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LPO and Antioxidant Defense in the Stomach of Albino Rats Injected with Angiotensin II and Enalapril Maleate

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The effects of components of angiotensin II system on LPO and antioxidant defense in the stomach of adult albino rats were studied using biochemical and chemiluminescent methods. Five intraperitoneal injections of angiotensin II in a dose of 100 μg/kg activated LPO and inhibited antioxidant processes in the studied tissues. Oral therapy with enalapril maleate (inhibitor of angiotensin-converting enzyme) in a daily dose of 10 mg/kg for 2 weeks normalized stress-activated LPO processes in gastric tissue.

Key Words: *angiotensin II; enalapril; lipid peroxidation; antioxidant defense; stomach*

Angiotensin II (AT II) is the main effector peptide of the renin-angiotensin-aldosterone system. Injection of AT II induces a wide spectrum of physiological reactions in the organism, including nontarget organs. AT II stimulates proliferative processes in epithelial tissues, including the gastric mucosa [4].

Reactive oxygen species rapidly forming after induction with AT II are regarded as second messengers in the transfer of regulatory signals both in health and disease. Intensive generation of superoxide anions stimulated by AT II is an important mechanism promoting the development of atherosclerosis and arterial hypertension [9,14].

We evaluated the effects of AT II and AT-converting enzyme inhibitor on LPO and antioxidant defense (AOD) system in the gastric tissue of adult rats.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats weighing 160-200 g. AT II was prepared at the Laboratory of Peptide Synthesis, Cardiology Research Center (Moscow), by classical and solid-phase methods. The mean content of AT II in a sample was 97.8%. Experimental identification of the peptide was carried out using ^{1}H-NMR spectroscopy. The animals were intraperitoneally injected with AT II in a dose of 100 μg/kg for 5 days at 10.00-11.00. Controls were injected with an equivalent volume of the solvent (sterile isotonic saline).

In the second experimental series the animals received AT-converting enzyme inhibitor enalapril maleate (ednit, Gedeon Richter) in a dose of 10 mg/kg via a gastric tube for 2 weeks. According to published data, this dose is most often used for evaluating the effects on various functions in rats [6,11]. Controls received an equivalent volume of isotonic saline. The preparation was given daily between 10.00 and 11.00. Intact animals served as an additional control.

The rats were decapitated 24 h after the last dose. A total of 50 animals were used in experiments.

LPO and AOD processes were studied in stomach homogenates. The concentrations of total lipids were measured using Lachema kits. The content of primary (lipid hydroperoxides (HP) [2] and intermediate LPO products (MDA [1]) were measured routinely. The concentration of α-tocopherol was measured as described previously [7].

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TABLE 1. LPO—AOD System in Gastric Tissues of Adult Rats Repeatedly Injected with AT II (*M*±*m*)

Parameter		Control	AT II
Total lipids, mg/g tissue		273.47±16.19	221.91±10.26***
α-Tocopherol, μg/g tissue		8.11±0.42	6.02±0.39*
HP, opt. density units/mg lipids		0.042±0.004	0.066±0.007**
MDA, arb. units/mg lipids		5.98±0.49	8.54±0.81***
Total yield of	spontaneous CL	0.33±0.04	0.46±0.04***
	induced CL	0.92±0.11	1.26±0.11***
Maximum flash intensity		0.63±0.06	0.94±0.07*

Note. *p<0.01, **p<0.02, ***p<0.05 compared to the control.

The integral assessment of free-radical oxidation in homogenates was carried out by luminol-dependent chemiluminescence (CL) induced by H_2O_2 [3]. CL was recorded on an LS 50B luminescent spectrometer (Perkin Elmer), the signal was standardized using Finlab software. The total yield of spontaneous (over 1 min) and induced CL (over 2 min) and the amplitude of the maximum flash were measured, standardized per mg lipids in a sample, and expressed in arb. units. The data were processed using Student's *t* test.

RESULTS

Repeated injection of AT II to adult rats caused significant changes in the LPO-AOD system in gastric tissues. The content of total lipids and α-tocopherol decreased by 19 and 26%, respectively.

Injections of AT II activated lipid peroxidation in gastric tissues, which was confirmed by accumulation of HP and MDA. The content of lipid HP increased by 1.6-fold. HP is the main primary product of nonenzymatic peroxidation of organic compounds. Enhanced generation of HP results in a 1.4-fold increase in the concentration of MDA, a secondary LPO product. Decreased content of α-tocopherol in gastric tissues can be due to its high consumption for inactivation of fatty acids peroxide radicals. On the other hand, phenol antioxidants, in particular, α-tocopherol, affecting some components of LPO cannot provide complete many-level defense from active oxygen metabolites, which is confirmed by accumulation of peroxidation products in the studied tissues.

The direction of changes in biochemical parameters coincided with CL findings. The total yield of spontaneous CL increased 1.4 times, indicating enhanced spontaneous endogenous free-radical oxidation. Moreover, induced CL and the maximum flash intensity increased 1.4- and 1.5-fold, which attests to intensification of LPO and impairment of AOD in gastric tissues (Table 1).

Intragastric administration of isotonic saline and manipulations associated with this procedure intensified LPO and induced AOD strain in the gastric tissue of control animals (Table 2). The level of α-tocopherol decreased by 27.2%, spontaneous CL increased 1.4 times, and induced CL and maximum flash intensity increased 1.2-fold. These changes attested to intensification of endogenous free-radical oxidation, increased oxidizability of lipids, and decreased content of natural antioxidants in the studied biological substrate. The later conclusion was confirmed by deficiency of fat-soluble antioxidant α-tocopherol (Table 2). The absence of changes in other parameters of the LPO—AOD system evaluated by biochemical

TABLE 2. LPO—AOD System in Gastric Tissues of Adult Rats Repeatedly Injected with Enalapril (*M*±*m*)

Parameter		Control	Intact control	Enalapril
Total lipids, mg/g tissue		222.64±8.37	234.67±8.72	223.42±8.16
α-Tocopherol, μg/g tissue		5.91±0.46*	8.12±0.63	8.18±0.57
HP, opt. density units/mg lipids		0.068±0.006	0.055±0.002	0.054±0.003
MDA, arb. units/mg lipids		5.64±0.24	5.22±0.2	4.67±0.26
Total yield of	spontaneous CL	0.73±0.07**	0.54±0.04	0.62±0.03
	induced CL	3.07±0.11**	2.5±0.22	2.39±0.26
Maximum flash intensity		3.91±0.15**	3.32±0.22	2.92±0.22

Note. *p<0.02, **p<0.05 compared to intact rats.

methods can be explained by higher sensitivity of CL compared to biochemical tests.

Injection of enalapril (AT-converting enzyme inhibitor) reduced CL parameters and increased α-tocopherol content in gastric tissues compared to the control. On the other hand, there were no appreciable differences between experimental and intact animals.

We found no reports concerning *in vivo* effect of exogenous AT II on the LPO-AOD system in gastric tissues of adult rats. The results of *in vitro* studies on cultured vascular smooth-muscle cells from animals [12] and humans [15] indicate a stimulatory effect of the peptide on the formation of reactive oxygen species. Conclusions on the capacity of AT II to enhance oxidative stress *in vivo* are based mainly on the results of investigation of peroxidation processes at the level of vascular tissues and serum [5,10]. Our findings, also obtained *in vivo*, indicate that exogenous AT II can promote activation of free-radical oxidation in gastric tissues.

Inhibition of free-radical oxidation under the effect of AT-converting enzyme inhibitors was observed both *in vitro* and *in vivo*. This effect was observed, for example, in cultured mesangial cells [13] and in isolated hearts [8]. These data suggest that AT-converting enzyme inhibitors directly modulate free-radical processes *in vivo*.

Treatment with enalapril virtually did not restore the balance in the gastric LPO-AOD system. According to published data [4], a similar scheme of enalapril treatment led to normalization of proliferative processes in the stomach of rats exposed to stress.

Hence, these results confirm and to a certain measure explain our previous findings and indicate that AT II is effective in the treatment of not only cardiovascular diseases.

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