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Changes in the TBARs content and superoxide dismutase, catalase and glutathione peroxidase activities in the lymphoid organs and skeletal muscles of adrenodemedullated rats

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Abstract

Thiobarbituric acid reactant substances (TBARs) content, and the activities of glucose-6-phosphate dehydrogenase (G6PDh), citrate synthase (CS), Cu/Zn- and Mn-superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) were measured in the lymphoid organs (thymus, spleen, and mesenteric lymph nodes (MLN)) and skeletal muscles (gastrocnemius and soleus) of adrenodemedullated (ADM) rats. The results were compared with those obtained for sham-operated rats. TBARs content was reduced by adrenodemedullation in the lymphoid organs (MLN (28%), thymus (40%) and spleen (42%)) and gastrocnemius muscle (67%). G6PDh activity was enhanced in the MLN (69%) and reduced in the spleen (28%) and soleus muscle (75%). CS activity was reduced in all tissues (MLN (75%), spleen (71%), gastrocnemius (61%) and soleus (43%)), except in the thymus which displayed an increment of 56%. Cu/Zn-SOD activity was increased in the MLN (126%), thymus (223%), spleen (80%) and gastrocnemius muscle (360%) and was reduced in the soleus muscle (31%). Mn-SOD activity was decreased in the MLN (67%) and spleen (26%) and increased in the thymus (142%), whereas catalase activity was reduced in the MLN (76%), thymus (54%) and soleus muscle (47%). It is particularly noteworthy that in ADM rats the activity of glutathione peroxidase was not detectable by the method used. These data are consistent with the possibility that epinephrine might play a role in the oxidative stress of the lymphoid organs. Whether this fact represents an important mechanism for the establishment of impaired immune function during stress remains to be elucidated.

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- TBARs

Introduction

Oxygen free radicals (superoxide, [O_2^-] and hydroxyl radical [$OH^\bullet$]) and hydrogen peroxide (H_2O_2), called reactive oxygen species (ROS), play a significant role in the antibacterial and antitumorigenic capacity of macrophages and neutrophils, but they are also capable of presenting a toxic action on self tissues causing lipid peroxidation (1,2). In fact, the deficiency of vitamin E has been reported to facilitate the increase in ROS concentrations leading to functional changes in the immune system (1,3). For instance, chemical antioxidants such as ß-carotene (4), vitamin E (5), vitamin C (6) and reduced glutathione (GSH) (7) improve the proliferative capacity of lymphocytes, and increase host defense and immunoglobulin synthesis (8). Evidence has been presented that stress may influence the function of the immune system of humans and experimental animals (9) and modify the pro-oxidant capacity of macrophages and neutrophils. These effects may cause a decrease in the function of these cells and modify the immune response to viruses and bacteria. Most of the investigations on this subject have focused on the role of glucocorticoids (10-12). Indeed, glucocorticoids present a high capacity for inhibition of ROS production by macrophages and neutrophils (13). However, during stress the sympathetic nervous system is stimulated and epinephrine is secreted from the adrenal medulla (14). Furthermore, as is the case for all cells responsive to epinephrine via cAMP generation, immune cells display ß-adrenoceptors (15,16). In fact, epinephrine increases the proliferative capacity of CD4+ and CD8+ cells via α-receptors, but inhibits that of CD4+ cells via ß-adrenoceptors (17-19). Thus, it was proposed that when cAMP concentrations increase in the lymphocytes, the proliferative capacity of the latter is inhibited (20). Cannon (21) suggested that cAMP might not be considered only an immune inhibitory agent. Munck et al. (22) also reported that "the increase in the concentration of cortisone in the plasma during prolonged exercise may serve to protect the body against the excessive activation of its immune defenses during stress". This means that epinephrine may act as a modulating agent of human immunity, in addition to glucocorticoids.

Recent work from our laboratory has shown that epinephrine stimulates H_2O_2 production in incubated rat macrophages (23). However, the effect of epinephrine observed *in vitro* does not imply that this hormone plays a physiological role for the control of immune function. In addition, whether the generation of H_2O_2 by epinephrine is linked to changes in the activity of antioxidant enzymes remains unknown. Taking into account the involvement of ROS in the immune function, it is important to investigate the effect of epinephrine on antioxidant enzyme activities, the major form of cell defense against acute oxygen toxicity (24), and lipid peroxidation. In the present study, the physiological effect of epinephrine (by bilateral removal of adrenal medulla (ADM)) on the activity of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) of the lymphoid organs (mesenteric lymph nodes (MLN), spleen and thymus) was examined. For comparison with nonimmune tissues, skeletal muscles (soleus and gastrocnemius) were also studied. Enzyme activities involved in the generation of reducing power were also measured: glucose-6-phosphate dehydrogenase (G6PDh), indicative of the flux of substrates through the pentose-phosphate pathway (25), and citrate synthase (CS), an indicator of the flux of substrates through the Krebs cycle (26). As an indication of the occurrence of lipid peroxidation under these conditions, the content of thiobarbituric acid reactant substances (TBARs) was also determined.

Material and Methods

Reagents and equipment

All chemicals and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO) and Boehringer Mannheim (Germany). The solutions were prepared with twice-distilled, Millipore Milli Q deionized water. All measurements were performed using Zeiss DMR-10 and Gilford (Model Response) spectrophotometers.

Animals

Male Wistar rats weighing 180 g (about 2 months of age) were obtained from the Institute of Biomedical Sciences. The rats were maintained at 23°C on a 12-h light:12-h dark cycle.

Adrenodemedullation

The rats were anesthetized by ether inhalation and the kidneys were exposed through the dorsal side. A small cut in the cortex of the adrenal glands was made so that the medulla could be removed by light pressure on the gland. Enzyme activities and lipid peroxidation were measured 35 days after adrenodemedullation. A similar procedure has been previously used (27).

Experimental procedure

The rats were always killed between 8:00 and 11:00 a.m. by cervical dislocation without anesthesia. The thymus, spleen, MLN, gastrocnemius (white portion, type IIb fibers) and soleus (type I fibers) muscles were then excised and maintained in liquid nitrogen prior to measurements of the enzyme activities and TBARs content.

Enzyme assays

The extraction medium for the measurements of Cu/Zn- and Mn-SOD, catalase and GPX activities contained 0.10 M sodium phosphate, pH 7.0. For the SOD assay (28), the homogenate was centrifuged at 10,000 *g* for 30 min. Cu/Zn- and Mn-SOD activities were measured by following the dismutation of KO_2 at 250 nm. The procedures used for catalase and GPX assays were similar to those used by Beutler (29) and Maral et al. (30), respectively. Catalase activity was determined by measuring the decomposition of hydrogen peroxide at 230 nm. GPX activity was measured by following the rate of oxidation of the reduced form of glutathione. The formation of oxidized glutathione was monitored by a decrease in the concentration of NADPH, measured at 340 nm, due to the addition of glutathione reductase to the medium.

The extraction medium for CS and G6PDh contained 50 mM Tris-HCl and 1 mM EDTA, pH 7.4. CS was assayed as described by Cooney et al. (31) and G6PDh according to Bergmeyer and Bernt (32). The assay medium for CS consisted of 100 mM Tris-HCl, 0.2 mM 5,5'-dithio-bis-2-nitrobenzoic acid, 15 mM acetyl-CoA, and 0.5 mM oxaloacetate, pH 8.1. The assay medium for G6PDh consisted of 86 mM Tris-HCl, 6.9 mM $MgCl_2$, 0.4 mM NADP, 1.2 mM glucose-6-phosphate, and 1.2 U/ml 6-phosphogluconate dehydrogenase, pH 7.6. All spectrophotometric measurements were carried out at 25°C. The enzyme activities are reported as µmol/min per g tissue fresh weight.

Determination of TBARs

Substances that react with TBARs were measured as described by Winterbourn et al. (33) in the same extraction medium as for the antioxidant enzyme assays.

Statistical analysis

Two-way ANOVA with *post hoc* contrasts was used to compare groups. The level of significance was set at $P<0.01$.

Table 1 - Content of thiobarbituric acid-reactive substances (TBARs) in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol/g tissue fresh weight)		
MLN	10.1 ± 2	8.5 ± 0.8	6.1 ± 0.3*
Thymus	13.2 ± 2	15.4 ± 1.2	9.2 ± 0.6*
Spleen	39.3 ± 5	42.0 ± 4.5	24.2 ± 8.4*
GC	4.8 ± 0.3	5.4 ± 0.4	1.8 ± 0.03*
Soleus	6.5 ± 0.4	6.5 ± 0.5	7.5 ± 0.4

Table 2 - Glucose-6-phosphate dehydrogenase (G6PDh) activity in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	14.3 ± 1.4	13.6 ± 1.3	22.7 ± 2.5*
Thymus	21.3 ± 1.9	22.8 ± 3.3	22.5 ± 2.7
Spleen	54.7 ± 5.6	51.9 ± 4.3	36.1 ± 2.4*
GC	4.4 ± 0.2	4.8 ± 0.3	3.7 ± 0.3
Soleus	6.4 ± 0.6	6.3 ± 0.6	2.1 ± 0.1*

Table 3 - Citrate synthase (CS) activity in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	107.3 ± 8	101.2 ± 5.3	25.2 ± 1.2*
Thymus	15.4 ± 1.2	13.9 ± 1.0	21.7 ± 1.5*
Spleen	71.9 ± 4.7	73.2 ± 0.8	21.0 ± 0.7*
GC	54.2 ± 2.8	56.8 ± 4.1	21.9 ± 1.6*
Soleus	55.4 ± 2.3	56.8 ± 4.1	32.0 ± 0.2*

Results

The results for sham-operated rats did not differ from those obtained in controls. Thus, the results for adrenodemedullated rats were compared with those of sham-operated rats only. The content of TBARs (Table 1) was diminished by adrenodemedullation in the lymphoid organs (mesenteric lymph nodes (28%), thymus (40%) and spleen (42%)) and gastrocnemius muscle (67%). In relation to the sites of production of reducing power, G6PDh activity (Table 2) was enhanced in the MLN (69%) and reduced in the spleen (28%) and soleus muscle (75%) due to removal of the adrenal medulla. CS activity (Table 3) was lowered in all tissues (MLN (75%), spleen (71%), gastrocnemius (61%) and soleus (43%)), except in the thymus which displayed an increment of 56% as a consequence of the absence of the adrenal medulla.

The antioxidant enzyme activities were also markedly changed by the removal of the adrenal medulla. Cu/Zn-SOD activity (Table 4) was increased in the MLN (126%), thymus (223%), spleen (80%) and gastrocnemius muscle (360%) and was reduced in the soleus muscle (31%). Removal of the adrenal medulla decreased Mn-SOD activity in the MLN (67%) and spleen (26%) and increased it in the thymus (142%) (Table 5), whereas catalase activity was lowered in the MLN (76%), thymus (54%) and soleus muscle (47%) (Table 6). It is particularly noteworthy that in ADM rats the activity of glutathione peroxidase was not detectable by the method used for all tissues studied (Table 7).

Discussion

Whether epinephrine plays a role for the establishment of an impaired immune response as reported for several modalities of stress is an intriguing point. In this study, changes in the oxygen metabolism of the

lymphoid organs (mesenteric lymph nodes, thymus and spleen) were investigated by the removal of the adrenal medulla. This experimental procedure was chosen in order to avoid possible misinterpretation of the results that may occur in pharmacological experiments (e.g. administration of adrenergic drugs). The results were compared with those obtained for skeletal muscle.

Taken as a whole, the effect of adrenodemedullation on TBARs content and enzyme activities in the lymphoid organs was not markedly different from that observed in the skeletal muscles.

The removal of adrenal medulla reduced the content of TBARs as shown in Table 1. Therefore, circulating catecholamines may play a role in the lipid peroxidation process. Catecholamines may increase the content of TBARs due to stimulation of the activities of enzymes involved in the sites of production of the reducing power required for NADPH oxidase activity (pentose-phosphate pathway and Krebs cycle) or by inhibition of antioxidant enzyme activities. These possibilities were examined in the present study. The removal of the adrenal medulla produced tissue-specific effects on G6PDh activities. This enzyme can provide a qualitative index of NADPH production via the pentose-phosphate pathway. There was an increase in G6PDh activity in the MLN and a reduction in the soleus muscle and spleen. However, adrenodemedullation provoked a marked decrease of CS activity in all tissues studied except the thymus (Table 3). This decrease was particularly marked for the MLN and spleen, suggesting a lower flux through the TCA cycle in cells of the lymphoid tissues, in the absence of adrenaline (26). Lymphoid cells may compensate for this change by increasing ATP synthesis via anaerobic glycolysis while also decreasing flux through the pentose-phosphate pathway (as a consequence of maximizing carbon flux through the ATP generating reactions of glycolysis). In fact, in our previous study it was found

Table 4 - Cu/Zn-superoxide dismutase (Cu/Zn-SOD) activity (x10^3) in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	24.3 ± 1.6	23.4 ± 2.2	56.1 ± 3.4*
Thymus	16.4 ± 2.1	16.2 ± 2.1	51.3 ± 3.1*
Spleen	41.4 ± 1.9	43.1 ± 2.0	75.2 ± 4.6*
GC	16.9 ± 0.8	15.4 ± 1.0	71.4 ± 6.5*
Soleus	50.9 ± 2.3	52.4 ± 2.7	34.2 ± 3.2*

Table 5 - Mn-superoxide dismutase (Mn-SOD) activity (x10^3) in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	0.27 ± 0.02	0.32 ± 0.02	0.10 ± 0.01*
Thymus	0.20 ± 0.02	0.22 ± 0.01	0.52 ± 0.06*
Spleen	0.62 ± 0.04	0.60 ± 0.02	0.43 ± 0.08*
GC	0.22 ± 0.01	0.21 ± 0.01	0.24 ± 0.02
Soleus	0.52 ± 0.04	0.53 ± 0.02	0.67 ± 0.03*

Table 6 - Catalase activity (x10^3) in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	1.19 ± 0.10	1.17 ± 0.20	0.28 ± 0.01*
Thymus	0.65 ± 0.01	0.61 ± 0.01	0.28 ± 0.01*
Spleen	1.50 ± 0.12	1.30 ± 0.13	1.13 ± 0.02
GC	0.14 ± 0.03	0.12 ± 0.01	0.19 ± 0.01
Soleus	0.74 ± 0.04	0.71 ± 0.02	0.38 ± 0.01*

Table 7 - Glutathione peroxidase (GPX) activity (x10^3) in the lymphoid organs and muscles of adrenodemedullated (ADM), sham-operated and control rats.

Values are reported as means ± SEM for 8 rats in each group. *P<0.01 for comparison between ADM and sham-operated rats (Student *t*-test). MLN, Mesenteric lymph nodes; GC, gastrocnemius white portion; ND, not detected.

	Control	Sham-operated	ADM
	(µmol min^{-1} g tissue fresh $weight^{-1}$)		
MLN	3.10 ± 0.19	3.21 ± 0.17	ND
Thymus	4.00 ± 0.64	3.80 ± 0.54	ND
Spleen	4.40 ± 0.10	4.22 ± 0.07	ND
GC	5.40 ± 0.14	5.23 ± 0.12	ND
Soleus	6.50 ± 0.18	6.33 ± 0.20	ND

that adrenaline markedly stimulates glucose consumption and lactate production in incubated macrophages (34). The combined reduction in TCA cycle and pentose-phosphate pathway activity will reduce NADPH generating capacity. This may explain the observed reduction in TBARs concentration in the lymphoid organs and gastrocnemius muscle of ADM rats (Table 1). Epinephrine may stimulate metabolic pathways for NADPH production, which could increase TBARs concentration in the tissues. In addition, we have evidence that epinephrine can greatly stimulate macrophage H_2O_2 production via cAMP within one hour of addition of the hormone (23). Thus, epinephrine may increase NADPH production by allosteric (in this example) as well as by transcriptional/translational mechanisms.

Adrenodemedullation also markedly affected the activities of anti-oxidant enzymes. In summary, among the enzymes studied, the absence of the adrenal medulla raised the activity of Cu/Zn-SOD (Table 4), diminished that of catalase (Table 5) and abolished that of glutathione peroxidase (Table 7). The findings of a decrease in catalase activity and abolition of glutathione peroxidase activity due to adrenodemedullation led us to speculate that epinephrine physiologically regulates the activities of these enzymes in the lymphoid organs. In fact, there is evidence that epinephrine activates glutathione peroxidase in the heart, liver and kidney (35). Whether this effect is mediated by the increase in the concentration of oxygen reactive species (36) or is directly caused by the action of the hormone itself remains to be determined.

The present observations are consistent with the possibility that epinephrine might play a role in the oxidative stress of the lymphoid organs. Whether this fact represents an important mechanism for the establishment of impaired immune function during stress remains to be elucidated.

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