ULTRASTRUCTURAL DISTRIBUTION OF ENZYMES OF CYCLIC NUCLEOTIDE METABOLISM IN THE MAMMALIAN BRAIN

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Neurotransmission in the CNS takes place with the aid of mediators, which are cyclic nucleotides. The regulation of these processes, as we know, is very complex and the principal role in mediator transmission is played by purine cyclic nucleotides; cAMP is involved in adrenergic and cGMP in cholinergic neurotransmission, and the nucleotides themselves perform independent functions in the CNS [7]. It is an interesting fact that cGMP induces interaction of acetylcholine with muscarinic receptors, thus confirming the hypothesis that this nucleotide is a mediator in the muscarinic action of acetylcholine [9, 10]. However, cAMP has a wider spectrum of action, and all adrenergic neurotransmission is effectively carried out with its aid. Activation of enzymes of the cyclic nucleotide system is the key factor in the triggering of these processes.

Discovery of the distribution of enzymes participating in synthesis and hydrolysis of cyclic nucleotides, namely adenylate cyclase (AC), guanylate cyclase (GC), and cAMP- and cGMP-dependent phosphodiesterase (PDE), which may be unique markers of synapses responsible for adrenergic and cholinergic neurotransmission, is therefore a promising approach to the study of the synaptoarchitectonics of the brain.

This paper describes a study of the localization of the above-mentioned enzymes in the mammalian brain, in the synaptosomes of the rat brain, and in preparations of isolated synaptic membranes.

EXPERIMENTAL METHOD

Experiments were carried out on 15 rats, 12 mice, 8 rabbits, 9 guinea pigs, and 7 dogs. Synptosomes were isolated from the rat brain by the method in [8] and synaptic membranes by the method in [6] with modifications [4]. The preparations thus obtained, as well as pieces of brain tissue (cortex, caudate nucleus, hippocampus, cerebellum), were prefixed by the immersion method in a mixture of 1% glutaraldehyde and 4% paraformaldehyde made up in 0.05 M cacodylate buffer with glucose, selected, incubated in the appropriate medium, washed in 0.08 M Tris-maleate buffer with glucose, fixed in a 1% solution of OsO_4 in 0.05 M cacodylate buffer with glucose, then dehydrated and embedded in Vestopal. The material was incubated at 37°C for 20-50 min in appropriate incubation media. The medium for detection of AC: 80 mM Tris-maleate buffer, 8% glucose, 2 mM magnesium sulfate, 2 mM theophylline, 0.5 mM adenylyl imidodiphosphate, 2 mM lead nitrate or acetate. The controls for detection of AC were: first — with activation: addition of 10 mM sodium fluoride, 10^{-6} M glucagon, or 10^{-4} M adrenalin to the incubation medium; second — with inhibition: addition of 10 mM alloxan or insulin to the incubation medium; third — incubation in medium without substrate [2]. The medium for detection of GC was: 80 mM Tris-maleate buffer, 8% glucose, 0.1 mM manganese chloride, 1 mM theophylline, 0.1 mM guanylyl imidodiphosphate, 2 mM lead or barium nitrate. The controls for detection of GC were: first — with activation: addition of 1 mM sodium azide or nitrate to the incubation medium; second — with inhibition: addition of 10^{-6} M chloromercuriphenylsulfonate to the incubation medium; third — incubation in medium without substrate [5].

To carry out the reaction for PDE, it was first necessary to preincubate in medium with 5'-nucleotidase: lyophilized toxin of *Crotalus atrox*, dissolved in 0.08 M Tris-maleate

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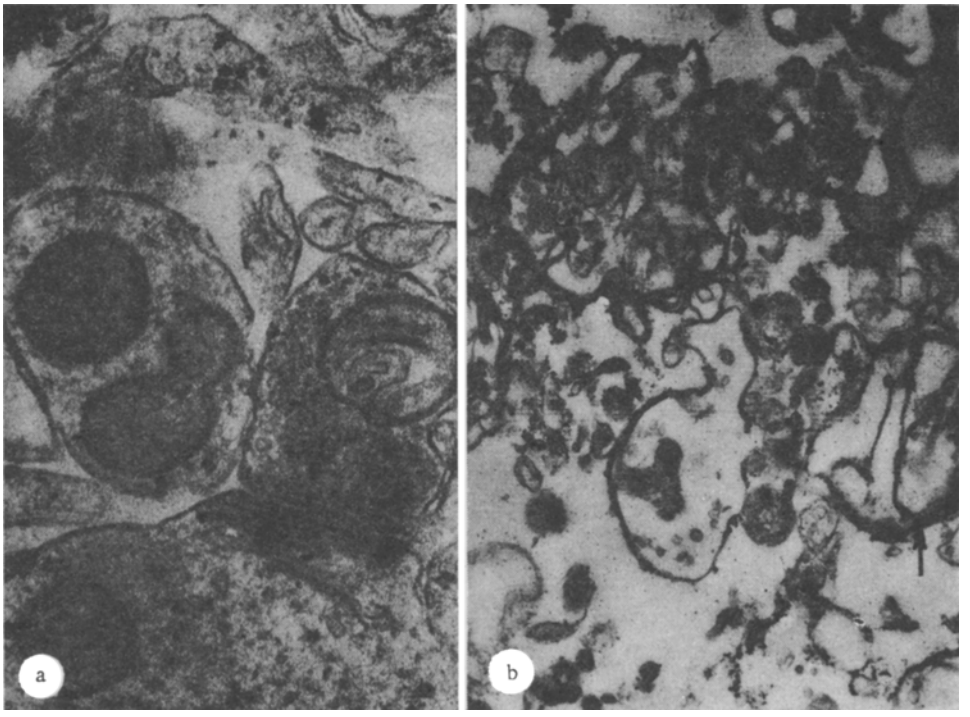

Fig. 1. Location of AC in synaptic formations from rat caudate nucleus: a) in pre- and postsynaptic formations of axodendritic synapse. 50,000×; b) in synaptosomal membranes (arrows). 26,000×.

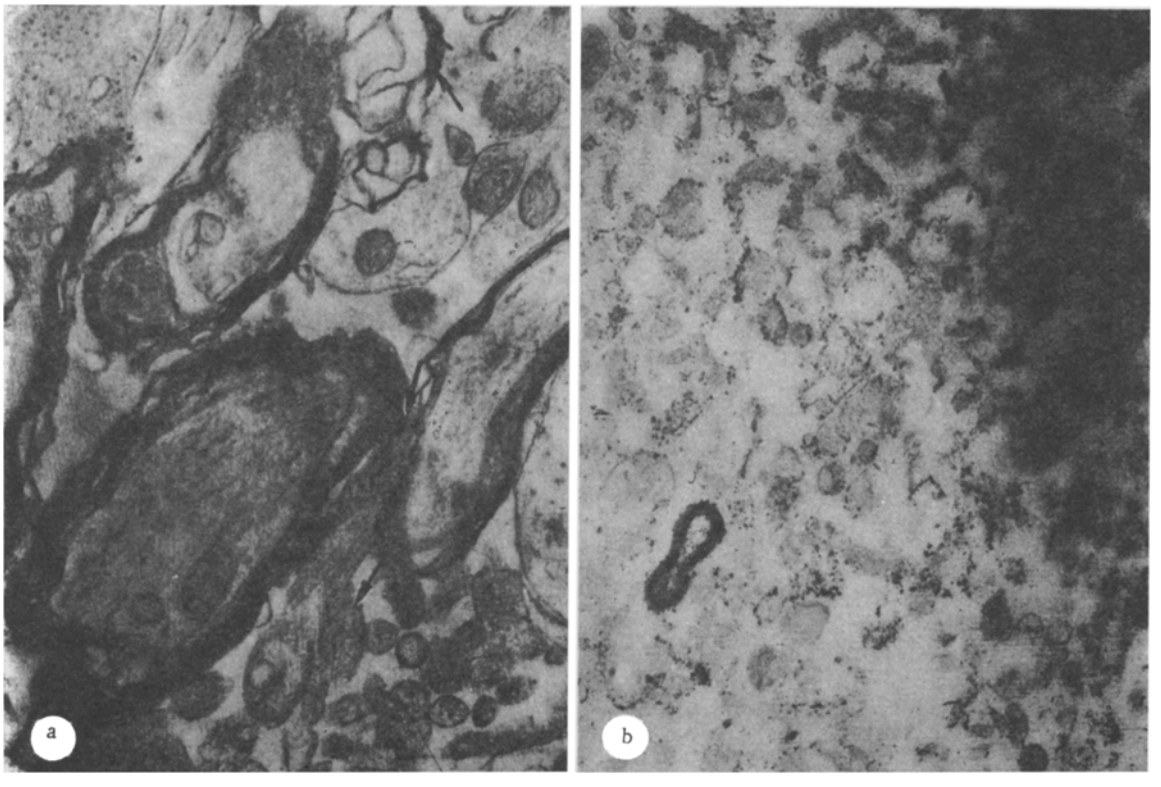

Fig. 2. Localization of GC in synaptic and myelin structures from rat cerebral cortex: a) in synaptosomal membranes, in matrix of synapses, and in membranes of myelin structures (arrows). 30,000×; b) In synaptosomes and myelin structures. 26,000×.

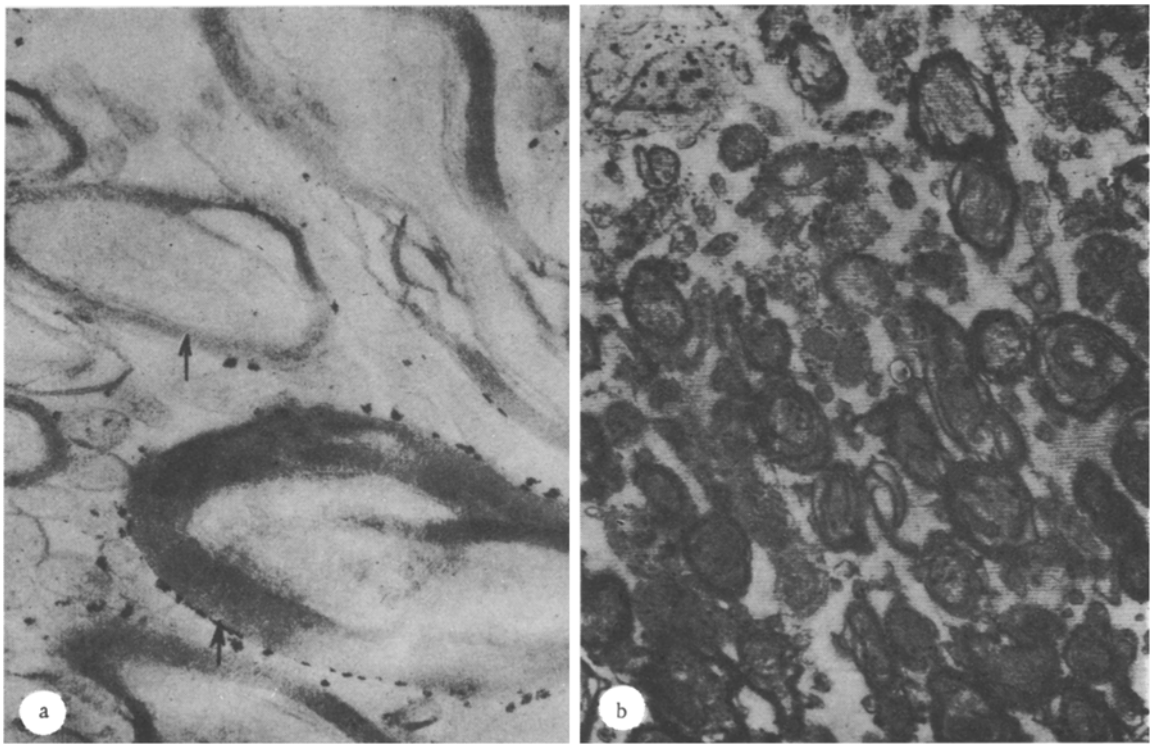

Fig. 3. Localization of PDE in membranes of myelin structures of rat brain: a) in cerebral cortex (arrows). 40,000×; b) in caudate nucleus. 17,000×.

buffer (pH 7.4) — 3 mg/ml, 4% glucose, 2-3 mM magnesium chloride. Preincubation was carried out at 37°C for 15-20 min. The material was then incubated in medium of the following composition: 0.08 M Tris maleate buffer, 4% glucose, 2 mM magnesium chloride, 0.5 mM cAMP, 2 mM lead nitrate, 1-3 mg/ml of *Crotalus atrox* toxin. The controls for detection of PDE were: first — with activation: addition of 10 mM imidazole or triazole to the incubation medium; second — with inhibition: addition of 30 mM theophylline to the incubation medium; third — preincubation without *Crotalus atrox* toxin, principal incubation in Tris-maleate buffer with glucose, 2 mM lead nitrate, and 3 mM 5'-AMP; fourth — incubation in medium without substrate [1].

EXPERIMENTAL RESULTS

The highest AC activity in different parts of the mammalian brain was found in synaptic formations in the cortex and caudate nucleus. In these regions precipitates were observed most frequently in the postsynaptic condensations of axo-dendritic synapses, less frequently in presynaptic formations. In some synapses, precipitates reflecting the location of AC were found in both membranes. Quite often the reaction product for AC could be found on membranes of synaptic vesicles (Fig. 1a). Distribution of the enzyme could be observed in membranes of endotheliocytes and erythrocytes from the capillaries carrying blood in the brain. AC was found to be present in 70-80% of preparations of isolated cerebral cortical synaptic membranes, an indication of high affinity of this enzyme for membranes. The distribution of AC in synaptosomes from different brain regions was the same as in whole brain tissue (Fig. 1b), evidence of the specificity of the reaction. It was shown by an electron-cytochemical method that GC is located both in membranes and, in the free state, in the cytoplasm of neurons. GC activity was much lower than AC in the tissues of the brain structures investigated. The enzyme was found in synaptosomal membranes, in the matrix of the synapses, in the cytoplasm of nerve cells, and also in myelin structures (Fig. 2a). A weak reaction for GC was found in the cytoplasm of the endothelial cells of the blood capillaries in the brain. The enzyme was found to be present in 30-40% of all isolated cortical synaptic membranes. The character of the GC distribution in synaptosomal preparations was similar to its distribution in sections through corresponding brain regions (Fig. 2b).

The highest PDE activity in mammals was found in the region of the cortex and caudate nucleus (Fig. 3a, b). An active reaction for the enzyme was found in the nuclei of the

neurons and on membranes of synapses and myelin structures. PDE was present both on membranes of synaptosomes and in the neurosecretory granules observed in these fractions.

These findings are important in connection with the intensive study of cytoreception currently in progress, for most neurotransmitters, like peptide hormones, exert their action through contact with specific receptors, mainly located on the plasmalemma, less frequently in the cytoplasm. Some enzymes, linked with receptors, undertake transduction of the action of hormones and neurotransmitters [3]. The enzymes which we studied belong to this category. The data on the localization of AC, GC, and PDE provide some information on the distribution of cytoreceptors, corresponding to particular neurotransmitter systems, in the CNS.

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