EFFECT OF STIMULATION OF CALCIUM METABOLISM ON MORPHOLOGY AND FUNCTION OF THE MAMMALIAN BRAIN

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UDC 612.822.014.2-06:/612.822.1.015.31:
546.41/.014.46:615.357.447

KEY WORDS: parathormone; calcium ions; ultrastructure; neocortex; neurosecretion

Metabolism of Ca^{++} ions in the CNS is a subject of interest to research workers because of the leading role of these ions in the regulation of most physiological processes of excitable tissues. Ca^{++} ions participate directly in the change of permeability of the outer cell membrane [10], in mechanisms of secretion of neurotransmitters [2], and jointly with the cyclic nucleotide system they are the main connecting link between central hormonal influences and the concrete spike response on their cells [3, 6]. Changes in the intracellular Ca^{++} concentration may lead to long-term changes in the integrative properties of their cells [4]. It has been shown, for instance, that prolonged administration of parathormone, which increases the intracellular concentrations of cAMP and Ca^{++} [8], increases the Na,K-ATPase activity of cell membranes [7] and produces long-term changes in the evoked response [1] and in the character of the electroencephalogram [9]. However, there are virtually no reports in the literature on morphological and functional analysis of the state of the CNS in response to intervention in Ca metabolism.

The aim of the present investigation was accordingly to study ultrastructural characteristics reflecting the state of the axon terminals, neocortical synaptic systems, and hypothalamic neurosecretory structures during stimulation of Ca metabolism by systemic parathormone administration.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male Wister rats weighing 180-200 g. Daily for 7 days the experimental animals were given an intramuscular injection of 1.8-2 ml parathormone in a dose of 10 U/100 g. Four rats receiving intramuscular injections of 2 ml of 0.14 M NaCl solution for 1 week and four intact rats served as the control. On the 7th day after the beginning of the experiments the animals were anesthetized with pentabarbital and killed. Pieces from the sensomotor and visual cortex, the supraoptic nuclei, the median eminence of the hypothalamus, and the neurohypophysis of the experimental and control rats were excised for electronmicroscopic analysis. Material was fixed by immersion in a 2.4% solution of glutaraldehyde in 0.08 M cacodylate buffer (pH 7.3) and in a 2% solution of OsO_4 in the same buffer. The pieces of brain were dehydrated and embedded in Araldite. Layers 2-3 of the neocortex, the supraoptic nuclei, and the hypothalamic median eminence were distinguished in semithin sections 1 μ thick, stained with methylene blue. Ultrathin sections were stained with lead citrate and examined in the TESLA BS-500 electron microscope.

EXPERIMENTAL RESULTS

In all parts of the brain of the experimental animals studied during stimulation of Ca metabolism ultrastructural changes, similar in character but more marked in the synaptic junctions of the neuropil, were observed. For instance, increased density of distribution of synaptic vesicles was observed in axon terminals of the neuropil in the sensomotor and visual areas of the neocortex and in the supraoptic nuclei of the hypothalamus of the experimental animals, filling the whole volume of the terminals, but unlike in the control, they were separated from the presynaptic membrane by a distance of 30-50 nm. Reduced electron density of the pre- and postsynaptic membranes, and a decrease in the width of the synaptic space and the postsynaptic condensation also were observed (Fig. 1a). In addition, destructive and degenerative changes

Central Research Laboratory, Medical Faculty, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 104, No. 8, pp. 231-233, August, 1987. Original article submitted December 29, 1986.

 0007-4888/87/0008-1150 $12.50

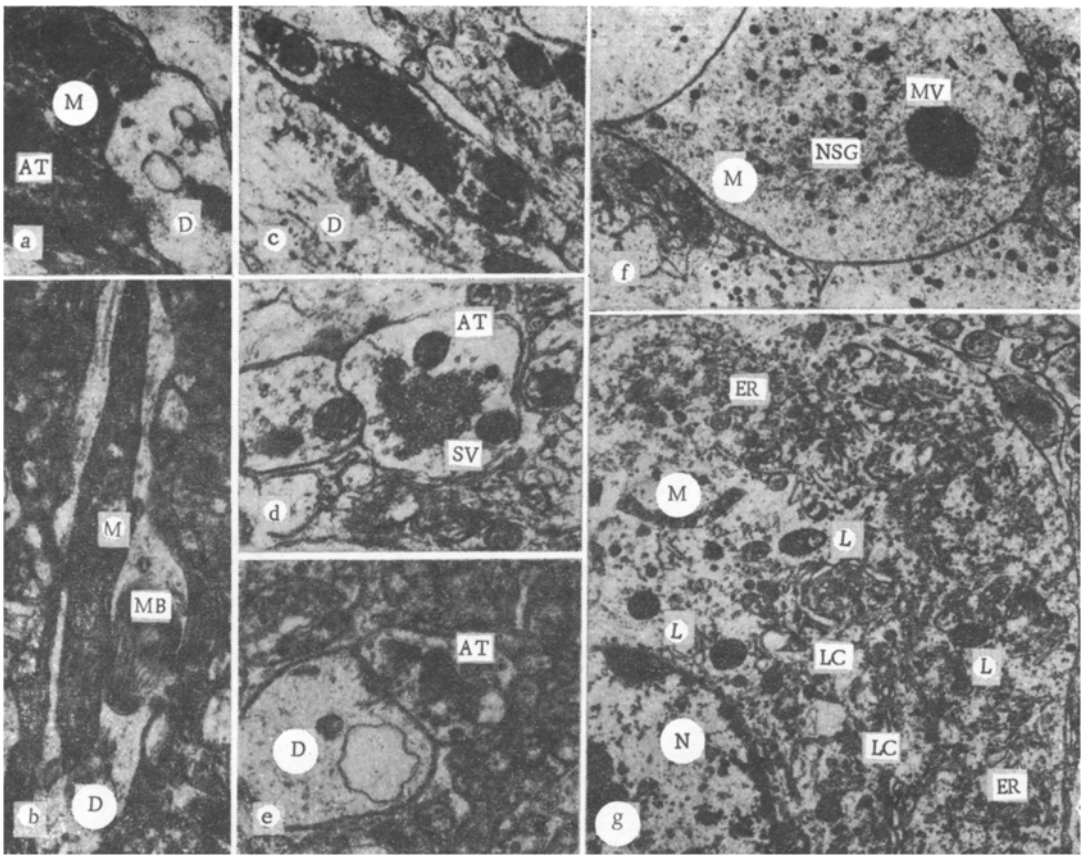

Fig. 1. Ultrastructural changes in rat brain after administration of parathormone: a) increase in number of synaptic vesicles in axon terminals of neocortex (39,000 ×); b) destructive changes in small neocortical dendrites (28,000 ×); c) axon terminal in neuropil of supraoptic nucleus of hypothalamus (23,000 ×); d) concentration of synaptic vesicles in central part of a terminal (27,000 ×); e) degenerative changes in axon endings of supraoptic nuclei (27,000 ×); f) destructive changes in neurosecretory terminal of median eminence of hypothalamus (18,000 ×); g) neurosecretory cell of supranucleus (12,000 ×). AT) Axon terminal; D) dendrite; M) mitochondria; SV) synaptic vesicles; MB) membraneous body; NSG) neurosecretory granules; MV) microvesicles; N) nucleus; LC) lamellar complex; L) lysosomes; ER) tubules of endoplasmic reticulum.

also were observed in the pre- and postsynaptic structures in the parts of the brain studied. Besides an increase in the number of synaptic vesicles in the axon terminals, they were found to be agglutinated in the central part of the endings (Fig. 1d). Concentration of synaptic vesicles in the region of the presynaptic membrane was not found (Fig. 1e). Destructive changes in the mitochondria, namely translucency of the matrix, shortening of the cristae, and their focal expansion, were found in pre- and postsynaptic structures. In the postsynaptic structures destruction of the specialized spinous apparatus was observed, with the appearance of vacuoles and electron-dense and membranous bodies (Fig. 1b). Degenerating structures as a rule were isolated from other elements of the neuropil by processes of astrocytes, which surrounded the modified axons and dendrites in several layers.

Increased condensation of nuclear chromatin, and widening of the perinuclear space could be seen in the neurosecretory cells of the supraoptic nuclei by comparison with the control. The perikaryon contained many short, dilated tubules of the endoplasmic reticulum and many free ribosomes, and destructive changes were found in the mitochondria. Meanwhile, cisterns of the lamellar complex were dilated, the number of neurosecretory granules was reduced, but the number of lysosomes and multivesicular bodies was increased (Fig. 1g). Concentration of synaptic vesicles and morphological and functional evidence of blocking of synaptic transmission were observed in axon terminals forming axosomatic synapses on the surface of neurosecretory cells.

A definite sequence of changes, from an increase in the number of microvesicles and a decrease in the number of neurosecretory granules to degenerative changes with agglutination of microvesicles in the central part of the neurosecretory terminals could be made out in the ultrastructure of the neurosecretory terminals of the median eminence and neurohypophysis of the experimental animals, just as in the neuronal axon endings (Fig. 1f).

The results are thus evidence that during stimulation of Ca metabolism by administration of parathormone the principal changes in the neurons and neurosecretory cells investigated were observed in the ultrastructure of the mitochondria and microvesicles involved in the maintenance of intracellular Ca homeostasis [14, 15]. In view of data in the literature, this fact can be explained on the grounds that microvesicles in neurosecretory terminals take part in the initial stages of regulation of intracellular Ca metabolism. Meanwhile, in the presence of more marked changes, when the Ca^{++} concentration in the cytosol exceeds 0.3 μM [14], mitochondria take part in the mechanisms of this regulation. In this case, translucency of the mitochondrial matrix and shortening of the cristae take place [13]. An increase in the intracellular Ca^{++} concentration also activates proteinases and phospholipases, and this may cause the development of intracellular destructive processes [11].

The ultrastructural changes discovered in this investigation are also evidence of the reduced physiological activity of axon terminals because of their overexcitation and blocking of the conduction of nervous impulses. The evidence of concentration of synaptic vesicles in the terminals and the conduction block to synaptic transmission, which were found are also evidently connected with Ca^{++} accumulation within the terminals, where they play a leading role in the mechanisms of transmitter secretion and resynthesis in the nervous system [2]. Morphological and functional disturbances of synaptic transmission revealed by the present investigation following administration of parathormone may also lie at the basis of the pathogenetic mechanisms of the conduction block to excitation observed in the neuromuscular synapses of patients with primary hyperparathyroidism [12], and also the conduction block to transmission in the axon terminals of the mossy fibers of the cerebellum induced in experimental animals in a state of weightlessness [5].

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