ANTIOXIDATIVE ACTIVITY OF CYCLOHISTIDYLPROLINE

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The antioxidative defense system is an important physiological system responsible for adapting living organisms to existence under aerobic conditions. This system protects vitally important compounds (lipids, proteins, DNA, RNA, etc.) against the damaging action of oxygen and of its activated forms [7]. Disturbances of the antioxidative defense system lead to severe pathological changes and to reactions of free radical oxidative destruction of membrane lipids and other biomolecules through the influence of activated forms of oxygen [7, 8]. The highest level of oxidative metabolism in vertebrates is characteristic of skeletal muscles and brain. These tissues also are distinguished by a relatively high content of various bioantioxidants, including α-tocopherol (vitamin E), ascorbic acid (vitamin C), glutathione, and also carnosine and its related compounds [1, 9, 10].

Cyclohistidylproline (CHP) was discovered in 1976 [13] and until now it has been regarded purely as a metabolite of thyrotrophin-releasing hormone (TRH) and as a cyclic peptide, possessing hormonal and neurotropic activity [12]. CHP is found in man in different parts of the brain, in the gastrointestinal tract, the adrenals, and blood serum. The possibility of CHP formation from TRH has been demonstrated experimentally. However, the CHP level in brain tissues, CSF, and blood is significantly higher than the TRH level. TRH metabolism cannot therefore be the sole source of CHP formation. There is indirect evidence that CHP may be formed from other precursors, and also that biosynthesis of CHP de novo from histidine and proline is possible [12]. The principal route of elimination of CHP from vertebrates is excretion by the kidneys.

It has been shown that CHP inhibits prolactin secretion by cells of the adenohypophysis, affects temperature regulation, prolongs sleep, and modifies behavioral responses of experimental animals [12]. However, the biological role of CHP and its physiological functions still remain unexplained.

In view of the structural similarity of CHP and TRH, the view has become widely accepted that CHP is a hormonal peptide, related to TRH, and involved in regulation of the prolactin level in mammals. Another view, which takes into account the presence of neurotropic activity in CHP, has led to its being regarded as a regulatory neuropeptide.

According to recent data, certain histidine-containing peptides possess antioxidative activity in vitro [1, 4, 6, 9] and in vivo [2]. It has also been shown [3] that some neuropeptides, if administered to animals, cause generalized inhibition of free-radical lipid oxidation, and this effect is most marked in brain tissues. These findings led us to propose a possible biological role for CHP as an endogenous antioxidant. Experimental data confirm the validity of this hypothesis, for we found that CHP exhibits high antioxidative activity in tests in vitro and in experiments in vivo.

EXPERIMENTAL METHOD

We used two tests to characterize the antioxidative activity of CHP in vitro. One was to investigate induced (Fe^{++} + ascorbate)-dependent lipid peroxidation (LPO) of membranes of the sarcoplasmic reticulum from rabbit skeletal muscles [6]. As buffer solution in the control

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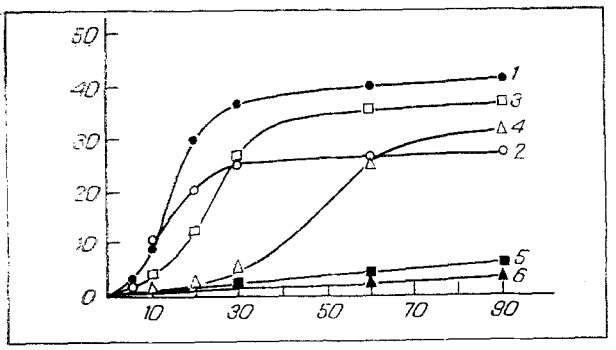

Fig. 1. Effect of CHP and carnosine on ascorbate-dependent LPO of membrane preparations of sarcoplasmic reticulum, measured as the increase in malonic dialdehyde concentration in the course of the reaction. 1) PESA, 50 mM (control); 2) carnosine, 20 mM; 3-6) CHP, 5, 7, 7.5, 10, and 20 mM respectively. Abscissa, incubation time (in min); ordinate, malonic dialdehyde concentration (in nmoles/mg protein of reticulum).

experiments we used piperazine-N,N'-2-ethanesulfonic acid (PESA) [4]. The second test consisted of measuring free-radical oxidation of cumene in the presence of azo-bis-isobutyronitrile [2]; dimethylformamide was used as the solvent for CHP.

The action of CHP on the antioxidative status in vivo was investigated in experiments on noninbred albino rats. The experimental animals were given CHP by intraperitoneal injection in a dose of 15 mg/kg (0.1 ml of a solution of CHP in dimethylformamide). Control animals received 0.1 ml of dimethylformamide only. The animals were decapitated after 1 h, the brain was isolated, and blood collected. Concentrations of LPO products in homogenate of the cerebral cortex and in the blood serum were determined by the reaction with thiobarbituric acid (TVA) [3, 5, 11].

EXPERIMENTAL RESULTS

The experiments in vitro showed that CHP possesses distinct antioxidative activity even in low concentrations. Antioxidative activity of CHP is much greater than that of an endogenous antioxidant of peptide nature such as the linear histidine-containing dipeptide carnosine. The corresponding experimental data are shown in Fig. 1

It will be clear from Fig. 1 that the antioxidative activity of CHP in the ascorbate-dependent LPO test was greater than that of carnosine. As might be expected, the inhibitory action of CHP on ascorbate-dependent LPO of the reticulum membranes rises with an increase in the CHP concentration. Measurements of activity of the Ca-pump of these membrane preparations in parallel experiments showed that activity of the Ca-pump is inhibited with an increase in the malonic dialdehyde concentration. Inhibition of the Ca-pump in the control (50 mM PESA) is observed 15 min after the beginning of peroxidation in medium with 20 mM carnosine and the Ca-pump was inactivated after 30 min. By this time, in medium with the same CHP concentration, activity of the Ca-pump of the sarcoplasmic reticulum was still at the 60% level.

In the free-radical oxidation of cumene test CHP also proved to be active. The antioxidative effect of 0.3 mM CHP, calculated as the time of delay of cumene oxidation, was 17.5 min, the same as the effect of 1.2 mM carnosine. Consequently, in this system also, the antioxidative activity of CHP was greater than that of carnosine.

The study of the antioxidative activity of CHP in experiments in vivo showed that after 1 h CHP lowered the level of TBA-active LPO products to 44% in the brain and to 53% in the blood serum (compared with the control). This effect still continued with time; 6 h after injection of CHP the level of TBA-active products was lowered to 20.5% in the brain and to 48.4% in the blood serum. The higher degree of inhibition of LPO in the brain is in good agreement with previous observations indicating that high (millimolar) concentrations of CHP are found in various parts of the brain [12] – an organ characterized by a high intensity of reactions of oxidative metabolism, involving the participation of active forms of oxygen, and a high concentration of lipids, rich in polyunsaturated fatty acids [7, 8].

This investigation thus showed for the first time that CHP possesses high antioxidative activity and can participate in protection of the brain and other organs and tissues of aerobic organisms against the damaging action of peroxidation. One essential function of CHP may be to inhibit processes of free-radical oxidation of lipids and of other biomolecules in membrane structures. CHP can be regarded as a new and highly effective endogenous antioxidant.

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