INHIBITION BY IONOL AND γ-HYDROXYBUTYRIC ACID OF LIPID PEROXIDATION WHEN ACTIVATED IN EMOTIONAL-PAIN STRESS

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It was shown previously [3, 4] that primary and secondary lipid peroxidation (LPO) products accumulate in the tissues of rats as a result of severe emotional-pain stress (EPS). This activation of LPO is most marked in heart muscle, and it is substantial, although less so, in skeletal muscle and brain. It has been suggested that this considerable accumulation of LPO products may play a role in the injury to membranous structures of cells in EPS [3]. It was considered useful to find ways of abolishing the activation of LPO in EPS, first, by inhibition of the central mechanisms responsible for the realization of stress and, second, by direct inhibition of LPO in the tissues with the aid of antioxidants.

Accordingly, in the investigation described below, the effect of γ-hydroxybutyric acid (GMBA), which has an inhibitory action at higher levels of the brain [5], and of ionol (4-methyl-2,6-di-tert-butylphenol), an inhibitor of free-radical lipid oxidation reactions, on stress-induced activation of LPO was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g were used. The animals were divided into six groups: 1) control, 2) control + GHBA, 3) control + ionol, 4) EPS, 5) GHBA + EPS, 6) ionol + EPS. Each group consisted of between nine and 14 animals. EPS was produced in the form of an "anxiety neurosis" [8]. GHBA was given per os in 10% glucose in a dose of 100 mg/kg 30 min before EPS, and again 2 h after the beginning of EPS. Ionol was injected intraperitoneally in a dose of 120 mg/kg daily for 3 days before EPS. Lipids were isolated from the brain, heart, aorta, and gastrocnemius muscle by the method of Folch et al. [9]. Accumulation of hydroperoxides in polyene lipids was assessed by two methods: UV-spectroscopy and polarography. UV-absorption spectra of solutions of lipids in a methanol–hexane (5:1) system were recorded on the MPS-50L (Shimadzu) spectrophotometer, assuming the coefficient of molar extinction at $\lambda_{max} = 232$ nm to be 2.1×10^4 moles · cm^{-1} [6]. Lipid peroxides were determined polarographically in a mixture of organic solvents methanol–benzene (2:1) on the P-8 polarograph (Tanagimoto) by the method described previously [21] [sic]. The concentrations of hydroperoxides were calculated from the value of the diffusion current for a halfwave potential ($E_{0.5}$) in the region from 0.9 to 1.1 V. End products of LPO were recorded as fluorescence spectra of solutions of lipids in chloroform with fluorescence excitation maximum at 360 nm and emission maximum in the region of 420-440 nm [7] on an Aminco-Bowman spectrofluorometer. Before each series of experiments the instrument was calibrated against a standard solution of quinine sulfate (1 μg/ml in 0.1 N H_2SO_4).

EXPERIMENTAL RESULTS

Changes in the content of fluorescent Schiff bases in lipids of heart muscle, the aorta, skeletal muscle, and brain of the rats after EPS are given in Table 1. It will be seen that 2 h after EPS the content of fluorescent LPO products in the heart, aorta, and brain was increased almost threefold, and in skeletal muscle it was doubled. During the next 2 days the quantity of secondary LPO products in the heart and aorta began to fall, and by the 5th day it reached the control level. The content of Schiff bases in the brain and skeletal muscle continued to rise in the course of 2 days and the control level was not reached until 10 days after EPS.

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TABLE 1. Dynamics of Accumulation of Lipid Peroxidation Products (as fluorescence of Schiff bases, in relative units) in Tissues of Rats after EPS (M ± m)

Tissue	Control	Time obtaining materials after EPS			
		2 h	2 days	5 days	10 days
Heart muscle	10,5±3,5	29,2±4,6	27,6±4,3	12,9±1,9	9,0±1,1
Skeletal muscle	10,3±0,9	20,6±2,8	27,0±9,7	10,8±1,7	9,0±1,2
Brain	7,8±0,9	20,7±3,2	26,9±3,3	23,7±4,1	10,6±2,2
Aorta	11,5±1,9	31,2±7,5	23,0±2,8	10,5±2,6	—

TABLE 2. Effect of Preliminary Administration of GHBA and Ionol on Accumulation of LPO Products after EPS (M ± m)

Test object	Accumulation of hydroperoxides based on results of UV-spectroscopy,			Accumulation of hydroperoxides based on results of polarography, nmoles/mg lipid			Changes in intensity of fluorescence of Schiff bases, relative units		
	physiological saline	ionol	GHBA	physiological saline	ionol	GHBA	physiological saline	ionol	GHBA
Heart muscle	30,9±2,9	11,9±2,4	11,9±2,4	6,7±1,5	0,3±0,3	0,4±0,3	18,7±5,9	2,6±2,2	4,6±1,6
P	<0,001	<0,002	<0,002	<0,02	>0,1	>0,1	<0,01	>0,1	<0,05
Skeletal muscle	19,0±3,4	2,4±3,4	2,4±3,4	0,8±0,1	0,4±0,3	0,2±0,1	10,3±2,9	5,2±4,2	1,0±0,9
P	<0,001	>0,1	>0,1	<0,02	>0,1	>0,05	<0,01	>0,1	>0,1
Brain	14,3±2,6	4,8±2,1	4,8±2,1	1,3±0,3	0,1±0,1	0,5±0,3	12,9±3,3	4,5±2,4	3,7±1,9
P	<0,001	>0,05	>0,05	<0,02	>0,1	>0,1	<0,002	>0,05	>0,05

Legend. Data given in this table are difference between content of LPO products in experiment (after EPS) and in corresponding control.

Activation of LPO after exposure to EPS thus continued for a considerable time – for different organs this could vary between 2 and 5 days.

The results of determination of primary molecular products of LPO (hydroperoxides of polyene lipids) and of the terminal products of LPO (fluorescent Schiff bases) in the brain, heart, and skeletal muscle 2 h after EPS, and data on the effect of preliminary administration of GHBA and ionol on activation of LPO in these tissues, are given in Table 2.

It also follows from the data in Table 2 that significant accumulation of both primary and terminal products of LPO can be found by all three methods in the brain, heart, and skeletal muscles after exposure to EPS. In addition to the data given in Table 2, it should be pointed out that the direct polarographic determination of concentrations of lipid hydroperoxides in the tissues of the heart muscle and brain and, to a lesser degree, of skeletal muscle reveals activation of LPO after EPS more clearly than determination of hydroperoxides as diene conjugants. For instance, according to the results of polarography, the content of hydroperoxides per milligram lipids after EPS in the tissues of the heart and brain was increased by more than 5 and 3.5 times: 8 ± 1.5 nmoles (control 0.3 ± 0.3 nmole) and 1.9 ± 0.3 nmoles (control 0.5 ± 0.1 nmole) respectively. Meanwhile, according to the optical density at 232 nm the content of hydroperoxides per milligram lipids in these tissues was increased more than threefold after EPS: 47.6 ± 2.4 nmoles (control 16.7 ± 1.4 nmoles) for the heart and 21.4 ± 2.4 nmoles (control 7.1 ± 1.2 nmoles) for the brain respectively.

Preliminary administration of GHBA or ionol completely abolished activation of LPO or significantly reduced it in all the tissues studied from animals exposed to the action of EPS.

Activation of LPO after EPS can thus be prevented both by GHBA, which limits excitation of the adrenergic and hypophyseo-adrenal systems, and by ionol, which is an effective trap for peroxide radicals [1] and so interrupts the process of free-radical LPO.

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