LOCALIZATION OF PROTEINS SYNTHESIZED DURING BENACTYZINE ADMINISTRATION IN THE BRAIN CELLS

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Brain proteins whose synthesis was induced by the central muscarinic cholinolytic drug benactyzine were located mainly in membrane-containing structures of nerve cells: plasma membranes, axons, and dendrites.

In previous investigations the writers [2] showed that administration of the central muscarinic cholinolytic benactyzine to animals (2-4 mg/100 g body weight) activates the synthesis of protein and RNA in the brain. The effect is most marked at the height of blocking of the central muscarinic cholinergic receptors and it disappears gradually as the blocking is removed.

One way to solve the problem of the functional role of a particular synthesis and the nature of the proteins synthesized under the influence of a cholinolytic drug is to study their intracellular localization. The present investigation was carried out for this purpose.

EXPERIMENTAL METHOD

In experiments on male rats weighing 150-200 g benactyzine was injected intraperitoneally in a dose of 4 mg/100 g body weight. In this dose benactyzine completely blocks central muscarinic cholinergic receptors for up to 5 h [3]. During this period conditioned-reflex activity of the animals is disturbed and they are unable to learn. Control animals received water only. Groups of control and experimental animals in each series consisted of at least eight animals, and the total number of series was ten. 1-C^{14}-Glycine (25 μCi/100 g) or 1-C^{14}-acetate (20 μCi/100 g) was injected intraperitoneally 1 h after the benactyzine or water, and 1 h later the animals were sacrificed by decapitation. The brain was removed and homogenized in ten volumes 0.25 M sucrose. (Here and later all volumes were calculated per weight of original tissue.) The method used to isolate the nuclei and membrane fragments of the cells (debris) was based on Chauveau's method [4]. The nuclei and membrane fragments were sedimented from the homogenate by spinning for 10 min at 800 g. The residue was washed by gentle homogenization in 5 volumes 0.32 M sucrose followed by centrifugation (10 min at 800 g). The washed residue was resuspended in 3 volumes 2 M sucrose and centrifuged for 40 < min at 75,000 g. Two fractions were obtained: the bottom residue (nuclei) and the floating residue (debris). Mitochondria, synaptosomes, and myelin were isolated by Whittaker's method [5]. To separate the microsomes and hyaloplasm the supernatant (after sedimentation of large structures at 20,000 g, 15 min) was centrifuged for 60 min at 75,000 g.

EXPERIMENTAL RESULTS

Under the influence of benactyzine a slight tendency, not statistically significant, toward activation of incorporation of C^{14}-amino acids into proteins (100-120% of the control level) was observed in membrane-containing cell structures: nerve endings (synaptosomes), mitochondria, and nuclei. Incorporation of C^{14}-amino acids into myelin proteins during benactyzine administration did not exceed, or was below, the control level (80-100%).

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Maximal and statistically significant activation of incorporation of C^{14}-amino acids into proteins was found in the debris: after injection of C^{14}-glycine activation by 48% (control 9.5 ± 1.7, experiment 14.2 ± 3.2 pulses/min/mg protein); after administration of C^{14}-acetate activation by 31% (control 6.5 ± 1.16, experiment 8.5 ± 1.69 pulses/min/mg protein). The debris was the total membrane fraction, consisting of large fragments of myelin, plasma membranes, and fragments of axons and dendrites. Bearing in mind that no increase in incorporation of the label was found in isolated myelin it can be assumed that proteins newly synthesized under the influence of the cholinolytic were localized predominantly either in the plasma membranes or in the axons or dendrites.

Activation of incorporation of C^{14}-amino acids into proteins of the hyaloplasm, i.e., the soluble part of the cytoplasm of nerve cells and microsomes (endoplasmic reticulum), was not statistically significant, namely 110 and 105% of the control level respectively.

The results thus suggest that brain proteins whose synthesis is induced by the cholinolytic are localized in the membranes, most probably in the outer plasma membrane, as reflected in the maximal and statistically significant level of activation in the total membrane-containing fraction (the debris).

This finding is of fundamental importance for the following reasons. First, interaction between neurons, which is blocked by the cholinolytic, takes place ultimately by contact between membranes (synapse), and the conduction of the nervous impulse is a membrane process; second, the structure of benactyzine (the diethylaminoethyl ester of diphenylglycolic acid) suggests that it may possibly interact with lipoprotein membranes on account of the hydrophobic groups in its molecule. In particular, Abood [6] has demonstrated interaction of this type between diphenylglycolates and membranes on model systems. The activation of protein synthesis in membranes during the action of benactyzine can be regarded as a repair process on the protein structures of neuron membranes which have been put out of action.

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