EFFECT OF WATER-SOLUBLE ANTIOXIDANTS OF THE SCREENED PHENOL CLASS ON THE β-ADRENERGIC SYSTEM OF RAT CARDIOMYOCYTE PLASMA MEMBRANES IN VITRO

A. P. Khokhlov and K. N. Yarygin

UDC 616.128-008.939.15-39-02:615.272.014.425

KEY WORDS: synthetic antioxidants; β-adrenoreceptors; rat cardiomyocytes; adenylate cyclase; lipid peroxidation

Experimental and clinical data obtained in recent years suggest that changes in lipid peroxidation (LPO) in cell membranes play an important role in the pathogenesis of several diseases. For instance, cytotoxic effects in myocardial ischemia and hypoxia are largely due to depression of activity of the biochemical systems that protect the heart from active forms of oxygen, and in turn, this suggests that induction of LPO is a factor involved in cardiomyocyte injury [9, 10]. It has been suggested [13] that the entry of Ca^{++} into cells as a result of structural disturbances of their membranes, caused by activation of phospholipases and proteases, is an important factor in myocardial damage. The role of other consequences of LPO activation and, in particular, of modification of membrane receptor activity [3], is not clear. The possibility of using various substances to compensate the depressed activity of components of the antioxidant system of the heart in the treatment of ischemic disturbances in heart muscle has been discussed in the literature [1]. Synthetic antioxidants [4, 6], which are relatively nontoxic inhibitors of radical reactions [7], are promising in this respect.

The aim of this investigation was to study the effect of changes in the level of LPO, induced by the action of synthetic antioxidants, on signal conduction in the β-adrenergic system of rat cardiomyocyte plasma membranes, i.e., in the receptor system which plays an important role in the pathogenesis of myocardial infarction [2], was studied.

EXPERIMENTAL METHOD

The followign water-soluble synthetic antioxidants beloning to the screened phenol class were used: phenosan [γ-(4-hydroxy-3,5-di-tert-butylphenol)propionic acid] and its potassium salt (phenosan-1K). Both compounds were synthesized in V. V. Ershov's laboratory at the Institute of Chemical Physics, Academy of Sciences of the USSR.

LPO induction was monitored by measuring accumulation of conjugated dienes at 234 nm; absorption spectra were recorded on the Hitachi-557 spectrophotometer (Japan). Fractions of cardiomyocyte plasma membranes of Wistar rats were isolated by the method described previously [5]. Adenylate cyclase activity was determined by the method in [12]. The incubation medium contained (in mmoles/liter): Tris-HCl (pH 7.5 at 37°C) 50, cAMP 1, $MgCl_2$ 5, phosphocreatine 20, ATP 0.1, theophylline 0.2, and also 0.5 mg/ml of creatine phosphokinase and 10^6 cpm of α-^{32}P-ATP (specific activity 3000 Ci/mmole, from Amersham Corporation, England). Samples were incubated for 15 min at 37°C and the final concentration of protein of the membrane preparation in the same was 0.05 mg/ml.

When complex formation of ^{3}H-dihydroalprenolol (specific activity 78 Ci/mmole, Amersham) with cardiomyocyte β-adrenoreceptors was investigated, specific binding was determined as the fraction of total binding which could be blocked by 1000-fold excess of L-alprenolol (from Sigma, USA). Cardiomyocyte membranes (500 μg protein/ml) were incubated for 60 min at 25°C in medium of the following composition (in mmoles/liter): imidazole 20, Na_2EDTA

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 415-417, October, 1986. Original article submitted March 11, 1986.

0007-4888/86/0010-1355$12.50

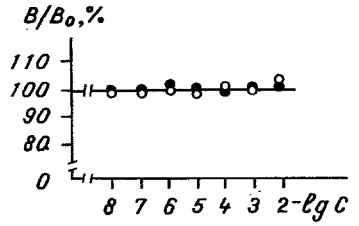

Fig. 1

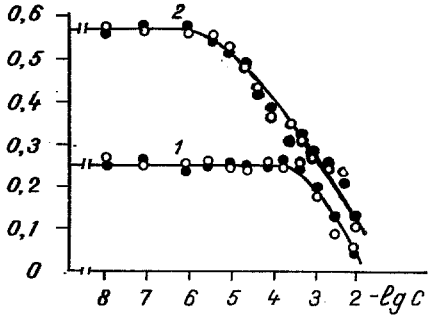

Fig. 2

Fig. 1. Dependence of specific binding of ^{3}H-dihydroalprenolol (10 mM) with rat cardiomyocyte plasma membranes in vitro under the influence of phenosan (filled circles) and phenosan-1K (empty circles). Incubation time 60 min (at 25°C). C) Concentration of antioxidants (in M), B_0) specific binding of ^{3}H-dihydroalprenolol (in cpm), B) specific binding of ^{3}H-dihydroalprenolol in presence of antioxidants (in cpm).

Fig. 2. Effect of phenosan (filled circles) and phenosan-1K (empty circles) in vitro on basal (1) and 10 μM isoproterenol-stimulated (2) adenylate cyclase activity of rat cardiomyocytes. C) Concentration of antioxidants (in M). Ordinate, quantity of synthesized cAMP (in nmoles/min/mg protein).

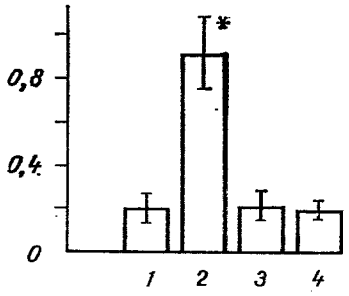

Fig. 3. Concentration of conjugated dienes during incubation of rat cardiomyocyte plasma membranes in vitro in the absence and in the presence of synthetic antioxidants. Abscissa: 1) initial level, 2) incubation without antioxidants, 3) incubation with phenosan (10 μM); 4) incubation with phenosan-1K (10 μM); ordinate: concentration of conjugated dienes (nmoles/mg phospholipid). Incubation time 15 min (37°C). *P < 0.01 compared with initial level.

1, $MgCl_2$ 5, pH 7.4. Bound and free ligand were separated by vacuum filtration through GF/C filters (Whatman, England).

EXPERIMENTAL RESULTS

Specific binding of the β-ligand was found not to be dependent on antioxidant concentration in the incubation medium, even if the latter were present in very high doses – about 10 mM (Fig. 1).

Raising the level of peroxide reactions in brain membrane lipids is known to activate the adenylate cyclase system [8, 11]. With this in mind, depression of adenylate cyclase activity would be expected under the influence of inhibitors of radical processes of membrane lipids. In fact, inhibition of isoproterenol-stimulated cAMP formation was recorded in vitro in cardiomyocyte membranes in the presence of antioxidants in concentrations over 10 μM, whereas basal adenylate cyclase activity was inhibited only in the presence of very high concentrations of phenosan and phenosan-1K – over 1 mM (Fig. 2).

Analysis of the intensity of LPO showed that after only 15 min of incubation of the cardiomyocyte plasma membranes at 37°C diene conjugates accumulated, evidently on account of activity of endogenous oxidaiton enzyme systems, whereas in the presence of the antioxidants, the formation of these membrane lipid peroxidation products was not observed (Fig. 3).

Physicochemical characteristics of membrane lipids, such as microviscosity, are known to have a significant influence on activity of membrane-bound adenylate cyclase systems [14], and the writers showed previously that the action of synthetic water-soluble antioxidants in vivo leads to a change in both lipid composition and structural characteristics (including microviscosity) of the lipid phase of various cell membrane fractions [1].

It can be concluded from the facts described above that the action of water-soluble synthetic antioxidants belonging to the screened phenol class on the β-adrenergic system of mammalian cardiomyocytes is realized, not at the level of complex formation between specific ligands and the receptor, nor at the level of regulation of activity of the immediate catalytic subunit of adenylate cyclase, but rather at the level of coupling of binding of β-receptor agonists with adenylate cyclase activation. In our view this may take place as a result of a change in the physicochemical state of the lipid bilayer of the membrane.

LITERATURE CITED

1. E. B. Burlakova and A. P. Khokhlov, Biol. Memb., 2, No. 6, 557 (1985).
2. Z. I. Vedeneeva, Byull, Éksp. Biol. Med., No. 10, 50 (1967).
3. M. L. Libe, E. D. Bogdanova, A. E. Rozenberg, et al., Byull. Éksp. Biol. Med., No. 11, 552 (1981).
4. A. Mieégombyn, A. Kh. Kogan, N. I. Losev, and A. N. Kudrin, Proceedings of the All-Union Conference on Bioantioxidants [in Russian], Chernogolovka (1983), pp. 77-78.
5. V. A. Tkachuk and G. N. Baldenkov, Biokhimiya, No. 6, 1097 (1978).
6. R. A. Frol'kis, V. A. Tsiomik, L. S. Mkhitaryan, et al., Proceedings of the All-Union Conference on Bioantioxidants [in Russian], Chernogolovka (1983), pp. 80-81.
7. N. M. Émanuél', Biofizika, No. 4, 706 (1984).
8. A. Baba, E. Lee, A. Ohta, et al., J. Biol. Chem., 256, 3679 (1931).
9. Y. Gauduel and M. A. Duvelleroy, Oxygen Transport to Tissue, New York (1984), pp. 421-431.
10. R. H. M. Julicher, L. B. M. Tijburg, L. Sterrenberg, et al., Life Sci., 35, 1281 (1984).
11. E. Lee, A. Baba, A. Ohta, and H. Iwata, Biochim. Biophys. Acta, 689, 370 (1982).
12. M. P. Panchenko and V. A. Tkachuk, FEBS Letters, 174, 50 (1984).
13. H. Rhee and J. Cooper, Oxygen Transport to Tissue, New York (1984), pp. 389-402.
14. R. Salesse and J. Garnier, Mol. Cell. Biochem., 60, 17 (1984).