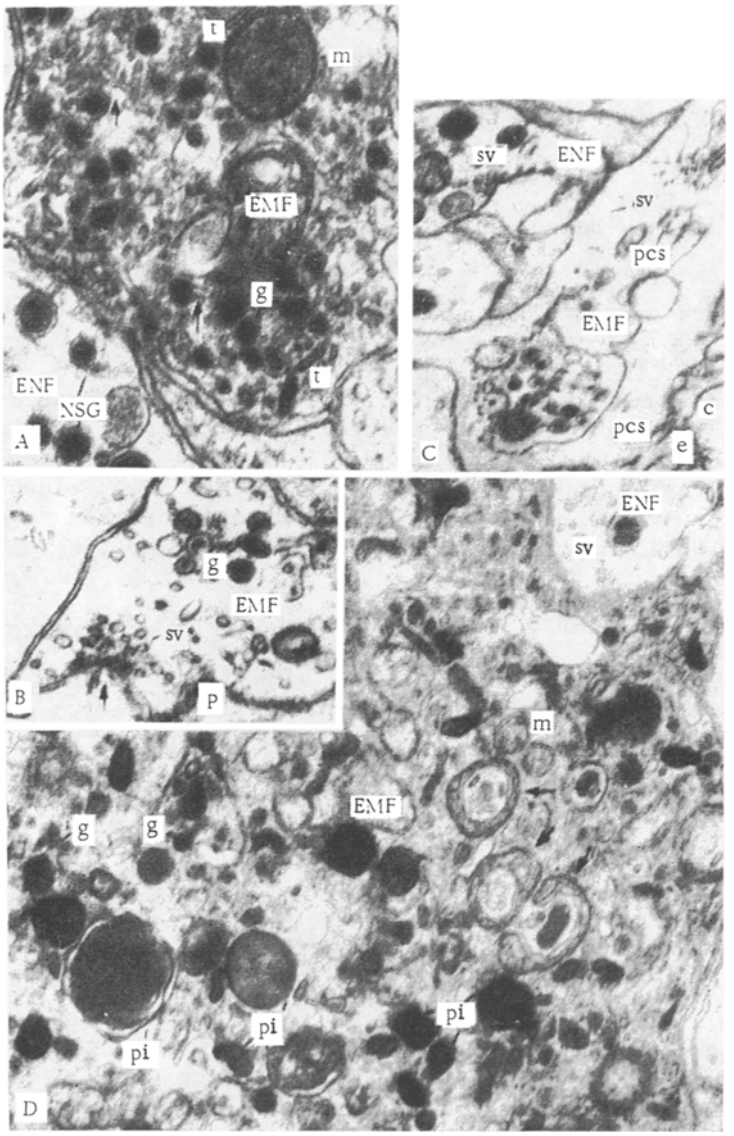

Fig. 1. Expansion of a monoaminergic fiber (EMF). A) EMF containing granules (g) of different types, mitochondria (m), and short tubules (t). Membranes of granules with tubular evaginations (arrows). ENF) Expansion of neurosecretory fiber, NSG) neurosecretory granules, 51,500×. B) Synaptoid contact (arrow), EMF with pituicyte (P), 48,000×. C) EMF located in pericapillary space (PCP); BM) basement membrane; c) capillary; sv) synaptic vesicles; e) endothelium 34,100×. D) Large EMF with numerous polymorphic inclusions (pi). All intermediate stages of "coiling" of mitochondria can be seen (arrows), 50,000×.

same size lying freely in the neuroplasm (Fig. 1A). The possibility cannot be ruled out that these patterns reflect the formation of new granules in the monoaminergic expansions.

Only solitary monoaminergic fibers were located around the capillaries — in the pericapillary space and at the boundary with it — both in the depth of PLP and chiefly where it joins the stalk. Most expansions of monoaminergic fibers were found to be some distance away from the capillaries and to be surrounded by neurosecretory structures and processes of pituicytes. These findings are in good agreement with the results of fluorescence-

microscopic analysis of PLP [4, 13, 14]. Since usually no specialized structures were found in the region of contacts between them (Fig. 1A) it can be assumed that these were expansions of monoaminergic fibers passing through, possibly to the pars intermedia of the pituitary [3, 5, 7]. The fact that no monoaminergic fibers could be detected in intact animals can be explained, first, by their small number and, second, by the accumulation of elementary granules, the size of which was used in this investigation to identify monoaminergic structures, in them during salt loading. This second explanation is more likely because thickenings of such fibers and an increase in the intensity of monoamine fluorescence has been observed under the fluorescence microscope during stress [1].

Collections of synaptic vesicles (Fig. 1B) were found extremely rarely in the expansions of the monoaminergic fibers at the boundary with processes of pituicytes, but at the boundary with one of the neurosecretory expansions there was a collection of electron-dense material near the plasmalemma. Despite the absence of true synapses, the influence of monoaminergic fibers on neurosecretory structures and pituicytes could perhaps be exerted at the places of these specialized contacts [13].

Expansions of monoaminergic fibers on the boundary with the pericapillary space contained relatively few granules but a fair number of synaptic vesicles. These formed a collection near the plasmalemma in contact with the outer basement membrane. A concentration of osmiophilic material was found in the neuroplasm at such places. On the 14th day of the experiment expansions of monoaminergic fibers measuring about 0.5 μ were found in the pericapillary space. In some places their neuroplasm was denser as a result of the accumulation of "ghost granules." Synaptic vesicles were not present in all expansions of the fibers in the pericapillary space, and short pale tubules were rare (Fig. 1C). One such expansion was surrounded by a pericyte. The functional role of the expansions of the monoaminergic fibers in the pericapillary space, as yet discovered only in PLP [11], is still unexplained although it is accepted that adrenergic fibers innervate capillaries [12].

On the 20th day of the experiment an expansion of a monoaminergic fiber measuring 5 × 9 μ, filled with all types of granules among which there were particularly many coarse-grained, disintegrating, and "ghost granules," were seen not far from the pituitary stalk (Fig. 1D). These expansions evidently corresponded to the drop-like structures observed under the fluorescence microscope [13, 14]. The neuroplasm of this expansion had increased electron density because of the high concentration of 750-Å tubules filled with moderately osmiophilic material. The length of the tubules in the sections reached 0.9 μ. Continuous series of intermediate forms between tubules and granules could be seen. Vesicles of synaptic as well as short pale tubules could be seen in places. Small, round mitochondria measuring about 0.3 μ and long and narrow mitochondria, up to 1.1 μ in length, mainly had fragmented cristae and a moderately osmiophilic matrix. Intermediate forms from horse-shaped mitochondria, constricted to 730 Å but with pinhead-swollen ends, to circular structures containing short tubules and vesicles with moderately osmiophilic material were seen (Fig. 1D). The structures described, just as in cells of the adrenal cortex, evidently reflect successive stages of degeneration of mitochondria [16]. Besides "twisted" mitochondria, other polymorphic inclusions also were frequently seen: electron-dense spheres 0.4 μ in diameter, lamellar bodies, and vacuoles with single thick walls. Polymorphic inclusions are known to be degeneration products of organoids and of secretory inclusions [6, 9, 15]. Some of the smaller expansions of monoaminergic fibers also contain polymorphic inclusions. Expansions of monoaminergic fibers with numerous polymorphic inclusions, also observed previously in intact rats [13], probably reflect processes of organoid renewal which are continuously taking place in nerve cells, and also aging of nerve cells [8, 15].

A few monoaminergic fibers located in the region of the junction with the pituitary stalk, were thus found electron-microscopically in the rat PLP; occasional fibers were found in the pericapillary space and at its boundary.

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