The increase in the number of MC during long-term immobilization preceded by injection of terbutaline may perhaps be connected with reduction of the degranulation of these cells, which may also be one of the mechanisms of reduction of venular permeability.

It was thus shown that during short-term and long-term immobilization, regulation of venular permeability by catecholamines is effected mainly through β-adrenoceptors of the venular endothelium and of the mast cells.

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CARNOSINE PREVENTS ACTIVATION OF FREE-RADICAL LIPID OXIDATION DURING STRESS

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Carnosine (β-alanyl-L-histidine), a dipeptide contained in muscle tissue in millimolar concentrations, is currently attracting ever-increasing attention of investigators. One reason for the increased interest in the study of the properties of carnosine is the fact that data have recently been obtained on new properties (immunoregulatory, antineoplastic, etc.) of this dipeptide which are of clinical importance. The discovery of the antioxidative activity of carnosine in systems in vitro [9], and also of the unique property of this dipeptide of lowering the concentration of free-radical oxidation (FRO) products through direct interaction with them [3], appears to be very important.

Antioxidants are known to possess an adaptogenic action, which is due to prevention of activation of FRO caused by stress-inducing factors [7]. On the basis of data on the effective antioxidative action of carnosine in vitro, and considering the extremely low toxicity of this dipeptide, it was logical to suggest that carnosine, administered in vivo, would possess marked stress-protective properties.

The aim of this investigation was to study changes in parameters of FRO in the brain and blood and also in the lipid composition of the brain of rats subjected to stress induced by painful electrical stimulation with or without previous administration of carnosine.

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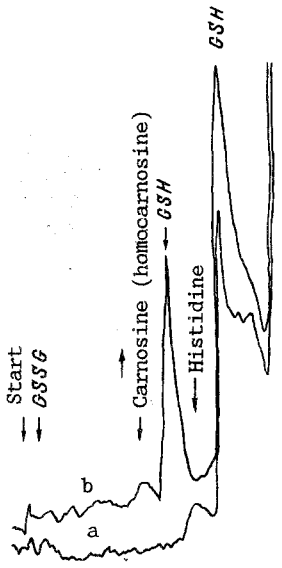

Fig. 1

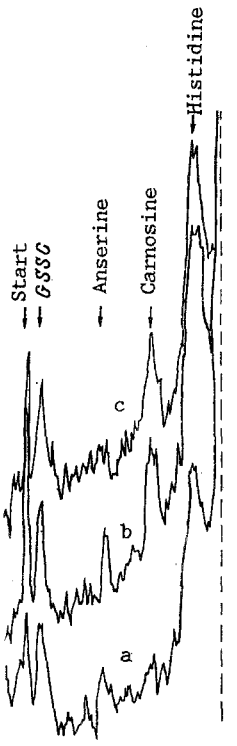

Fig. 2

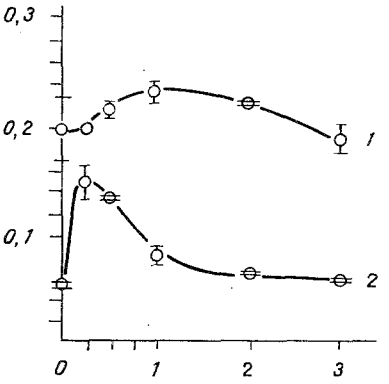

Fig. 3

Fig. 1. Chromatograms of protein-free extracts of blood (a) and brain (b) of intact rats, treated with NBD chloride.

Fig. 2. Chromatograms of NBD-derivatives of blood of rats receiving carnosine. a) Control; b, c) 15 and 30 min after administration of carnosine, respectively.

Fig. 3. Changes in concentration of substances giving positive diazo reaction in protein-free extract of blood (1) and brain (2) of rats after administration of carnosine. Ordinate, optical activity at 490 nm (optical density units). Abscissa, time (in h).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-180 g. Half of the animals were subjected to stress due to electric shock in a special chamber with electrically conducting floor, to which was applied a current of 3-4 mA for 2 h at the rate 1-2 stimuli in 10 sec. Half of the intact and half of the stressed rats were given an injection of carnosine in aqueous solution in a dose of 20 mg/kg before exposure, whereas the remaining animals were given an injection of the corresponding volume of isotonic NaCl solution. The animals were thus divided into four groups: intact (I), intact and receiving carnosine (IC), stressed (S), and stressed, receiving carnosine (SC).

After the end of exposure to stress the animals were decapitated, their blood collected, the brain quickly cooled, and the cerebral cortex removed. Serum was obtained from the blood by centrifugation, and the cerebral cortex was homogenized in 3 volumes of ice-cold isotonic saline. After centrifugation for 15 min at 2000g, the concentrations of products reacting with 2-thiobarbituric acid (TBA) [14], of conjugated dienes and ketodienes [5], and of Schiff bases [4] were determined, and the intensity of superoxide radical scavenging was estimated [13]. Lipids were extracted from part of the brain homogenate with a chloroform-methanol mixture after Folch [11], concentrations of cholesterol and total and individual phospholipids in the lipid extracts were determined by thin-layer chromatography [15] and the antioxidative activity was measured in a co-oxidation system with cumene [6].

Carnosine in the protein-free brain extract and in heparinized blood was determined colorimetrically by Pauli's diazo reaction [8] and densitometrically by one-way thin-layer chromatography of 7-chloro-4-nitrobenzo-2-oxy-1.3-diazole (NBD) derivatives [10].

TABLE 1. Parameters of FRO and Lipid Composition of Cerebral Cortex of Rats of Different Groups

Parameter	Group of animals		
	IC	S	SC
TBA-active products			
Brain	56,4 (48,5—66,7)	200,0 (189,4—215,2)	28,5* (24,2—31,8)
Blood serum	57,5 (43,8—67,2)	200,0 (192,2—204,7)	27,2* (25,0—31,3)
Conjugated dienes			
Brain	61,8 (50,0—77,8)	123,0 (111,1—133,3)	15,6* (11,1—27,8)
Blood serum	49,4 (35,7—78,3)	115,7 (102,4—126,5)	16,9* (6,0—30,1)
Ketodienes			
Brain	47,4 (13,2—56,8)	126,3 (105,3—157,9)	5,3* (0—13,2)
Blood serum	62,5 (41,7—100,0)	137,6 (83,3—166,7)	16,7* (0—20,8)
Superoxide dismutase activity (brain)	190,5	42,9	266,7*
Nonenzymic superoxide scavenging activity (blood serum)	218,2	27,3	245,5*
Schiff bases (brain)	57,5 (53,7—65,4)	155,6 (151,9—158,9)	58,4 (56,1—60,8)
Cholesterol	67,9 (65,0—70,4)	130,0 (122,7—137,2)	45,6* (37,9—54,2)
Total phospholipids	117,5 (115,7—119,4)	91,0 (87,7—93,3)	126,1* (125,0—126,9)
Cholesterol/phospholipids	57,8	142,9	36,1*
Sphingomyelin	110,7	60,0	114,8*
Phosphatidylcholine	68,8	133,6	62,9*
Phosphatidylethanolamine	71,5	121,0	68,3*
Phosphatidylinositol	127,0	50,3	127,0
Phosphatidylserine	135,9	80,7	138,5*
Cardiolipin	190,9	31,8	187,9
Phosphatidic acid	1339,2	0	1964,2*
Readily oxidized phospholipids/not readily oxidized phospholipids	133,9	81,7	137,2
Antioxidative activity	74,3 (71,4—78,6)	161,4 (142,9—171,4)	38,6* (32,1—42,9)

Legend. Results expressed in percent of corresponding parameters for group I, taken as 100%. Limits of variations given in parentheses. All differences between groups I—IC, I—S, IC—S, and SC—SC are significant at the $p < 0.01$ level; significant differences in IC—SC groups at the $p < 0.01$ level are marked by an asterisk.

EXPERIMENTAL RESULTS

The results are given in Table 1. Stress induced by painful foot shock caused activation of FRO in the brain and blood serum (as shown by an increase in the concentrations of FRO products), accompanied by inhibition of superoxide scavenging activity of the blood, accumulation of cholesterol in the brain, and a decrease in the total phospholipid concentration, mainly due to exhaustion of the fraction of readily oxidized phospholipids.

Administration of carnosine to intact animals had an action opposite to that of electric shock: the concentration of FRO products fell, the intensity of superoxide scavenging was increased, phospholipids accumulated (mainly on account of readily oxidized fractions), and the cholesterol concentration in lipid extracts of the brain fell.

Corresponding changes also were observed in the antioxidative activity of lipids: during stress accumulation of not readily oxidized phospholipids and cholesterol led to an increase, whereas after administration of carnosine the accumulation of readily oxidized phospholipids and a fall of the cholesterol level, led to a decrease in the antioxidative activity of the lipid extracts.

When carnosine was given before induction of stress by foot shock, the direction of the changes in the parameters studied corresponded to that after administration of carnosine to intact animals, but the degree of the changes in the parameters as a rule was greater in the SC group than in the IC group.

In an additional series of experiments changes in carnosine concentrations in the blood and brain of the rats were studied at different times after administration of carnosine to the animals to a dose of 20 mg/kg. Chromatograms of blood and brain extracts from control animals, treated with NBD-chloride, are shown in Fig. 1. The composition of extracts from these tissues differed significantly: appreciable amounts of oxidized glutathione and a substance with mobility close to that of histidine were detected in blood. The brain tissue contained oxidized and reduced glutathione and also homocarnosine, which has the same mobility as carnosine. Chromatograms of brain extract after administration of carnosine showed no significant change, whereas the composition of blood extracts was significantly changed. It will be clear from Fig. 2 that 15 min after administration of carnosine considerable amounts of carnosine and its methylated derivative, anserine, appeared in the blood. By the 30th minute the anserine had virtually completely disappeared although the carnosine level remained high. The results of colorimetric determination of carnosine in protein-free extract of blood and brain at different times after administration of carnosine are given in Fig. 3. The greatest increase in the concentration of diazo-reactive compounds was observed in blood during the first 30 min after injection of the dipeptide. Changes in the brain were less marked and were observed later. After 2-3 h (this period corresponds to the time of investigation of FRO parameters in the animals) the composition of the blood and brain (in the form of extracts) had virtually returned to normal.

The results are evidence that administration of carnosine prevents generalized activation of FRO and lipid conversions in the brain induced by stress, by giving rise to the opposite changes, namely inhibition of FRO and accumulation of readily oxidized lipids. Thus carnosine, which possesses the properties of an antioxidant in vitro [3, 9], can exert an effective antioxidative action when administered in vivo also. Like other well-known antioxidants [1, 7], carnosine prevents accumulation of FRO products, activates scavenging of superoxide radicals, lowers the cholesterol concentration and cholesterol/phospholipids ratio, thereby increasing the "flowability" of membranes and increases the concentration of readily oxidized phospholipids in membranes. The effective antioxidative action of carnosine may be mediated by several mechanisms, including direct interaction of the dipeptide with FRO products [3, 9], scavenging of free radicals [2], and activation of superoxide-scavenging activity of the body as a whole [12]. However, in the present experiment, during the period of considerable changes in FRO and in the lipid composition of the brain (Table 1) an excessive carnosine concentration was not recorded in the blood and brain (Figs. 1-3). It can be tentatively suggested that the antioxidative effects of carnosine observed were the result of its direct action on the state of FRO, exerted in the initial period after its administration, when the carnosine concentration in the tissues was raised. An alternative hypothesis, which requires further investigation, is the possibility of an indirect action of carnosine, namely the "activation" by an excess of carnosine of other mechanisms controlling the body level of FRO.

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CLINICAL AND ELECTROENCEPHALOGRAPHIC CHANGES IN MMP^+-INDUCED PARKINSONIAN SYNDROME IN RATS

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KEY WORDS: parkinsonian syndrome; electrical activity; caudate nuclei; MMP^+; generator of pathologically enhanced excitation

The parkinsonian syndrome induced by injection of tetanus toxin [1], cainic acid [3], acetylcholine [5], and antibodies to dopamine (DA) [6] into the caudate nuclei (CN), and also by systemic injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [4], which damages DA neurons of substantia nigra (SN), is connected with the formation for a generator of pathologically enhanced excitation (GPEE) in CN [2]. Recent data indicate that the toxic effect of MPTP on neurons of SN is due to the end product of its oxidation, namely 1-methyl-4-phenylpyridinium (MPP^+), which has high affinity for DA neurons [9-11]. Injection of MPP^+ directly into SN induces marked degeneration of the DA neurons of SN, reduces the concentration of striatal DA and motor disturbances characteristic of the parkinsonian syndrome, in rats and mice [7, 12, 13].

The aim of this investigation was to compare the severity of behavioral disturbances in a parkinsonian syndrome induced by intranigral injection of MPP^+, and the EEG changes in various brain structures and to elucidate the generator mechanisms of this syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats aged 9-10 months and weighing 440-550 g. Under hexobarbital anesthesia, and taking coordinates from a stereotaxic atlas [8], metal cannulas with bipolar electrodes for recording electrical activity (EA) were implanted bilaterally into the compact zone of SN. Bipolar nichrome electrodes were implanted simultaneously in the rostral zones of CN, the globus pallidus (GP), and the ventrolateral thalamic nucleus (VLT). The animals were kept in individual cages under standard animal house conditions and on the ordinary diet. By means of a Hamilton microsyringe, 7-8 days after the operation 10 μg of MMP^+ in a volume of 2 μliters of physiological saline was injected once through the cannula into each nucleus of SN (MPP^+ consisted of yellow crystals, m.p. 164-165°C, and was characterized by its IR and PMR spectra). The corresponding concentration of sodium iodide in 2 μliters of physiological saline was injected into animals of the control gorup, for the iodide of 1-methyl-4-phenylpyridinium was used in the experiment. EA was recorded on a "Neurograph-18" electroencephalograph (O.T.E. Biomedica, Italy), and it was subsequently processed on a BAS-161 computer from the same firm. EA was recorded before injection of the neurotoxin, continuously for 5 h, and for 1 h daily during the first 3 days. EA was recorded and microinjections of MPP^+ given while the animals were unrestrained. Oligokinesia, rigidity, and tremor were assessed on a point system [4]. The experimental results were subjected to statistical analysis.

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