ELECTRON-HISTOCHEMICAL AND X-RAY SPECTRAL STUDY OF THE REACTION PRODUCT FOR CALCIUM IN THE CORTEX AND CAUDATE NUCLEUS OF THE RAT BRAIN AFTER ADMINISTRATION OF GLYPIN

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Systematic investigations of the psychopharmacological changes caused by central cholinolytics have been based in recent years on the hypothesis of adreno-cholinergic interrelations [8].

The phenomenon of excitation of the adrenergic system under the influence of antidepressants of the cholinolytic series (i.e., during cholinergic blockade) has recently been explained both by an increase in the liberation of catecholamines [2] and by a reduction in their uptake by the presynaptic endings [9]. After administration of the central muscarinic (M) cholinolytic glypin (10 mg/kg), increased activity of sensomotor cortical neurons in rats was ascribed by Gromov and Lokhnitskaya [1] to an increase in the content of catecholamines.

Psychopathological analogs of adrenergic cholinergic processes (the dominant focus) – namely depressive and manic states – are associated by some workers with hypo- and hypercalcemia [6]. Under clinical conditions, during systematic electroconvulsive therapy of depressive diseases, a decrease in the total calcium concentration in the CSF and plasma has been observed [6]. On the basis of all these observations, adreno-cholinergic imbalance has been linked with calcium metabolism in the CNS.

At the synaptic level, the obligatory role of calcium ions in neurotransmission is generally accepted. It is equivalent to their role in the secretion of catecholamines and acetylcholine [9]. At the same level, in postsynaptic processes calcium homeostasis depends mainly on adrenergic neurotransmission [12].

In connection with the above observations it became necessary to study the spatiotemporal dynamics of the product of the reaction for calcium in the CNS during administration of the central M-cholinolytic glypin.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male Wistar rats weighing 180 g. The cytochemical reaction was carried out by the method of Berridge et al. [5] on slices (40 μ) of the temporal cortex and caudate nucleus of 8 rats in a solution of lead nitrate (1 mM for 1 h at 4°C). Four rats were given glypin by intraperitoneal injection in a dose of 16 mg/kg body weight. The animals were decapitated 30 min later. Four rats served as the control. The cytochemical reaction of 8 rats was carried out by Berridge's method in our own modification, i.e., with the addition of 1 mM ouabain to the incubation medium. Four of these rats were exposed to poisoning with glypin (16 mg/kg) for 30 min, and the other four served as the control.

Sections subjected to histochemical treatment were postfixed in 1% OsO_4 solution in cacodylate buffer for 1 h at 4°C. After dehydration in acetone the sections were embedded in a mixture of Epon and Araldite. Ultrathin sections were examined in the JEM-100B electron microscope.

X-ray Spectral Microanalysis

Sections not exceeding 500 nm in thickness were used for microanalysis; they were mounted on a copper grid and subsequently sprayed with carbon. X-ray spectral analysis of these preparations was carried out

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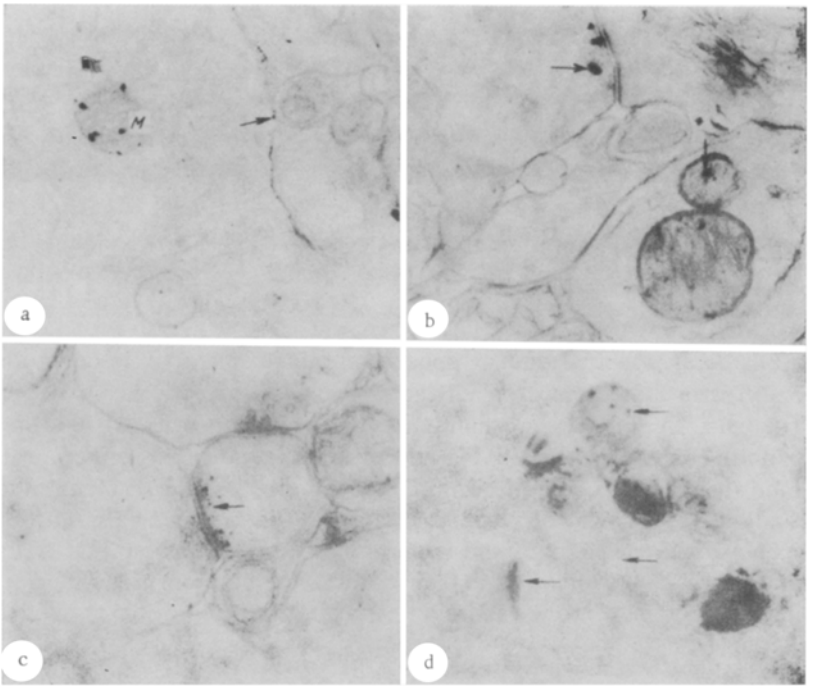

Fig. 1. Electron-microscopic detection of reaction products for calcium in sections through rat cerebral cortex and caudate nucleus by the method of Berridge et al. (unstained sections). a) Localization of deposit of large granules on mitochondrial matrix (M) and of fine granules on inner surface of neurons (arrow), 35,000×; b) localization of residue of large granules on presynaptic vesicles and matrix of mitochondria of neuropil (arrow), 33,000×; c) localization of finely granular residue on inner surface of postsynaptic membrane of synapse (arrow) in caudate nucleus of experimental rat (16 mg/kg, 30 min). Sections treated with ouabain (1 mM), 36,000×; d) fragment of section (40 μ) of rat caudate nucleus (poisoning with glypin, 16 mg/kg, 30 min), treated with ouabain (1 mM). X-ray spectral analysis from surface of membrane freed from residue (1 arrow), precipitate of large crystals in mitochondria (double arrow [sic]), and finely granular precipitate of postsynaptic thickening (triple arrow [sic]), 400,000×.

by representatives of the "JEOL" and "KEVEX" firms on the JEM-100S electron microscope and the KEVEX-7000 microanalyzer.

EXPERIMENTAL RESULTS

On investigation of material from the control animals (without the addition of ouabain) an electron-dense deposit consisting of large crystals of lead phosphate was found on the mitochondrial membranes of the neurons (Fig. 1a), and neuropil and on membranes of the smooth vesicles (Fig. 1b). In addition, a finely granular residue was found on the inner surface of the synaptolemma, axolemma, and neurilemma (Fig. 1a). In sections obtained from the experimental animals (lysine, 16 mg/kg, 30 min) the character of the cytochemical reaction was similar in principle with that in the control.

On investigation of sections obtained from the control animals, with the addition of 1 mM ouabain, residues of large granules also were found but were localized only in the mitochondrial matrix and in the presynaptic smooth vesicles. However, by contrast with the experiments of series I the fine-grain residue on the inner surface of the neurilemma, axolemma, and synaptolemma was practically invisible. A marked increase in the number of synapses (from 2 to 4 per ultrathin section) with a finely granular residue on the inner surface of the postsynaptic membrane was observed in the experiment (Fig. 1c).

The addition of ouabain to the incubation medium thus enabled ATPase activity – the main factor in the formation of phosphate on neuron membranes – to be ruled out.

To interpret the composition of the residues discovered it had to be considered that lead, the marker in Berridge's reaction, can give sufficiently stable precipitates with endogenously formed phosphate anions [5, 13].

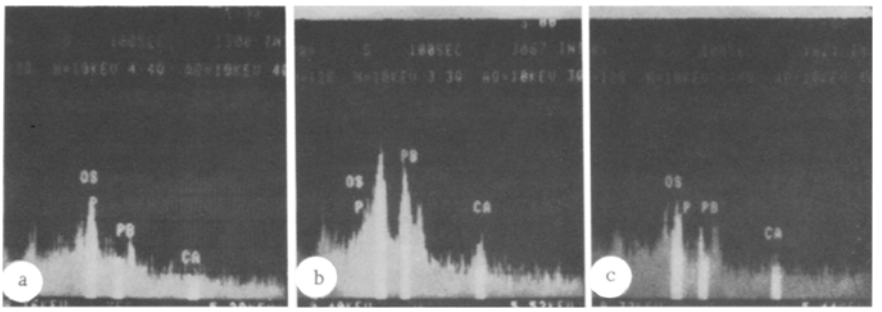

Fig. 2. X-ray spectral energy spectra obtained from points on sections (Fig. 1d) with indication of quantitative composition of element (0A – phosphorus, 1A – lead, 2A – calcium). a) energy spectra for calcium, lead, and phosphorus atoms obtained from point taken from membrane surface free from residue (peaks of osmium and phosphorus coincide and indicate osmium background); b) energy spectrum of calcium, lead, and phosphorus atoms obtained from points taken on precipitate of large crystals in mitochondria; c) energy spectra for calcium, lead, and phosphorus atoms obtained from point taken in region of postsynaptic thickening.

It should be noted that lead pyrophosphate, formed in the reaction for detection of adenylate cyclase activity, for which the substrate is exogenous, is similar in character. In all probability the fine-grain residue on the inner surface of the postsynaptic membrane remaining after treatment with ouabain, and frequently found during cholinolytic poisoning, was a compound of lead with pyrophosphate – the product of the adenylate cyclase-dependent conversion of ATP into cyclic AMP. The residue of large crystals, investigated by the microanalyzer in salivary gland mitochondria [5], which gave a well-marked peak for calcium, was interpreted by Berridge et al. as a calcium-containing residue.

X-ray structural analysis, carried out to verify the composition of the residues discovered in the sections, treated with ouabain, of the rat caudate nucleus showed that the precipitate of large crystals (Fig. 1d), localized in the mitochondria, gave significant peaks for calcium, phosphorus, and lead (Fig. 2b). Microanalysis of the fine-grain precipitate in the postsynaptic thickening of the same section showed similar but less-marked energy spectra (peaks) of calcium, phosphorus, and lead atoms (Fig. 2c). For control purposes, a microanalysis was made of a point on the membrane surface with no residue (Fig. 1d). In that case the level of the energy spectra of calcium, phosphorus, and lead did not exceed background level and no peaks of these elements were present (Fig. 2a).

Treatment with cholinolytics is known to lead not only to blocking of acetylcholine receptors, but also to a change in the liberation of acetylcholine by the nerve endings. Some investigations have shown similarity between the action of central M-cholinolytics and Ca^{++} under these circumstances [2, 4]. This calcium-like action of M-cholinolytics has been explained [12] by competitive interaction (a reduction in the charge) of the drugs with calcium ions on the membrane surface of these vesicles, leading to the blocking of exocytosis [8]. In this connection it can be suggested that in cholinolytic poisoning the calcium ions necessary for exocytosis are mainly utilized for exocytosis of catecholamine-containing vesicles which, in turn, leads to activation of the adenylate cyclase system in the postsynaptic neuron. In our investigations activation of this sort was found in the form of pyrophosphates, which themselves can perform the role of an active buffer system for Ca^{++}. On the other hand cyclic AMP, which activates protein kinase, leads to an increase in phosphorylation of the specific membrane protein [12]. Recent investigations [14] have shown that under conditions of phosphorylation of this protein, permeability for sodium and potassium is unchanged, whereas the inflow and outflow of ^{45}Ca through the synaptosomal membrane are sharply reduced (the so-called closure of the calcium pores). This phenomenon must also be taken into account during the discussion of our results (the formation of calcium complexes in the postsynaptic thickening).

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