ADAPTOGENIC EFFECT OF DIMETHYL SULFOXIDE IN RATS WITH CHRONIC EMOTIONAL-PAINFUL STRESS

N. V. Gulyaeva and I. P. Levshina

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The key role of activation of lipid peroxidation (LPO) in realization of the harmful action of acute and chronic emotional-painful stress (EPS) has been conclusively demonstrated in recent years [2, 4, 6]. Nevertheless, the concrete causes of activation of free-radical oxidation reactions during EPS have not yet been discovered. Recently, during the discussion of mechanisms of initiation of free-radical oxidation the question of whether hydroxyl radicals (OH·) can be formed *in vivo* has been raised, and the majority of investigators have come out in support of OH· radical generation in living cells [9]. Since OH· radicals are among the most reactive oxygen radicals, it can be postulated that these radicals play an essential role in the realization of the harmful action of EPS [3]. Direct verification of this hypothesis is complicated by factors of a methodologic character, due to the short (under 10^{-3} sec) life of OH· and the toxicity for animals of the spin traps used for the binding and determination of these radicals, and also to the poor permeability of the cells for the least toxic interceptors (mannitol, for example) [8]. The least toxic intereceptor, with the greatest ease of penetration into cells, is dimethyl sulfoxide (DMSO), which intercepts radicals effectively in a concentration of 0.5-10 mM [8, 10], and with LD_{50} for laboratory animals of 4-50 g/kg body weight (depending on the species of animal and mode of injection) [14].

The aim of this investigation was to study the role of OH· radicals in the development of pathological changes induced by EPS, with the use of DMSO as the specific OH· interceptor.

EXPERIMENTAL METHOD

Experiments were carried out on 52 non-inbred male rate weighing 200-250 g. Chronic EPS was induced by the scheme described previously [4], involving combined exposure to white noise and painful electrical stimulation for 3 weeks. Before the beginning and after the end of EPS the animals' behavior in an open field was tested, and their blood pressure (BP), respiration rate (RR), and heart rate (HR) were measured as described previously [1, 4]. The animals were divided into four groups: 1) control; 2) EPS (the rats of these groups received daily intraperitoneal injections of 1 ml of physiological saline); 3) control, receiving DMSO; and 4) stress & administration of DMSO. DMSO was injected daily into the rats of the last two groups in a dose of 1 ml of a 20% aqueous solution, equivalent to 1 g/kg body weight (this dose produces an average DMSO concentration of 10-15 mM, with which efficient scavenging of radicals takes place). DMSO was injected 30-60 min before EPS in order to obtain complete distribution of the compound in the animal's tissues [15]. After the end of exposure and testing the animals were decapitated, the brain removed, and the malonic dialdehyde (MDA) concentration [12] and superoxide dismutase activity in a system of phenazine metasulfate—NADH-nitroblue tetrazolium [11] were determined in homogenate of the cortex and hippocampus. These same parameters were determined in blood serum collected during decapitation.

EXPERIMENTAL RESULTS

EPS led to an increase in weight of the adrenals by 26% ($P < 0.01$), a tendency for the weight of the thymus to decrease, and multiple gastric ulcers. Open field behavior of ani-

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TABLE 1. Values of BP, RR, and HR during Immobilization for 2 h after EPS and Injection of DMSO (M ± m)

Group of animals	BP, mm Hg			RR, cycles/sec
	0 h	1 h	2 h	0 h
1 (control)	107,5±5,6[d]	103,1±6,2	127,2±7,1	1,1±0,1
P	<0,05			
2 (EPS)	147,6±7,0[a,b,c]	144,8±7,3[a,b,c]	152,9±8,1[a,b,c]	1,4±0,1[a]
P	<0,01	<0,01	<0,01	<0,05
3 (control +DMSO)	110,9±5,9	103,1±6,2	104,5±5,3	1,3±0,1
4 (EPS + DMSO)	108,8±7,1	110,4±6,0	106,9±6,3	1,3±0,2

Group of animals	RR, cycles/sec			HR, beats/sec	
	1 h	2 h	0 h	1 h	2 h
1 (control)	0,9±0,1	1,1±0,1	7,7±0,3	7,4±0,2	8,0±0,5
P					
2 (EPS)	1,1±0,1[a,c]	1,3±0,1[a,c]	8,4±0,4[a,b,c]	7,8±0,3[a,c]	8,2±0,4[c]
P	<0,05	<0,05	<0,01	<0,05	<0,01
3 (control + DMSO)	1,0±0,1	1,2±0,1	7,1±0,3	7,4±0,4	7,9±0,3
4 (EPS + DMSO)	0,8±0,1	1,0±0,1	7,1±0,3	7,2±0,3	7,6±0,3

Note. a, b, c) Significant differences between group 2 and groups 1, 3, or 4; d) significant differences between values of BP in group 1 for periods of 0-1 h and 0-2 h.

mals subjected to EPS showed a number of pathological features, including an increase in the number of squares crossed by 3.7 times ($P < 0.01$) and in the number of standing up by 8.3 times ($P < 0.01$) and a decrease in the number of exits into the center by 5.3 times ($P < 0.01$). These changes are evidence of an increased feeling of anxiety and unease as a result of exposure to EPS. Injection of DMSO into the control rats has no effect on the state of the animals' internal organs or the parameters of their open field behavior, but in the case of prophylactic treatment before sessions of EPS, it abolished the development of gastric ulcers and completely prevented the behavioral changes induced by EPS. DMSO abolished the hypertension that developed as a result of EPS and effectively prevented the rise of BP caused by immobilization of the control animals for 2 h (Table 1). Considerable normalization of function of the cardiovascular system and also of the reactivity of the autonomic nervous system, as reflected in values of RR and HR, was observed in stressed rats treated with DMSO.

In agreement with data obtained previously [1, 4, 5] EPS caused activation of LOP in the cerebral cortex and hippocampus of the rats, which was recorded as elevation of the MDA level by 31-32% ($P < 0.01$). No MDA could be found in the brain homogenates of rats receiving DMSO. Additional experiments on brain homogenates *in vitro* showed that the added DMSO inhibits MDA formation proportionally to the logarithm of DMSO concentration, and accordingly it is impossible to judge the level of LPO unequivocally in tissues of rats receiving DMSO on the basis of these results. SOD activity in the cortex and hippocampus was lowered by 23% ($P < 0.01$) and 13% ($P < 0.05$) respectively, but after injection of DMSO it increased by 29 and 30% in these parts of the brain of the control rats ($P < 0.01$) and by 43 and 38% in stressed animals. Inhibition of SOD as a result of EPS has been described previously [1, 6], but its activation by DMSO was demonstrated by the present writers for the first time. SOD is ascribed a key role in protection of the animal against the harmful action of free oxygen radicals [7], and for that reason activation of this enzyme may have an essential role in the adaptogenic action of DMSO. Additional experiments showed that chronic administration of DMSO induces SOD in rat tissues and that direct addition of DMSO to homogenates or samples containing a purified preparation of SOD activates the enzyme, to a degree that depends on the DMSO concentration. Thus, the activation observed *in vivo* is the sum of two effects: induction and direct acceleration of the superoxide dismutase reaction under the influence of DMSO in the tissue.

Changes in the MDA level and SOD activity similar to those observed in the brain were also observed in the rats' blood serum. The MDA level was raised by 30% after EPS ($P < 0.01$) and was below the sensitivity of the method of measurement in groups of rats receiving DMSO. Serum SOD activity was reduced by 9% ($P < 0.05$) after EPS and increased by 18% ($P < 0.01$)

and by 45% ($P < 0.001$) after injection of DMSO into the control and stressed rats respectively.

The results are thus evidence of the effective antistress action of DMSO at all the levels studied: It prevents behavioral disturbances induced by EPS, normalizes function of the cardiovascular and autonomic nervous systems, and activates the antiradical protection system of the body. It is particularly important to note that, in the dose used, DMSO itself had no significant effect on any of the physiological parameters (this is in agreement with data in the literature [13]) and that the same dose provides a concentration in the tissues adequate for scavenging OH· radicals. Considering the abundant and convincing data on participation of LPO reactions in the development of pathological changes in the body during EPS, the results of the present investigation can be regarded as evidence of the important role of OH· in realization of the action of EPS. The fact that DMSO has an antistress action, which is not less effective than that of a phenolic antioxidant (a nonspecific radical interceptor), also confirms the key role of OH· [1, 2, 4].

The results of SOD determination in the brain and blood point to its activation by DMSO as an additional mechanism of antistress action of the compound, besides interception of OH· radicals.

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