EFFECT OF ANTITHEINES WITH DIFFERENT EFFECTS ON MEMORY ON cAMP PHOSPHODIESTERASE, LIPID PEROXIDATION, AND RNA SYNTHESIS IN RAT BRAIN NEURONS

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Among the various physiological and metabolic effects of the antitheines, attention has been drawn to the dissimilar action of structural analogs of ethylnorantitheine (ethimizole) on long-term memory, which is largely linked with their effects on the genetic apparatus of neurons [2, 7]. However, the problem of structural and functional changes in the outer cell membrane under the influence of these drugs remains virtually unstudied.

The aim of this investigation was to discover how processes of lipid peroxidation (LPO) and activity of cAMP phosphodiesterases (PDE) in neuronal membranes are affected by memory stimulators, namely ethimizole and its demethylated derivatives (M1 and M2) [1], and also allyl- and propylnorantitheines — substances with a negative effect on preservation of an acquired skill [2], and to compare the results with the action of the above-mentioned compounds on the RNA-synthesizing activity of neurons.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-200 g. The test substances were injected intraperitoneally in a dose of 3 mg/kg 15 or 90 min before decapitation of the animals. In the experiments in vitro the incubation medium contained the test substances in concentrations of 10^{-7} to 10^{-3} M. Preparations of the crude mitochondrial fraction, containing a large part of the plasma membranes and nucleus from rat cerebral cortical neurons, were obtained as described previously [6, 9]. The velocity of LPO was estimated from the accumulation of malonic dialdehyde (MDA) in the course of incubation of a membrane suspension (t = 37°C) with continuous mixing. MDA was determined by the reaction with thiobarbituric acid (2-TBA) [9]. Activity of cAMP PDE in membranes containing 5′-nucleotidase was determined by the formation of [^{3}H] AMP and [^{3}H] adenosine from [^{3}H] cAMP, as described previously [9]. The reaction products were identified with the aid of thin-layer chromatography on "Silufol" plates (Czechoslovakia). The RNA-synthesizing activity was determined from incorporation of [^{3}H] UTP into the nuclear suspension, as described previously [6]. Radioactivity was counted in Bray's scintillator on a "RackBeta" counter (LKB, Sweden). The protein content was measured by the method in [10] and the DNA content as in [11]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Structural and functional changes in the outer cell membrane were accompanied by changes in enzyme activity controlling the secondary messenger level [31. The substances we chose for testing structurally resemble the alkylxanthines, whose effects are realized through involvement of cAMP PDE and adenosine receptors [8]. Since ethimizole does not affect adenylate cyclase activity, either directly or through adenosine receptors [9], the action of its analogs was studied on activity

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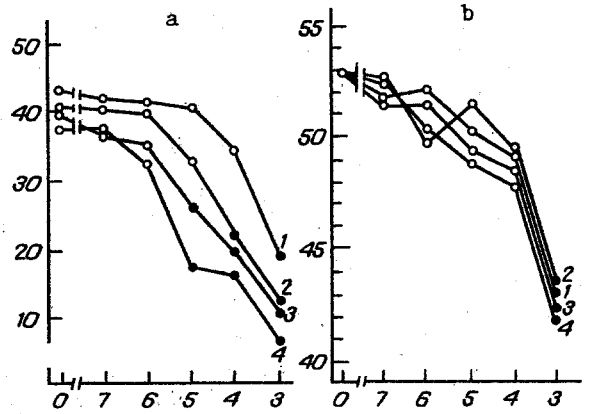

Fig. 1. Action of antitheines on cAMP PDE activity with high (a) and low (b) substrate affinity of rat brain membrane fraction. 1) M2; 2) ethylnorantitheine; 3) allylnorantitheine; 4) propylnorantitheine. Abscissa, logarithm of concentration of substance (in M); ordinate, PDE activity (in pmoles cAMP/mg protein/min). Filled circles: $p < 0.05$ compared with control.

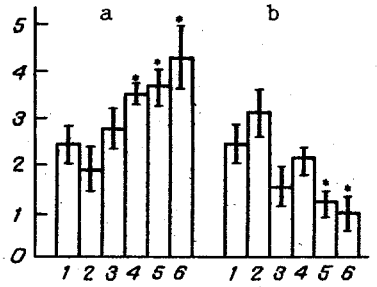

Fig. 2. Effect of antitheines on LPO in rat brain membranes 15 min (a) and 90 min (b) after injection. 1) Control; 2) M1; 3) M2; 4) ethylnorantitheine; 5) allylnorantitheine; 6) propylnorantitheine. Ordinate, increase in MDA (in nmoles/mg protein/20 min of incubation). *$p < 0.05$ compared with control.

of PDE with high affinity, controlling the basal cAMP level in the cell, and on activity of PDE with low affinity, to which an important role is ascribed in regulation of the cAMP level under extremal conditions. All the preparations studied had an inhibitory action on membrane PDE, but did not produce changes in 5'-nucleotidase activity (Fig. 1). Allyl- and propylnorantitheines were most effective: in a concentration of 10^{-5} M, when neither ethimizole nor M2 had any action, they significantly reduced activity of the enzyme by 34 and 59% respectively. In a concentration of 10^{-4} M these substances also inhibited PDE with high affinity more strongly than did ethimizole and M2. Activity of the enzyme with low affinity was significantly reduced by the action of all the substances tested in a concentration of 10^{-3} M. Appreciable inhibition of high-affinity PDE by ethimizole and its structural analogs may be significantly reflected in the cAMP level in brain tissue and may be the cause of the pharmacologic and metabolic effects of antitheines. This is shown by the considerable increase in the values of several parameters (electrical activity of brain structures, electrical activity of the neuron membranes, characteristics of glycolysis, and so on) under the influence of the most effective of the series of PDE inhibitors we are examining, namely allyl- and propylnorantitheines [2, 5]. The rule thus established also was observed when the effects of the substances on LPO was investigated. It was shown that both ethimizole and allyl- and propylnorantitheines, substances with

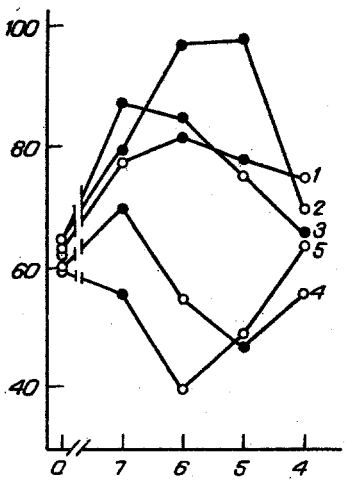

Fig. 3. Effect of antitheines on RNA-synthesizing activity in isolated nuclei of rat brain neurons. 1) M1; 2) M2; 3) ethylnorantitheine; 4) allylnorantitheine; 5) propylnorantitheine. Abscissa, logarithm of concentration (in M); ordinate, incorporation of [^{3}H] UTP (in pmoles/mg DNA/min).

dissimilar action on long-term memory, can induce structural and functional changes in brain cell membranes as early as 15 min after systemic injection. On incubation of a suspension of these membranes for 20 min under aeration conditions an increase was observed in TBA-active products by 15-18% (Fig. 2a). Longer exposure to the substances in experiments in vivo led to the opposite effect, but only under the influence of allyl- and propylnorantitheines (Fig. 2b). Demethylated ethimizole derivatives M1 and M2, substances with the weakest pharmacologic activity, but capable of considerably enhancing the efficacy of skill preservation, caused no changes in LPO after either 15 or 90 min; their action on PDE also was weakest.

M1 and M2, and also ethimizole, in a concentration of 10^{-6}-10^{-5} M, increased the RNA-synthesizing activity of nuclei from rat brain neurons, whereas allyl- and propylnorantitheines depressed it (Fig. 3), in direct correlation with the effect of these substances on long-term memory. The results are evidence that effects relating to the activity of the genetic apparatus have greater importance than their membrane effects in the molecular mechanisms of action of antitheines on skill preservation. If the substances used are regarded as a special kind of molecular probes for studying memory mechanisms, it can be tentatively suggested that activation of the genetic apparatus of neurons by endogenous neuroactive agents is the key stage in the process of formation and storage of the engram in situ.

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