ELECTRON-CYTOCHEMICAL STUDY OF RAT BRAIN ADENYLATE CYCLASE

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Adenylate cyclase (E.C. 4.6.1.1) is an enzyme which, although with low activity, has high sensitivity toward many hormones, mediators, nucleotides, polypeptides, and other substances. Through their action on adenylate cyclase, these substances modify the intracellular cyclic AMP concentration and thus induce specific responses in the cell. The importance of this reaction makes it essential to obtain histochemical data on the localization of adenylate cyclase in brain tissues. Reik et al. [6] first used a lead technique [7] for the electron-cytochemical detection of adenylate cyclase in liver cells. They used ATP as substrate. Later, the introduction of a synthetic ATP analog, namely adenylyl imidodiphosphate (AMP-PNP) into this reaction [4] considerably improved its specificity and increased the reliability of the method, for unlike ATP, this substrate is resistant to ATPase and is not utilized by most ATP-hydrolyzing enzymes.

The object of this investigation was to study the ultrastructural localization of adenylate cyclase in various cells of the rat cerebral cortex and caudate nucleus.

EXPERIMENTAL METHOD

Pieces of albino rat brain tissue measuring 3-4 mm^3 were fixed for 20 min in a freshly prepared 4% solution of paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3). The specimens were then cut into small pieces measuring 1 mm^3 and washed for 4-24 h in 0.1 M Tris-maleate buffer (pH 7.3). To detect adenylate cyclase activity pieces of brain tissue were incubated for 30 min at 30°C in medium of the following composition: 80 mM Tris-maleate buffer (pH 7.3), 0.6 mM adenylyl imidodiphosphate (from Böhringer, West Germany), 2 mM $MgSO_4$, 2 mM $Pb(NO_3)_2$, and 5 mM theophylline. Adenylate cyclase activity was activated with 10 mM NaF and 1 mM isoproterenol, in the presence of 10^{-5} M guanylyl imidodiphosphate. Control fragments were incubated in medium without substrate and with the addition of 10 mM alloxan, an adenylate cyclase inhibitor. After incubation the fragments were washed in 0.1 M cacodylate buffer, fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4 for 30 min, dehydrated in ethanol, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on LKB (Sweden) and Reichert (Austria) ultramicrotomes and studied in the EM 201 (Philips, The Netherlands) electron microscope.

EXPERIMENTAL RESULTS

Examination of the distribution of adenylate cyclase in neurons of the cortex and caudate nucleus revealed deposition of lead phosphate, indicating a site of enzyme activity, on the outer surface of the plasma membrane, in the intercellular spaces, in the perinuclear space, and in the tubules of the endoplasmic reticulum and lamellar complex (Fig. 1a). A similar distribution of adenylate cyclase activity was observed in the glial cells of the cortex and caudate nucleus.

Addition of 10 mM NaF or 1 mM isoproterenol to the incubation medium led to a marked increase in enzyme activity, expressed as an increase in the quantity of precipitate, but did not affect the distribution of the reaction product (Fig. 1b). Addition of 10 mM alloxan to the incubation medium led to the almost complete inhibition of enzyme activity.

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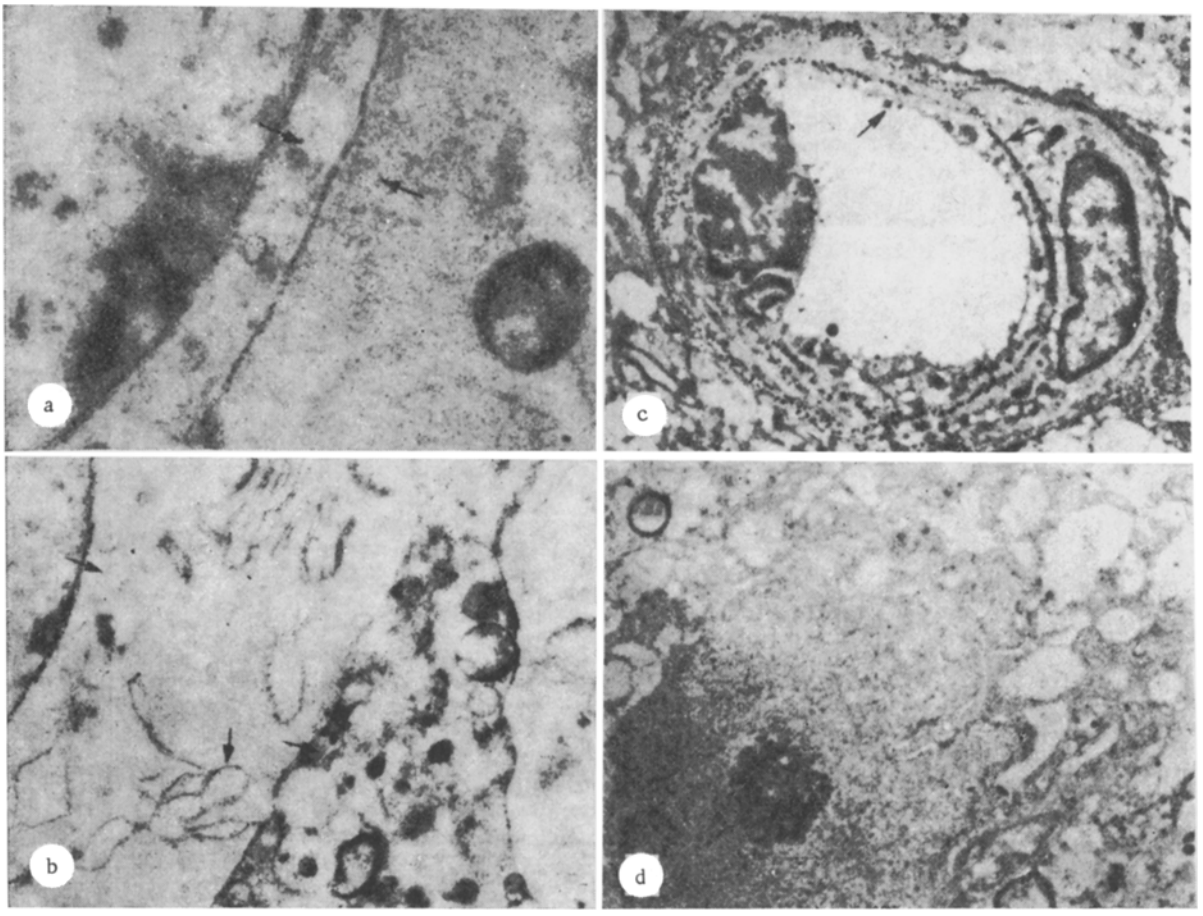

Fig. 1. Localization of adenylate cyclase activity in rat brain: a) on plasma membrane and in perinuclear space of cortical neuron, 20,000 ×; b) on plasma membrane, in endoplasmic reticulum and perinuclear space of cortical neuron. Incubation with isoproterenol, 7000 ×; c) on basement membrane and on luminal surface of capillary endothelial cell from caudate nucleus, 7000 ×; d) inhibition of adenylate cyclase activity in cortical neuron. Incubation with alloxan. Arrows indicate locations of adenylate cyclase activity.

No precipitate of lead, indicating the site of enzyme activity, could be found in the incubation medium without substrate (Fig. 1c).

Most synapses in this material were free from enzyme activity, but sometimes precipitate was found in the synaptic space, indicating that adenylate cyclase activity was present there. The apparent absence of adenylate cyclase in the synapses may be due either to the inactivating effect of the fixative or to the presence of adenylate cyclase not activated by isoproterenol and fluorine ions in the synapses. It is known, for example, that dopamine-sensitive adenylate cyclase is associated mainly with the synaptic membrane fraction [3].

Adenylate cyclase activity also was found in the brain capillaries. The reaction product was detected in the form of tiny spots along the luminal surface of the endothelial cells, in the micropinocytotic vesicles, and along the basement membrane (Fig. 1d). This localization of adenylate cyclase activity in the capillaries suggests that this enzyme is concerned in the regulation of brain capillary permeability.

Most workers who have studied the ultrastructural localization of adenylate cyclase in the liver [6], in pancreatic islet cells [4], and in the rat cerebral cortex [5], found the reaction product only on the outer surface of the plasma membranes of the cells. We found adenylate cyclase activity in the perinuclear space, the endoplasmic reticulum, and the lamellar complex. The increase in enzyme activity after stimulation by isoproterenol and fluorine ions and inhibition of activity in the presence of alloxan are evidence of the reliability of the method and the localization of significant amounts of enzyme not only on the plasma membrane, but also in the perinuclear space and the tubules of the endoplasmic

reticulum and lamellar complex. The failure of previous investigators to find adenylate cyclase activity in the intracellular structures of the cell, in our opinion, may be due to the effect of the prolonged fixation, the use of ATP as substrate, and also, perhaps, to other causes. Evidence of the intracellular localization of adenylate cyclase also was given by the results of other studies [1, 2, 8] in which activity of hormone-sensitive adenylate cyclase was found in the fraction of membranes of the endoplasmic reticulum and lamellar complex from hepatocytes and in isolated thymocytes.

The results of the present investigation thus indicate that adenylate cyclase is present not only on the plasma membrane, but also in the intracellular structures of brain neurons and glial cells.

LITERATURE CITED

1. I. B. Bukhvalov, in: Abstracts of Proceedings of the 11th All-Union Conference on Electron Microscopy [in Russian], Vol. 2, Moscow (1979), p. 86.
2. H. Cheng and M. G. Farquhar, J. Cell Biol., 70, 671 (1976).
3. Y. Clement-Cormier and R. George, Life Sci., 23, 539 (1978).
4. S. L. Howell and M. Whitfield, J. Histochem. Cytochem., 20, 873 (1972).
5. F. Joo, I. Thot, and G. Jancso, Naturwissenschaften, 62, 397 (1975).
6. L. Reik, G. L. Petzold, J. A. Higgins, et al., Science, 168, 382 (1970).
7. M. Wachstein and E. Meisel, Am. J. Clin. Path., 27, 13 (1957).
8. W. N. Yunghaus, Cytobiologie, 17, 212 (1978).

MORPHOMETRIC STUDY OF PURKINJE CELLS IN THE DOG CEREBELLAR CORTEX

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The cerebellum, which plays an important role in reflex responses of the brain stem and higher levels of the CNS, not only performs the function of "movement modulator" [3], but also, as experimental and clinical investigations [15] have shown, it participates in sensory integration, in learning skilled movements, in visual and auditory discrimination, regulation of emotions and the level of wakefulness, and pain perception. The Purkinje cells (PC) are the only neurons in the cerebellum along whose axons information leaves the cerebellum. That is why information on their number and structural state is essential in order to understand the functions of this part of the brain. It must be recalled that although close together, or even neighboring, PC have different specific functions [3], i.e., the PC population is heterogeneous. Investigations by various morphological techniques [12, 14] have revealed pale, dark, and intermediate PC. However, their arrangement in the cerebellar cortex is not yet known.

The object of this investigation was to study the distribution of different types of PC in three sagittal zones of the cerebellum, differing in their physiological characteristics [6].

EXPERIMENTAL METHOD

Experiments were carried out on seven mature mongrel dogs of both sexes. The brain was removed from the skull immediately after the animals had been killed under electrical anesthesia. The right half of the cerebellum was divided in the sagittal plane into three parts, corresponding to medial (I), intermediate (II), and lateral (III) zones [9]. After

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