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Oxidative Modification of Plasma Proteins during Hypothermia and after Dalargin Administration

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Short-term and prolonged (3 h) moderate (30°C) hypothermia intensified oxidative modification of plasma proteins, while deep hypothermia (20°C) decreased the intensity of this process to a control level. Preliminary intraperitoneal injection of dalargin had practically no effect on oxidative modification of plasma proteins during moderate hypothermia.

Key Words: *hypothermia; blood plasma proteins; oxidative modification of proteins; dalargin*

Reactive oxygen species (ROS) intensify lipid peroxidation (LPO) and promote oxidative modification of soluble and membrane-bound enzymes, thus disturbing their function [11]. The initial stages of hypothermia stimulate generation of ROS, which was confirmed by the intensity of LPO in the blood and various tissues [4,9]. The effect of hypothermia on oxidative modification of plasma proteins remains unknown. Previous studies showed that preliminary intraperitoneal injection of dalargin, a synthetic analogue of Leu-enkephalin, prevents LPO activation in various tissues under stress conditions [2], *e.g.*, during hypothermia [4]. It can be hypothesized that dalargin produces a protective antioxidant effects on proteins.

Here we studied the dependence of free radical oxidation of plasma proteins on the degree of hypothermia and evaluated the possibility for correction of this process with opioid peptide dalargin.

MATERIALS AND METHODS

Experiments were performed on outbred albino male rats weighing 170-200 g. For modeling moderate and deep hypothermia, the animals were maintained in a cold chamber until a decrease in rectal temperature to 30 and 20°C, respectively. In special experimental series the rats were exposed to moderate hypothermia (30°C) for 3 h. Dalargin in a dose of 100 μg/kg was injected intraperitoneally 30 min before hypothermia or decapitation (normothermic control). Control rats received an equivalent volume of physiological saline. Oxidative modification of plasma proteins was estimated by the number of carbonyl groups reacting with 2,4-dinitrophenylhydrazine [1]. We evaluated the initial content of carbonyl groups and their accumulation at 37°C over 15 min without prooxidants (spontaneous oxidation) and in the presence of 10^{-3} M Fe^{2+}, 10^{-3} M ethylenediaminetetraacetate (EDTA), and 3×10^{-4} M H_2O_2. The content of carbonyl groups measured at 370 nm was calculated using the molar extinction coefficient (22,000 liter/M/cm for aliphatic dinitrophenylhydrazones [12]) and expressed in nmol/mg protein. Protein content was measured by the method of Lowry [13].

RESULTS

The presence of carbonyl groups in control rats confirmed oxidative modification of plasma proteins under physiological conditions (Table 1). Incubation under conditions promoting ROS generation markedly increased the number of carbonyl groups in plasma

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TABLE 1. Number of Carbonyl Groups (nmol/mg protein) in Rat Plasma Proteins during Hypothermia and After Dalargin Administration ($M\pm m$, n=6-8)

Group	Initial content of carbonyl groups	Accumulation of carbonyl groups over 15-min oxidation	
		spontaneous	Fe^{2+}-dependent
Normothermic control	1.50±0.06	3.56±0.10	57.24±1.48
+dalargin	1.44±0.12	2.85±0.12*	52.63±1.28*
Hypothermia, 30°C	3.91±0.10*	3.72±0.13	58.82±1.42
+dalargin	3.07±0.19*+	3.03±0.13*+	53.56±1.67+
Prolonged hypothermia, 30°C, 3 h	4.47±0.14*+	6.43±0.18*+	94.19±3.91*+
+dalargin	4.47±0.19*	6.42±0.12*	90.13±4.76*
Hypothermia, 20°C	1.97±0.22*+	3.03±0.14*+	49.38±2.06*+
+dalargin	1.64±0.13	3.28±0.08*	49.64±1.46*

Note. p<0.05: *compared to the control, +compared to 30°C.

proteins. This effect was most pronounced in the presence of Fe^{2+}(EDTA)+H_2O_2, which is consistent with published data on primary formation of carbonyl groups during metal-catalyzed oxidation of proteins (E. R. Steadman [14]).

Under conditions of moderate hypothermia the number of carbonyl groups in plasma proteins increased by 162%, while the rate of *in vitro* protein oxidation remained unchanged (Table 1).

Long-term (3 h) moderate (30°C) hypothermia markedly increased the number of carbonyl groups in plasma proteins and the rate of their accumulation in model systems. Since *in vivo* and *in vitro* oxidative modification of proteins was intensified, it can be assumed that these processes are related to enhanced ROS generation and conformational changes in proteins. These changes improve the accessibility of hidden amino acid residues to prooxidants.

The drop of core temperature to 20°C decreased the number of carbonyl groups in plasma proteins compared to the previous stage of hypothermia (Table 1). The intensity of protein oxidation in the model system decreased. These results are consistent with published data that deep hypothermia inhibits oxygen consumption by tissues and respiratory processes [7,8] accompanied by intensive ROS formation [6]. However, it remains unclear why the number of carbonyl groups in proteins decreases with further decrease in body temperature. Oxidized proteins undergo aggregation and fragmentation [10]. Protein aggregation is associated with the formation of dityrosine cross-links, which prevents registration of carbonyl groups. Oxidized proteins undergo more rapid hydrolysis than native proteins [10]. This process results in the formation of low-molecular-weight oligopeptides. The decrease in the number of carbonyl groups in plasma proteins at 20°C probably attests to accelerated aggregation, fragmentation, and proteolysis of oxidatively modified proteins.

Dalargin had no effect on the initial content of carbonyl groups in proteins in control rats, but significantly decreased their accumulation in the model system (Table 1). Dalargin produced a weak protective effect during moderate hypothermia and was ineffective during prolonged moderate and deep hypothermia (Table 1).

Low protective effects of dalargin on plasma proteins during moderate hypothermia and the absence of antioxidant activity under conditions of long-term cold exposure are probably related to rapid cleavage of this substance with plasma proteolytic enzymes (7-13 min) [5]. Moreover, this peptide in a dose used in our experiments produces no direct antioxidant effects. Dalargin prevents stress-induced activation of catabolic processes and ROS generation [3]. The effects of dalargin are realized via interaction with specific receptors on cell membranes in various tissues, including blood cells [3]. Dalargin indirectly modulates oxidative modification of plasma proteins during hypothermia probably by inhibiting ROS accumulation and preventing efflux of oxidized proteins and prooxidants from cells and tissues to the plasma.

Thus, moderate and, especially, prolonged hypothermia markedly promotes oxidative modification of plasma proteins. Preinjection of dalargin produces no protective effects.

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