Stress-Protective Effect of a Novel Derivative of n-3 Polyunsaturated Fatty Acids

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Recently, the specialists working in different branches of experimental and clinical medicine have been closely studying docosahexaenoic and eicosapentaenoic acids (DHA and EPA) as widespread representatives of the series of n-3 highly polyunsaturated fatty acids in the human organism [10]. The spectrum of their biological activity is extremely broad [2,14]. There are also data indicating that EPA and DHA exert a pronounced effect on the morphofunctional state of the cell membranes [11,15], on the processes of myelinization [9], and on neurotransmission in the central nervous system (CNS) [13], as well as on memory and learning [14]. It is known that destabilization of membrane structures and disturbances of lipid-lipid and lipid-protein interactions resulting from hypercatecholaminemia underlie one of the leading pathogenetic mechanisms of development of the stress syndrome, culminating in the damage to internal organs [3,4,6]. Some possible ways of preventing stress damage to internal organs in experimental animals were studied in our laboratory, using pretreatment with a new biologically active substance (BAS) (assigned the number P-55 in our laboratory), an n-3 polyunsaturated fatty acid derivative obtained from the products of molecular distillation of certain grades of fish oil and stabilized with natural antioxidants.

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MATERIALS AND METHODS

The experiments were carried out on 72 albino male Wistar rats weighing 180-220 g. The animals were divided into 4 groups: 1) intact rats; 2) animals in which stress was reproduced; 3) a group with chronic stress against the background of a 30-day preventive administration of BAS P-55 in a dose of 0.2 g/kg; 4) a group with chronic stress against the background of a 30-day administration of an equal dose of stabilizing antioxidants mixed with sunflower oil in a total dose of 0.2 g/kg, per os. Chronic stress in the phase of decompensation was reproduced by a 4-day sleep deprivation after Juvet [12]. The dynamics of the animals' weight as well as of the weight of their main stress-competent organs (thymus, spleen, myocardium, and adrenals) was studied. The degree of the characteristic stress-induced changes in the adrenals [7] was assessed in accordance with the number and arrangement of the ascorbic acid-containing granules histochemically determined in the tissues after Bacchus [8]. The intensity of the histochemical reaction vis-a-vis the control was visually assessed according to a 4-point system: + - weak, ++ - moderate, +++ - marked, and ++++ - intensive [1]. The frequency, number, and length of ulcerous lesions in the gastric mucosa were also

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recorded. The state of the myocardium was judged by the ECG in the standard lead II, and the functions of the CNS were assessed according to the animals' behavior in the "open field" [5]. The reliability of differences between the groups was analyzed using Student's t test.

RESULTS

Chronic stress in the phase of decompensation manifests itself in pronounced changes of different morphometric parameters in experimental animals. In particular, a marked decrease in the body weight of stressed animals is observed: by the end of the 4-day period of sleep deprivation the mean weight constituted 78.1% of the initial value ($p<0.001$). Simultaneously, characteristic changes occur in the relative weight of the stress-competent organs: the weight of the myocardium and adrenals increases by 17.6% and 63.5%, respectively, while the weight of the lymphoid organs (thymus and spleen) drops 45.7% and 40.7%, respectively. In intact animals, ascorbic acid is found in all the cortical zones of the adrenals as well as in the medulla. In the glomeruli, the granules are large and located inside the cells. In the cells of the fascicular zone, small and large granules are either diffusely arranged in the cytoplasm or grouped around the nuclei (Fig. 1, *a*). A similar arrangement is observed in the cells of the reticular zone. In the cells of the medulla, the granules are small, diffusely and uniformly distributed throughout the cytoplasm. The intensity of the histochemical reaction in the glomeruli corresponds to ++, and in the fascicular and reticular zones of the cortex and medulla to +++. During stress, the intensity of the reaction for ascorbic acid drops to +. The granules in the glomeruli are small and are diffusely distributed through the cytoplasm. In the cells of the fascicular and reticular zones, separate small granules are encountered, the majority of them displaced from the cytoplasm toward the vessels. Here, they either are located in the vascular wall, or fill the lumen (Fig. 1, *b*). In the gastric mucosa of stressed animals, marked degenerative-dystrophic changes are observed: the folds of the mucosa smooth out; abundant ulceration is observed in 88.9% of animals (on the average, 5.3 per stomach, average length 8.2 mm). The ECG data confirm the presence of the myocardial hypertrophy morphometrically determined in stressed animals. This manifests itself in a 43%-increased amplitude of the R wave in the standard lead II ($p<0.01$). Chronic emotional stress raises the anxiety component in the behavior of experimental animals: their total locomotor activity in the "open field" increases by 82.6% in comparison with the activity of intact animals (Table 1).

According to our findings, the 30-day preventive administration of BAS P-55 in the indicated dose raises the rat organism's adaptability under the conditions of chronic emotional stress. On the 4th day of sleep deprivation, mortality is found to drop from 31.3% in intact animals to 12.5% in those given P-55. By the end of exposure to stress, the weight losses of stressed rats were smaller against the background of P-55 administration. The weight losses in the control group and in the group which received P-55 constituted 21.9% and

TABLE 1. Effect of Preventive Administration of P-55 on Intensity of Some Morphological and Physiological Manifestations of Chronic Stress in Rats ($M\pm m$, $n=18$)

Animal group	Statistical test	Weight loss, %	Relative weight of organs, mg/100 g				Ulceration			ECG R (II), mV	Locomotor activity (5 min in open field)
			adrenals (both)	myocardium	thymus	spleen	occurrence, %	number	size, mm		
1st (intact) –	–	20.4±0.7	347.4±21.7	170.6±7.4	320.8±15.5	–	–	–	0.77±0.07	63.28±2.23	
2nd (stress) –	21.9±3.2	34.3±1.0	408.5±36.2	92.7±5.1	190.4±5.4	88.9	5.3±1.4	8.2±2.2	1.10±0.07	116.4±6.9	
	% (2–1)	–	163.5	117.6	54.3	59.3	–	–	–	143	182.6
	p (2–1)	–	<0.001	<0.05	<0.001	<0.001	–	–	–	<0.01	<0.001
3rd (P–55+ + stress)	–	11.1±2.8	30.1±1.2	364.1±18.3	110.0±6.5	295.1±46.2	38.8	1.3±0.7	2.0±1.2	0.80±0.08	78.4±7.8
	% (3–1)	–	147.5	104.8	64.5	92.0	–	–	–	104	122.9
	p (3–1)	–	<0.01	<0.5	<0.01	<0.05	–	–	–	>0.5	<0.05
	% (3–2)	50.7	87.8	89.1	118.7	155.0	–	23.4	24.3	73	67.4
	p (3–2)	<0.01	<0.05	<0.05	<0.05	<0.05	–	<0.02	<0.05	<0.02	<0.01
4th (stabilizers+ + stress)	18.2±3.0	35.2±1.3	388.2±27.6	95.2±6.3	217.0±22.6	83.3	4.2±1.1	6.1±1.9	1.04±0.08	108.3±7.1	
	% (4–1)	–	172.5	111.7	55.8	67.6	–	–	–	135	169.7
	p (4–1)	–	<0.001	<0.05	<0.001	<0.02	–	–	–	<0.01	<0.001
	% (4–2)	83.1	102.6	95.0	102.7	114.0	–	79.2	74.4	95	93.0
	p (4–2)	<0.2	>0.5	<0.5	>0.5	<0.2	–	<0.2	<0.2	>0.5	<0.2
	% (4–3)	164.0	116.9	106.6	86.5	73.5	–	323	305	130	138.1
	p (4–3)	<0.05	<0.05	<0.1	<0.1	<0.02	–	<0.05	<0.1	<0.01	<0.02

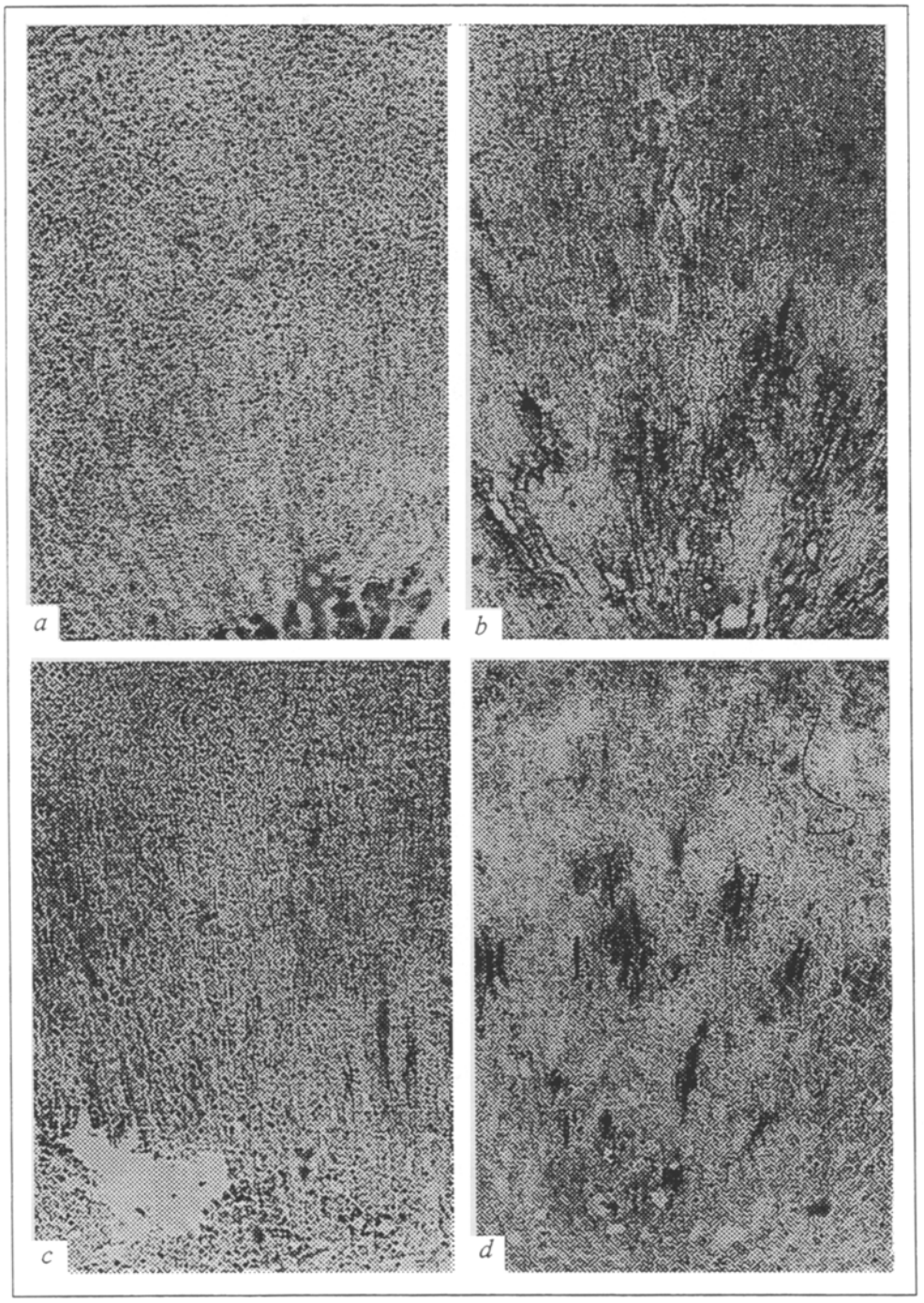

Fig. 1. Arrangement of ascorbic acid granules in adrenal cortex and medulla in male Wistar rats. *a*) intact animals; *b*) stressed animals; *c*) stressed animals pretreated with P−55; *d*) stressed animals given stabilizing antioxidants without concentrate of polyunsaturated fatty acids. Staining after Bacchus, ×100.

11.1%, respectively ($p<0.05$). A trend toward normalization of the relative weight of the stress-competent organs is also observed under the influence of preventive administration of P-55. For instance, myocardial and adrenal hypertrophy, as well as hypoplasia of the thymus and spleen are less pronounced in the animals given P-55 than in the control group (Table 1). In some portions of the adrenal cortex the intensity of the histochemical reaction for ascorbic acid increased during stress against the background of P-55 as compared with the control and constituted +++. In these portions the granules of ascorbic acid are located in the cell cytoplasm, as they are in intact animals. In places, the intensity of the reaction remains the same as in the control group (+) and the granules are lo-

cated in the vascular wall (Fig. 1, *c*). The ECG results show a reliably reduced amplitude of the R wave (II) in the animals given the compound as compared with the intact group ($p<0.02$), and the average R (II) amplitude approaches that in intact animals (Table 1), this also being evidence of a decreased myocardial hypertrophy during stress under conditions of preventive administration of P-55. During the study of the gastric mucosa of rats exposed to stress against the background of P-55, it was established that the occurrence of stress gastric ulcer among the animals of this group was 38.8%, whereas in the control it was 88.9%. The mean number of ulcers per stomach and the average length of an ulcer in a single stomach also proved to be reliably lower than the control values by 76.6% and 75.7%, respectively (Table 1). The animals which received the novel BAS were calmer in the "open field" after stress: their total locomotor activity was 32.6% lower than that in the control ($p<0.01$) and approached the level in the intact group (Table 1).

The results attest to the marked protective effect of long-term pretreatment with P-55 against stress. At the same time, the effect of the new BAS on stress manifests itself not only in the normalization of the characteristic morphological parameters in stressed animals, but also in the action upon their higher nervous activity, evidence of which is seen in the actometric data. The positive effect of P-55 during stress cannot be explained by the action of natural antioxidants added in small amounts to the preparation, because studies carried out on animals which received equal doses of the complex of antioxidants without DHA and EPA demonstrated a remarkably low effectiveness of such a combination under similar conditions, and, in most cases, reliable differences from the corresponding parameters in the animals given P-55 (Table 1, Fig. 1, *d*).

Possibly, the stress-protective effect of the compound P-55 is due to its capability (thanks to EPA and DHA) of altering the lipid-lipid and lipid-protein interactions in the cell membranes, (thereby modulating their permeability and the activity of membrane enzymes [3,4]), of affecting the processes of myelinization and neurotransmission in the CNS [6,11], as well as of preventing disturbances of the microcirculation owing to a reduction of the blood viscosity [15].

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