10. W. J. Fry, J. F. Brennan, and J. W. Barnard, Ultrasound Biol. Med., 3, 110 (1957).
11. S. A. Goss, Ultrasound Med. Biol., 5, 181 (1979).
12. J. Grayson, J. Physiol. (London), 118, 51 (1952).
13. P. P. Lele, Ultrasound Med. Biol., 5, 317 (1979).

EFFECT OF THE ANTIOXIDANT DIBUNOL ON EPR SIGNALS IN RAT TISSUES AT DIFFERENT AGES

E. N. Gorban' and V. K. Kol'tover

UDC 612.014.423:543.422.25].014.46:615.243.4

KEY WORDS: dibunol; resonance; metabolism; blood proteins.

Since the work of Harman [8] and Émanuél' [6] it has been known that the antioxidant butylated hydroxytoluene (ionol, dibunol) has a geroprotective action, i.e., it lengthens the life span of laboratory animals if added regularly to their diet.

In model reactions of free-radical peroxidation of lipids and other natural compounds dibunol exhibits antioxidant properties: It inhibits these reactions and intercepts active free radicals (FR), but the nature of its action *in vivo* has not yet been explained [5, 6].

The aim of this investigation was to study the effect of dibunol on function of enzymes of tissue oxidative metabolism and also on the blood proteins transferrin and ceruloplasmin in rats of different ages. Electron paramagnetic resonance (EPR), a direct method of detection and study of paramagnetic centers and electron-transport chains in the tissues [1], was chosen as the experimental method.

EXPERIMENTAL METHOD

Experiments were carried out on the tissues of adult (5-6 months) and old (28-30 months) male Wistar rats. Dibunol (4-methyl-2,6-di-tert-butylphenol), dissolved in a 10% aqueous solution of the solubilizer Tween-80, was injected intraperitoneally in a dose of 10 mg/100 g body weight. The rats were decapitated 6 h after injection of dibunol. The dose of dibunol and time of its administration were chosen on the basis of data showing the dynamics of the effect of dibunol on tissue antioxidative activity [3]. All animals were killed at the same time (3-4 p.m.). Tissue samples for EPR measurements were prepared in the form of cylindrical columns, frozen at 77°K, with a length (l) of 20 mm and a diameter (d) of 4.7 mm (in the case of heparinized whole blood), $l = 20$ mm and d = 3.0 mm (for liver, kidneys, myocardium, skeletal muscles, thyroid gland), and $l = 5$ mm and d = 3.0 mm (for adrenal cortex) [4]. EPR signals were recorded on an E-109 spectrometer (Varian, France), equipped with an internal standard, at a temperature of 77°K and with an amplitude of high-frequency modulation of the magnetic field of 10 G. The microwave power was kept constant at 50 mW for recording EPR signals of blood, 0.2 mW for recording signals with a g-factor of about 2.0, and 10 mW when recording all other signals. In each experiment eight experimental and eight control animals were used. The results were subjected to statistical analysis by the usual methods [2].

EXPERIMENTAL RESULTS

The EPR spectra of the tissues corresponded in shape of lines and average intensity to those observed previously [1, 4, 7]. To determine the quantitative characteristics of the spectra, the intensities (amplitude

Laboratory of Physiology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev. Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR, Chernogolovka, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 694-696, June, 1985. Original article submitted April 2, 1984.

0007-4888/85/9906-0745$09.50 © 1985 Plenum Publishing Corporation

TABLE 1. Intensity of EPR Signals (in relative units) in Tissues of Myocardium, Skeletal Muscle, Liver, Kidneys, and Thyroid Gland of Rats of Different Ages, in Control (I) and under Influence of Dibunol (II) ($M \pm \sigma$; $P < 0.05$)

Age of rats	EPR signal	Experimental conditions	Myocardium	Skeletal muscle	Liver	Kidney	Thyroid gland (pooled thyroid glands of eight animals)
Adult	ISP	I	75±3	—	76±2	77±2	—
		II	69±4	—	80±4	83±7	
	FR	I	81±2	22±2	92±4	74±8	26,0
		II	89±4	25±2	97±3	76±10*	26,5
	Cytochrome P-450	I	—	—	100±8	29±7	---
		II	—	—	102±7*	—	—
	SO	I	—	—	41±5	—	—
		II	—	—	44±5*	—	—
Old	ISP	I	75±7	—	68±6	61±5	—
		II	70±7*	—	66±4*	71±6	—
	FR	I	81±4	22±3	75±7	54±5	29,0
		II	88±2	24±3*	77±8*	59±7	30,0
	Cytochrome P-450	I	—	—	72±9	23±5	—
		II	—	—	60±12	——	—
	SO	I	—	—	36±6	—	—
		II	—	—	38±4*	—	—

Legend. *) Difference from control not significant at $P < 0.05$ level.

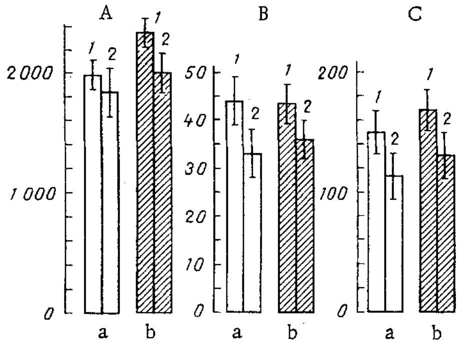

Fig. 1. Intensity of EPR signals of ISP (A), FR (B), and cytochrome P-450 (C) in adrenal cortex of adult (a) and old (b) rats. Ordinate, intensity (in relative units); 1) control, 2) dibunol.

from peak to peak) of the corresponding spectral lines were measured. Intensities of signals with $g \simeq 1.94$ and 1.97 are proportional to the content of mitochondrial iron-sulfur nonheme proteins (ISP) and sulfite oxidase (SO) in the reduced state respectively, whereas the intensity of the signal with $g \simeq 2.0$ is proportional to the content of mitochondrial flavoproteins in the free-radical semiquinone form (FR). The intensity of the signal with $g \simeq 2.5$ is proportional to the content of cytochrome P-450 in the tissue with heme iron in the oxidized, low-spin form. In blood preparations the intensity of the signal with $g \simeq 4.3$ is proportional to the content of Fe(III)-transferrin (β_1-globulin carrying iron atoms), whereas the signal with $g \simeq 2.05$ is proportional to the content of Cu(II)-ceruloplasmin (α_2-globulin performing the role of transport protein for copper and, possibly, the oxidase of certain substrates including biogenic amines) [1, 7].

Statistically significant age differences were found in the amplitudes of the ISP and FR signals in the liver and kidney tissues, signals of cytochrome P-450 and SO in the liver, and the SR signal in the thyroid gland. No significant age differences were found in myocardial or skeletal muscle tissues (Table 1).

Tissue of the adrenal cortex from old animals had a weaker ISP signal (Fig. 1), evidence of an increase in the content of adrenodoxin in the reduced form with age. The amplitudes of the FR and cytochrome P-450 signals of adult and old rats did not differ significantly.

Blood of the old animals showed a lower intensity of the transferrin signal and a higher intensity of the ceruloplasmin signal (Fig. 2).

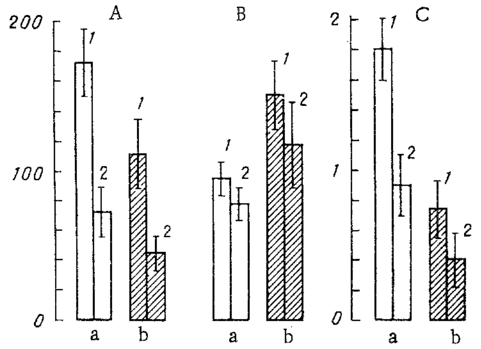

Fig. 2. Intensity of EPR signals of transferrin (A), ceruloplasmin (B), and ratio of intensities of transferrin to ceruloplasmin signals (C) in blood of adult (a) and old (b) rats. Legend as to Fig. 1.

Administration of dibunol caused significant changes in the intensity of the EPR signals in most tissues (Table 1). ISP signals were increased in the kidney tissues of animals of both ages and the liver of adult rats. The intensity of the ISP signal in the myocardial tissue of adult rats decreased after injection of dibunol. An increase in the intensity of FR signals under the influence of dibunol was observed in the myocardial and thyroid tissues of rats of both ages, in skeletal muscle and liver tissues of adult rats, and kidney tissues of old rats. The intensity of the cytochrome P-450 signal fell statistically significantly in the liver tissue of the old animals.

The most marked and consistent changes in EPR signals under the influence of dibunol were observed in the adrenocortical tissue and blood: the intensity of the EPR signals fell (see Figs. 1 and 2).

The results are evidence that dibunol, only a comparatively short time after its administration, significantly modifies the metabolic activity of most tissues studied. Since the most marked and consistent changes in EPR signals took place in the blood and adrenal cortex it can be concluded that dibunol has a significant effect on the hormonal regulation system of the body.

LITERATURE CITED

1. Ya. I. Azhipa, Medico-Biological Aspects of the Electron Paramagnetic Resonance Method [in Russian], Moscow (1983).
2. N. Bailey, Statistical Methods in Biology [Russian translation], Moscow (1963).
3. E. B. Burlakova, A. V. Alesenko, E. M. Molochkina, et al., Bioantioxidants in Radiation Lesions and Malignant Growth [in Russian], Moscow (1975).
4. R. A. Gus'kova, M. M. Vilenchik, and V. K. Kol'tover, Biofizika, 25, 102 (1980).
5. V. K. Kol'tover, Usp. Sovrem. Biol., 96, 85 (1983).
6. N. M. Émanuél', Izv. Akad. Nauk SSSR, Ser. Biol., No. 4, 503 (1975).
7. N. J. F. Dodd, in: Metal Ions in Biological Systems, Vol. 10, New York (1980), p. 95.
8. D. Harman, J. Geront., 23, 476 (1968).