thickness and had distinct outlines. The content and distribution of RNA, glycogen, and hyaluronic acid in the nerve cells was normal again.

Differences between the numbers of changed neurons in the ganglia of the experimental and control animals were not statistically significant ($P>0.5$).

Preparation of the teeth for crowning thus induces reversible changes in some neurons, intraganglionic nerve fibers, and synaptic endings in the trigeminal and superior cervical sympathetic ganglia and the inferior ganglion of the vagus nerve on the side of the operation. The nerve cells of the gasserian ganglion undergo more varied and intensive changes. This can evidently be explained by its anatomical and physiological features and by the velocity and level of flow of afferent impulses. The relative resistance of unmyelinated nerve fibers compared with myelinated was observed and may perhaps be due to differences in the velocity of conduction of nociceptive impulses in them. The results of the investigation demonstrate the high plasticity and functional adaptability of these ganglia and they can serve as original data for the experimental evaluation of the effectiveness of existing methods of anesthesia in orthopedic stomatology and methods in process of development.

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ELECTRON-HISTOCHEMICAL STUDY OF THE LOCALIZATION OF ADENYLATE CYCLASE AND ACETYLCHOLINESTERASE IN SYNAPSES OF THE CORTEX AND BASAL GANGLIA OF THE RAT BRAIN

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Synapses of the cerebral cortex and basal ganglia of rats were studied by electron-histochemical reactions for adenylate cyclase and acetylcholinesterase. On the basis of the characteristics of the presynaptic terminal vesicles and the localization of the two enzymes in the synapse receptor area three types of synapses were identified; cholinergic, adrenergic, and mixed.

KEY WORDS: types of synapses; adenylate cyclase; acetylcholinesterase.

During analysis of the cytopharmacological effect of neurotropic agents changes in the synapses play the leading role [2, 4]. The division of synapses into cholinergic and adrenergic [1, 4], adopted in modern enurobiology, is based on biochemical and pharmacological data and takes account mainly of the neuromediator present in the presynaptic terminal.

The object of the present investigation was to develop a cytochemical model of a central synapse on the basis of the results of detection of adenylate cyclase (model of an adrenergic receptor) [5] and acetylcholinesterase (model of a cholinergic receptor) [3]. The results were generalized with allowance for both the receptor discovered and the neurotransmitter contained in the vesicles of the presynaptic terminal.

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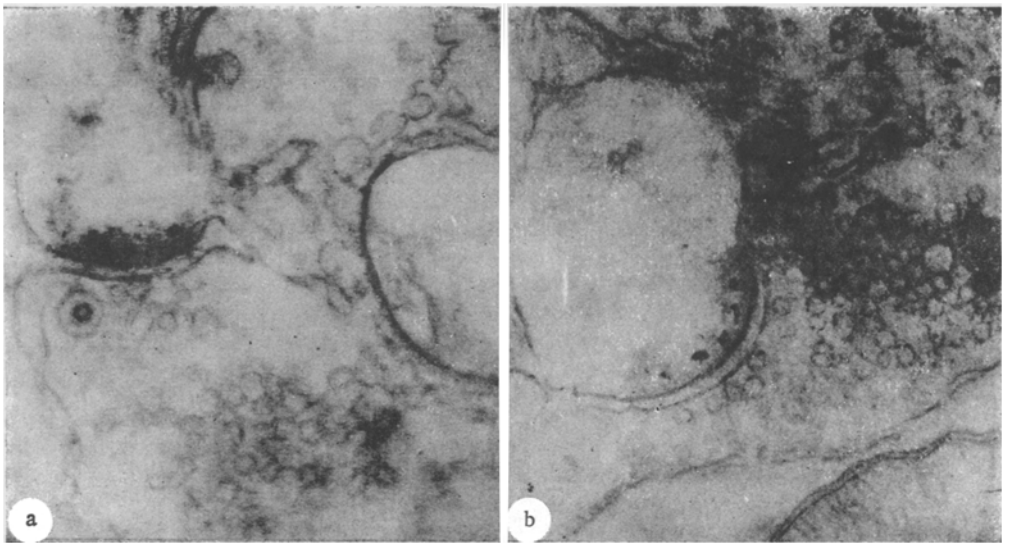

Fig. 1. Localization of adenylate cyclase activity in central synapses. Unstained sections: a) deposition of reaction product (arrow) in postsynaptic condensation of oxo–dendritic synapse of rat caudate nucleus. Granular vesicle (GV) can be seen in presynaptic terminal (60,000×); b) deposition of reaction product (arrow) in postsynaptic condensation of axo-dendritic synapse in rat cerebral cortex. Terminal contains only agranular vesicles (AV; 70,000×).

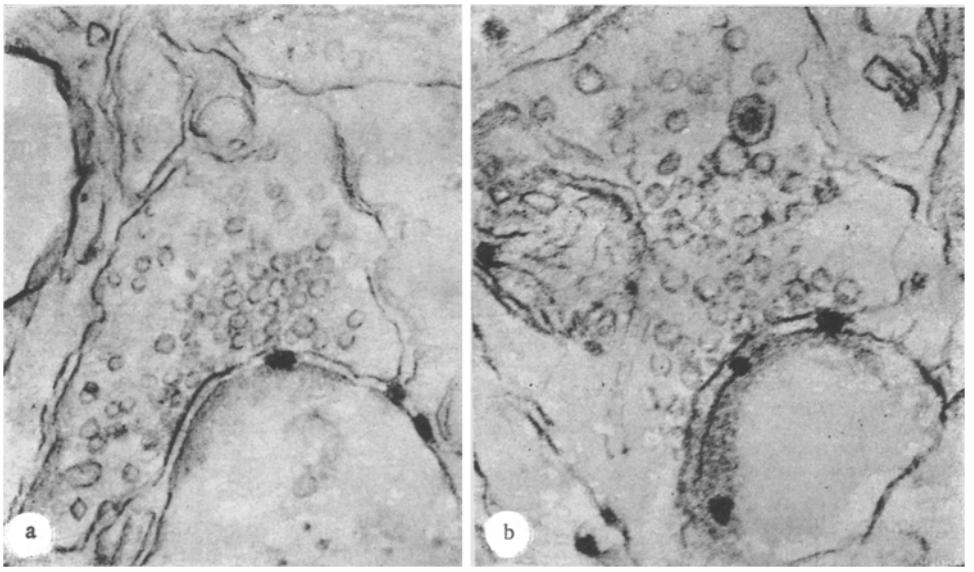

Fig. 2. Localization of acetylcholinesterase activity in central synapses. Sections stained with lead citrate: a) axo–dendritic synapse of rat cerebral cortex. Terminal contains only angular vesicles (AV). Reaction product can be observed in synaptic space (arrow; 60,000×); b) axo–dendritic synapse of rat caudate nucleus. Terminal contains granular vesicles (GV). Reaction product present in synaptic space (arrow; 80,000×).

EXPERIMENTAL METHOD

Adenylate cyclase activity was determined in 10 male Wistar rats weighing 180 g. The animals were decapitated. Pieces of the temporal cortex and central part of the caudate nucleus were fixed in 1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4), containing 4.5% glucose. Fixation continued for 1 h at room temperature. The tissue was then washed several times in the same buffer and allowed to stand overnight at 4°C. After sorting, suitable pieces

of tissue were incubated by the method of Howell and Whitfield [6], with the addition of 10 mM sodium fluoride to the incubation mixture as the control. After incubation the material was quickly washed in 0.05 M Tris-maleate buffer, pH 7.4, and then postfixed in 1% osmium tetroxide solution in 0.05 M cacodylate buffer, with glucose at the same pH for 1 h at 4°C. The tissue was then washed in 0.05 M cacodylate buffer with glucose (pH 7.4). A control for detection of enzyme activity was used at the same stages, butwithout the use of the incubation mixture. Pieces of tissue were dehydrated in acetones and embedded in a mixture of Epon and Araldite. Sections were examined in the electron microscope.

Acetylcholinesterase was determined in 8 male Wistar rats weighing 180 g. Sections obtained from pieces of cortex and ventral part of the caudate nucleus were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, by Karnovsky's technique [7]. Acetylcholine iodide was used as the substrate. Isopropyl pyrophosphoramide was added to the incubation medium in a concentration of $2 \cdot 10^{-4}$ M as an inhibitor of nonspecific esterases. Eserine sulfate ($2 \cdot 10^{-4}$ M) was used as the control for the specific acetylcholinesterase. Sections incubated without the substrate also served as controls. Incubation continued for 1 h at 4°C. After incubation the sections were postfixed in 1% osmium tetroxide solution in 0.1 M phosphate buffer, pH 7.4. The material was dehydrated in acetones and embedded in a mixture of Epon and Araldite. Ultrathin sections were examined in the electron microscope.

EXPERIMENTAL RESULTS

During analysis of the cytochemical reaction for adenylate cyclase activity in the test material it was found that in individual synapses of the cortex and caudate nucleus the reaction product [lead pyrophosphate (a fine-grained electron-dense precipitate)] appeared on the inner surface of the postsynaptic membrane (in the postsynaptic condensation). Under these circumstances the terminals of the synapses contained either agranular with solitary granular vesicles (Fig. 1a) or agranular vesicles only (Fig. 1b).

The final reaction product for acetylcholinesterase — iron ferrocyanide (the electron-dense precipitate) — was localized in the synaptic spaces. Moreover, either agranular vesicles only were contained in the terminals of the synapses with this reaction (Fig. 2a) or solitary granular vesicles were present along with agranular (Fig. 2b).

On the basis of these results three types of synapses can thus be conventionally distinguished: 1) cholinergic (synapses with acetylcholinesterase activity in the synaptic space and with agranular vesicles only), 2) adrenergic (synapses with adenylate cyclase activity in the postsynaptic condensation and with solitary granular vesicles in the terminal), 3) mixed (synapses without granular vesicles in the presynaptic terminal, but with adenylate cyclase activity in the postsynaptic condensation, and also synapses with acetylcholinesterase activity in the synaptic space and containing both agranular and solitary granular vesicles).

The existence of synapses of mixed type and their general typical features can be regarded as conditional, for absolute grounds do not exist for precise correlation between the morphological characteristics (agranular and granular vesicles) and the biochemical data (catecholamines and acetylcholine).

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