INVOLVEMENT OF SYNTHESIS OF BRAIN CELL STRUCTURAL PROTEINS IN THE ACTION OF ANTITHEINES ON LONG-TERM MEMORY

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An important role in the mechanisms of action of the known nootropic drugs is ascribed to macromolecular synthesis [3]. An increase in the intensity of uptake of labeled precursors into proteins of a cell suspension from certain parts of the brain has been demonstrated experimentally. In the modern view, long-term retention of the memory trace is connected with activation of protein synthesis in the region of synaptic boutons [1]. Research workers are interested not least in the role of intracellular formations, notably structures of the endoplasmic reticulum, in the mechanism of integrative functions of the neuron. There is evidence that weakly bound microsomal surface proteins of animals exhibit reactivity during learning [4].

The aim of this investigation was to study the effect of structural analogs of ethylnorantitheine (ethimizole), a substance with nootropic type of action, on protein metabolism in various intracellular structures of rat brain neurons.

EXPERIMENTAL METHOD

Male albino rats weighing 200-220 g were used. Nuclei were isolated from cerebral cortical neurons as described previously [5]. Fractions of the smooth and rough reticulum were isolated by differential centrifugation in medium containing 0.32 M sucrose, 0.025 M Tris-HCl, pH 7.5 and 0.005 M $MgCl_2$. Weakly-bound microsomal surface proteins were obtained by extraction with 0.14 M NaCl. Synaptic membranes and mitochondria were obtained by ultracentrifugation in a stepwise sucrose density gradient [9]. The purity of the synaptic membranes thus obtained was verified by electron microscope.

The test substances were injected intraperitoneally in a dose of 3 mg/kg body weight 3 h before decapitation. Control animals received physiological saline. Rats of the control and experimental groups received ^{3}H-leucine (450 TBq/mole) by intraperitoneal injection in a dose of 5 MBq/kg body weight. In some experiments a mixture of ^{3}H-leucine and ^{3}H-glycine, each in a dose of 2.5 MBq/kg, was used. Specific radioactivity was determined after treatment with TCA only in the TCA-insoluble part of the isolated cell structures in Bray's scintillator on a Rack-Beta counter (LKB, Sweden). Protein was determined by Lowry's method [10]. The results were subjected to statistical analysis by the Microstat program (Ecosoft Inc.), with calculation of Student's t.

EXPERIMENTAL RESULTS

The study of the molecular mechanisms of action of antitheines revealed definite correlation between their effects on memory and their effect on the RNA-synthesizing activity of rat brain neurons [6, 7]. Preparations lowering

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TABLE 1. Effect of Antitheines on Incorporation of ^{3}H-Leucine into Different Subcellular Fractions of the Rat Brain (M ± m, n = 4)

Substance	Incorporation of ^{3}H-leucine (pmoles/mg protein, % compared with control)					
	synapto-somes	mito-chondria	nuclei of neurons	SP of smooth reticulum	Smooth reticulum – SP	Rough reti-culum
Control	1851±86	2210±149	2879±131	1363±90	1650±94	3370±172
M_1	133*	92	89	92	73*	91
M_2	118*	83	84	90	71*	98
Ethylnorantitheine	106	76*	81*	78*	75*	93
Allylnorantitheine	107	68*	84*	91	80*	119*
Propylnorantitheine	107	73*	73*	86*	81*	124*

Legend. SP) Weakly bound surface proteins, *$p < 0.05$.

the level of RNA synthesis (allyl- and propylnorantitheins) impair long-term memory, whereas substances stimulating it (ethylnorantitheine and its demethylated derivates – M_1 and M_2), make skill storage more effective. To determine the ability of antitheines to cause changes in protein synthesis of importance for preservation of the memory trace, we studied incorporation of labeled precursors into various intracellular structures of cerebral cortical neurons in rats. The amino acids were injected 60 min after systemic injection of the antitheines (when marked changes were found in RNA-synthesizing activity [5], and fractionation of the intracellular structures began 60, 120, and 180 min thereafter).

A single injection of ethylnorantitheine and its structural analogs was accompanied by significant changes in protein metabolism in all cell structures after 120 min of exposure with labeled amino acids (and 180 min with the antitheines), i.e., within the time interval when maximal incorporation of amino acids takes place and when activity of catabolic processes is low [11] (Table 1). With shorter (60 min) or longer (180 min) exposure times with labeled amino acids and antitheines, a similar tendency was observed for changes in ^{3}H-leucine uptake. In most cortical and hippocampal cell structures studied no definite relationships could be found between the effect of antitheines on the intensity of ^{3}H-leucine and their action on the RNA-synthesizing activity of neurons and the efficiency of conditioned reflex storage. For instance, all the antitheines caused a decrease in incorporation into proteins of the smooth reticulum, ethyl-, allyl-, and propylnorantitheines significantly reduced the rate of incorporation of the label into nuclear and mitochondrial proteins, and only ethylnorantitheine reduced incorporation of the labeled precursor into the microsomal surface proteins. Allyl- and propylnorantitheines stimulated protein synthesis in the fraction of the rough reticulum.

There is evidence in the literature that the ability of ethylnorantitheine (ethimizole) to increase the length of skill preservation corresponds to the appearance of morphological features characterizing the active state of the synapses [2]. However, we were unable to find changes in ^{3}H-leucine incorporation into synaptosomal proteins under the influence of this substance. Only administration of M_1 and M_2 – substances with the strongest positive action on long-term memory and RNA synthesis [8], led to a significant increase in synthesis of synaptosomal proteins. Demethylated derivatives of ethimizole, unlike the original preparation, have virtually no effect on the cAMP level and possess weaker pharmacological activity [7]. In that case it can be postulated that the high membranotropic activity of ethimizole masks the activating action of the drug on the intensity of incorporation of labeled amino acids into synaptosomal proteins.

The results confirm modern views on the key role of synaptosomal protein synthesis in mechanisms of the higher integrative functions of the brain, namely learning and memory, and they are evidence of the need to study the effect of biologically active substances on protein metabolism not only in different parts of the brain, but also in its intracellular structures.

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ROLE OF CALCIUM IN THE ANALGESIC EFFECT OF NONNARCOTIC ANALGESICS AND CALCIUM CHANNEL BLOCKERS

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Evidence has recently been published that calcium ions are involved directly or indirectly in the control of nociceptive sensitivity [4, 5, 10]. For instance, the antinociceptive action of various inorganic and organic calcium antagonists has been demonstrated [6, 8, 10]. Meanwhile, no attempt has yet been made to analyze data on quantitative relationships between the strength of the analgesic action of different substances and their ability to block calcium transport.

The aim of this investigation was to compare the analgesic action of calcium antagonists (verapamil, fenigidin) and the promising nonnarcotic analgesic PV-107 with their effect on calcium transport.

EXPERIMENTAL METHOD

The analgesic activity of the substances was studied by the hot plate test [9]. Experiments were carried out on noninbred male albino mice weighing 22 ± 2 g, kept on a standard diet and receiving food and water ad libitum. The test substances, namely verapamil (USSR), fenigidin (East Germany), and PV-107 (a promising nonnarcotic analgesic developed at the Kiev Research Institute of Pharmacology and Toxicology, Ministry of Health of the Ukrainian SSR) were dissolved in isotonic sodium chloride solution with the addition of 5% Tween-20, and injected intramuscularly into animals in a volume of 0.2 ml.

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