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IN VITRO MODULATION OF STIMULUS-INDUCED CYCLIC AMP FORMATION BY A SYNTHETIC ANTIOXIDANT

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KEY WORDS: synthetic antioxidant; adenylate cyclase; membrane receptors; membrane lipids.

There is evidence in the literature of the important role of the composition and physicochemical properties of cell membrane lipids in regulation of the interaction between receptor and adenylate cyclase (AC) [12, 13]. Data of this kind have been obtained for AC in the membranes of peripheral organs [4, 7] and brain of mammals [3]. In particular, activation of AC of synaptic membranes of the rat brain has been found when lipid peroxidation (LPO) is intensified [2, 8].

In the investigation described below changes in basal and stimulus-induced AC activity were studied in membranes of various internal organs of rats when exposed *in vitro* to a synthetic water-soluble antioxidant.

EXPERIMENTAL METHOD

By the use of techniques described previously fractions of membrane preparations of rat internal organs were obtained: plasma membranes of hepatocytes [5], plasma membranes of enterocytes from the small intestine [10], and also brain synaptosomes [9]. The preparations used to stimulate cyclic AMP (cAMP) formation included glucagon (10 μM), isoproterenol (100 μM), and the opioid receptor agonist dalargin (D-Ala^2,Leu^5,Arg^6-enkephalin; 10 μM), obtained in the Laboratory of Peptide Synthesis (Head, M. I. Titov), All-Union Cardiologic Science Center, Academy of Medical Sciences of the USSR.

To determine AC activity the incubation medium for plasma membranes of the liver contained 50 mM Tris-HCl (pH 7.5), 2.5 mM cAMP, 3 mM $MgCl_2$, 1 mg/ml creatine phosphokinase, 50 mM creatine phosphate, 0.3 mM ATP, 0.25 mM GTP, 3 mM dithiothreitol, 0.5 mM theophylline, and [^{32}P]-ATP with activity of 0.5×10^6 cpm. In experiments with enterocyte membranes and brain synaptosomes, the incubation medium was of the same composition, but concentrations of some compounds were different: 0.3 mM dithiothreitol, 10 mM theophylline, and 1 mM EGTA. The final protein concentration of the membrane preparations was 0.05 mg/ml; protein was determined by the method in [11].

Membranes were incubated in the reaction medium for 15 min at 37°C. The reaction was stopped by addition of 0.5 N HCl. The samples were subjected to hydrolysis at 100°C for 7 min and then neutralized with 1.5 M imidazole. The labeled cAMP thus formed was removed by filtration on columns with neutral aluminum oxide [15]. A water-soluble antioxidant belonging to a class of screened phenols (phenozan-1K), synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR, was used. Interaction with the antioxidant took place during preincubation of the membrane preparation with phenozan-1K for 30 min at 25°C, after which the membranes were incubated for 15 min in the reaction mixture at 37°C.

EXPERIMENTAL RESULTS

The action of phenozan-1K *in vitro* increased both the basal and glucagon-stimulated AC activity of the hepatocyte plasma membranes, with a maximum when the phenozan-1K concentration was 10^{-5} M (Fig. 1, I).

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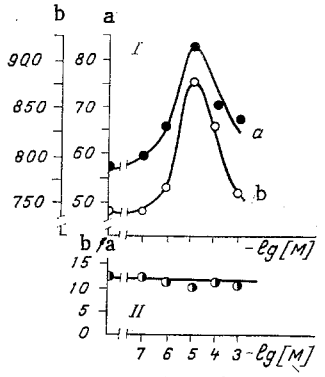

Fig. 1

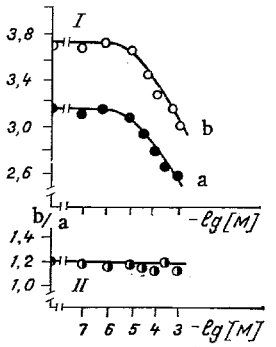

Fig. 2

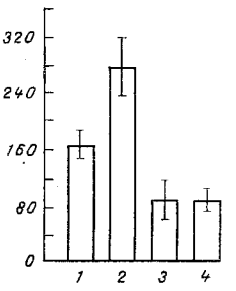

Fig. 3

Fig. 1. Effect of phenozan-1K on basal (a) and glucagon-stimulated (b) AC activity of rat hepatocyte membranes. Here and in Fig. 2: abscissa, $-\log_{10}$ of phenozan-1K concentration; ordinate, I) concentration of cAMP formed (in pmoles/min/mg protein); II) ratio of stimulated to basal AC activity.

Fig. 2. Effect of phenozan-1K on basal (a) and isoproterenol-stimulated (b) AC activity of rat brain synaptosomes.

Fig. 3. Effect of phenozan-1K and naloxone on dalargin-stimulated AC activity of enterocyte membranes of rat small intestime. Ordinate, concentration of cAMP formed (in pmoles/min/mg protein). 1) Basal AC activity; 2) dalargin (10^{-5} M); 3) dalargin (10^{-5} M) + phenozan-1K (10^{-3} M); 4) dalargin (10^{-5} M) + naloxone (10^{-3} M).

The antioxidant also caused a change in basal and isoproterenol-stimulated AC activity of rat brain synaptosomes (Fig. 2, I) but, unlike in the experiments with hepatocyte plasma membranes, in this case AC activity was reduced when phenozan-1K was present in concentrations above 10^{-5} M. The unchanged ratio of stimulus-induced to basal activity in both these cases shows (Fig. 1, II; Fig. 2, II) that the antioxidant realizes its effect not at the level of interaction of the ligands with brain membrane β-receptors and hepatocyte membrane glucagon receptors, but at the level of regulation of activity of AC associated in the receptor complexes.

The results are evidence that the action of phenozan-1K on AC activity differs in different receptor complexes, and it may evidently reflect the presence of interreceptor regulatory relationships (Figs. 1 and 2). There are also indications that β-adrenoreceptors and glucagen receptors, distributed differently on cell membranes, may influence different AC pools [6].

To explain the possible mechanisms of the effect of the synthetic antioxidant on AC activity of cell membranes, two possible ways of action of phenozan-1K can be suggested: 1) The antioxidant reduces to the intensity of LPO, whose level, according to data in the literature, is related to enzyme activity [2, 8]; 2) the antioxidant causes changes in the flowability of the lipid bilayer of the membranes [1], which also affects AC activity [14]. Whatever the case, the direct action of phenozan-1K on the enzyme can be ruled out, as is confirmed by the different changes in observed rat liver and brain cell membranes under the influence of the same biologically active substance. In our view the antioxidant affects AC activity through a change in the physicochemical characteristics of the lipid environment of AC.

It will be clear from Fig. 3 that phenozan-1K inhibits the dalargin-stimulated activation of AC in enterocyte plasma membranes of the rat small intestine. It is an interesting fact that the influence of the antioxidant is analogous to the action of naloxone, a specific antagonist of opioid receptors, but the points of application of these substances in their action on the receptor differ. Whereas naloxone interacts with a receptor binding site exposed to the aqueous phase, the antioxidant most probably acts on part of the receptor complex buried in the membrane bilayer.

It can be concluded from the data described above that AC activity associated in receptor complexes can be modulated in the required direction by means of synthetic water-soluble antioxidants, acting on the level of the lipid bilayer of cell plasma membranes of different mammalian organs.

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EFFECT OF 17α-ETHINYLESTRADIOL ON ACTIVITY OF RAT LIVER ENZYMES FOR SYNTHESIS AND HYDROLYSIS OF CHOLESTEROL ESTERS

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KEY WORDS: 17α-ethinylestradiol; cholesterol esters; acyl-CoA-cholesterol-O-acyltransferase; cholesterol esterase; bile.

Administration of estrogens is known to lower the concentration of cholesterol esters (ChE) in the blood vessel wall and may delay the development of atherosclerosis [9]. At the same time, we know that under the influence of esterogens redistribution of concentrations of free cholesterol (FCh) and ChE takes place in rats between the blood and liver [3], as a result of intensification of receptor-dependent uptake of low-density lipoproteins by the hepatocytes [11]. However, the mechanisms of the intracellular redistribution of FCh and ChE in the liver under these conditions have not been adequately studied.

The aim of this investigation was to study the effects of 17α-ethinylestradiol (ETE) on activity of enzymes involved in synthesis and hydrolysis of ChE by the liver, and levels of FCh and bile acids (BA) in the bile in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats weighing 180-200 g, divided into two groups: 1) 30 animals received ETE (from Gedeon Richter, Hungary) in a dose of 25 μg/100 g body weight daily for 3 days intraperitoneally; 2) physiological saline was injected at the same time and by the same method into 30 animals (control). Twenty four hours before decapitation 10 animals from groups 1 and 2 were given an intraperitoneal injection of ^{14}C-cholesterol (^{14}C-ChS) (from Izotop, Leningrad) in a dose of 100 μCi/100 g, incorporated with lipofundin-20 (Finland). The animals were decapitated after deprivation of food for 16 h and the liver was removed and washed with a cold solution of 0.25 M sucrose, 1 mM EDTA, 5 mM Tris-HCl, pH 7.4, to remove blood, and homogenized in buffer of the same composition in the ratio of 1:5 (w/v). Individual liver fractions were obtained by differential centrifugation [14]. The bile duct was cannulated [3] in 6 experimental and 6 control animals under pentobarbital anesthesia before sacrifice, and bile was collected for 30 min.

Activity of lysosomal [5] and cytoplasmic [10] cholesterol esterases (ChEases; EC 3.1.1.13) was determined by using cholesterol [1-^{14}C]-oleate as the substrate. Activity of acyl-CoA-cholesterol-O-acyltransferse (ACAT, EC 2.3.1.26) was determined [12] in the supernatant

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