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HORMONAL REGULATION OF SUPEROXIDE DISMUTASE, CATALASE, AND GLUTATHIONE PEROXIDASE ACTIVITIES IN RAT MACROPHAGES

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Abstract—This study examined the effects of glycocorticoids, insulin, thyroxine, and epinephrine upon the activities of CuZn- and Mn-superoxide dismutases (SOD), catalase, and glutathione peroxidase (GPX) and upon hydrogen peroxide production in rat macrophages obtained from the intraperitoneal cavity. The experiments were performed *in vivo* under conditions causing hormonal dysfunctions: adrenal demedullation, dexamethasone treatment, thyroidectomy, administration of L-tri-iodothyronine (T_3) and L-thyroxine (T_4), and diabetes. Macrophages were also cultured for 24 hr in the presence of dexamethasone, thyroid hormones, and insulin as to evaluate possible interferences caused *in vivo* by changes in other hormones. The results indicated that these hormones do control the activities of the antioxidant enzymes and hydrogen peroxide production both *in vivo* and *in vitro*. Insulin increased the activities of CuZn-SOD, catalase, and GPX and reduced that of Mn-SOD. Thyroid hormones raised the activities of CuZn- and Mn-SOD and decreased that of GPX, whereas glucocorticoids reduced both Mn-SOD and GPX. The removal of the adrenal medulla caused a decrease of Mn-SOD and GPX activities in the macrophages. Hydrogen peroxide production was increased by insulin and reduced by thyroid hormones and glucocorticoids. The changes in antioxidant enzyme activities caused by these hormones in macrophages may indicate important mechanisms for the establishment of impaired immune function in endocrine pathologies.

Key words: macrophages; superoxide Dismutase; Catalase; glutathione Peroxidase; hormones

Macrophages, like all cells of the immune system, are derived from stem cells in the bone marrow. Macrophages not only play a role in killing foreign organisms, but also initiate the immune response. These cells have high endocytic activity and also the capacity to secrete a large number of multifunctional compounds, including enzymes, prostaglandins, cytokines, and reactive metabolites of oxygen [1].

Early metabolic studies concerning the macrophage focused on events associated with phagocytosis or the respiratory burst [2]. The respiratory burst is characterized by activation of an NADPH-dependent membrane-associated oxidase to produce O_2^- from O_2, and H_2O_2 from the dismutation of O_2^- [3]. The reactive metabolites of oxygen that are generated at or near the cell surface and within the phagocytic vacuole exert antimicrobial and antitumor effects and have been associated with carcinogenesis and autoimmune diseases [4]. However, the macrophage itself has to be protected from the toxicity of reactive oxygen species generated intracellularly. This self-protection of macrophages is obtained, in great part, from the activities of the antioxidant enzymes: CuZn-SOD‡, Mn-SOD, catalase, and GPX. In spite of this, few studies have been performed on the regulation of the activities of these enzymes in macrophages.

Macrophages, as well as lymphocytes and neutrophils, contain receptors for many hormones including corticosteroids, insulin, and catecholamines [5]. In fact, these hormones can control immune function and inflammatory response. The plasma levels of glucocorticoids regulate the inflammatory response in several ways, the reduction in prostaglandins synthesis being one important mechanism for this process to take place [6]. Glucocorticoids are known to inhibit interleukin-1 synthesis immunocytes [7], and this could well involve an effect on the metabolism of these cells, as has been reported for lymphocytes [8]. The important role played by insulin in the inflammatory response has been evidenced by studies carried out in diabetic subjects [5], who present high incidences of infections. Recent studies have shown high oxygen radical production in macrophages from diabetic-prone BB rats [9]. The enhanced respiratory burst in the cells of the spontaneously diabetic rats is also accompanied by a similar increase in glucose metabolism [10]. Thyroid hormones and catecholamines also affect immune function, and changes in immune and inflammatory responses have been reported in diseases involving these two hormones [6]. In fact, thyroid hormones affect lymphocyte proliferation [11] and metabolism [12] and present antiviral effects [13], whereas epinephrine increases the production of hydrogen peroxide in incubated macrophages [14]. Although the importance of these hormones for the immune function and inflammatory response is widely reported, their effects on antioxidant enzyme activities of macrophages have not been investigated. Study of this subject is particularly important since oxygen metabolism is closely related to macrophage function.

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‡ Abbreviations: SOD, superoxide dismutase; GPX, glutathione peroxidase; T_3, L-tri-iodothyronine; and T_4, L-thyroxine.

The effects of glucocorticoids, insulin, T_4, and epinephrine upon the enzymatic antioxidant capacity of macrophages were investigated. For this purpose, the activities of CuZn-SOD, Mn-SOD, catalase, and GPX were determined in macrophages isolated from rats subjected to several hormonal dysfunctions and in cells cultivated in the presence of these hormones. The following conditions causing hormonal dysfunctions were studied: adrenal demedullation, dexamethasone treatment, thyroidectomy, administration of thyroid hormones (T_3 and T_4), and diabetes. Macrophages were also cultured for 24 hr in the presence of dexamethasone, thyroid hormones, and insulin to evaluate possible interferences caused *in vivo* by changes induced in other hormones.

MATERIALS AND METHODS

Chemicals and hormones

All chemicals and enzymes were obtained from Boehringer Mannheim, Lewes, East Sussex, U.K., and the Sigma Chemical Co., St. Louis, MO. U.S.A. except for T_4 and T_3 which were from Aché Laboratories, São Paulo, SP, Brazil. The solutions were prepared with bidistilled Millipore Milli Q deionized water.

Animals

Wistar adult male rats were used for all experiments. The animals were maintained in an environment at 23°C and a light/dark cycle of 12/12 hr (lights on from 7:00 a.m.).

Hormonal dysfunctions

For the establishment of the desired hormonal dysfunctions, the rats were subjected to the respective treatments for specific lengths of time in each case. Previous experiments had confirmed the adequacy of the procedures used for obtaining the hormonal dysfunctions used in this study.

Demedullation of the adrenal glands. Rats (N = 8) 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. The experiment was performed 35 days after the surgery, according to Waynforth [15]. A sham-operated group (5 rats) that received a small cut in the cortex of the adrenal only was included as a control.

Dexamethasone treatment. Dexamethasone at a dose of 5 mg/kg body weight was injected intraperitoneally into the rats (N = 8) for 5 days, following the protocol described by Serrano *et al.* [8].

Induction of hyperthyroidism. The rats (N = 8) were made hyperthyroidic by the daily administration of 500 mg T_4 and 125 mg T_3/kg body weight, in a small volume of water, for 7 days. The animals were killed 24 hr after administration of the last dose of the hormones [14].

Induction of hypothyroidism. Surgical thyroparathyroidectomy was carried out on the rats (N = 8) under ether anesthesia, 50 days before the experiment. Calcium lactate (2%) was added to the drinking water after surgery to prevent hypocalcemia.

Hyper- and hypothyroid states were confirmed by measuring circulating T_4 levels by radioimmunoassay [16], body weight gain, and heart rate. The values obtained for these measurements were similar to those reported previously [12, 14, 17].

A sham-operated group (5 rats) that did not have the thyroid removed was also included; the results obtained were not different from those of the control group, so they are not presented.

Induction of diabetes. Animals were fasted for 18 hr, and then were injected intravenously with a dose of 42 mg/alloxan in PBS/kg body weight [18]. The animals used in the experiment were those with glycemia above 200 mg/100 mL. A group of 5 rats was injected with a similar volume of PBS as a control. In addition to diabetic rats without treatment, diabetic rats treated with insulin (2 U/animal, for 8 days) and fasted for 24 hr (presenting normoglycemia but low insulinemia) were also included in this study.

Peritoneal cell preparation

The rats were killed between 8:00 and 10:00 a.m. by decapitation without anesthesia. After laparotomy, macrophages from the intraperitoneal cavity were collected. Resident macrophages were obtained by intraperitoneal lavage with 6 mL of sterile PBS, pH 7.2.

Macrophages were purified by adherence to plastic petri dishes. The cells were suspended in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM glutamine and 20 μg penicillin-streptomycin/mL and were added to 100 mm tissue culture petri dishes at a density of 1.0×10^7 cells/dish. After incubation for 4 hr at 37° in 5% CO_2/95% air, adherent macrophages were washed three times vigorously with PBS. The population of cells that remained adhered to the plate was at least 98% macrophages (as determined by differential staining [19]). The macrophages were removed from the plates by gentle scraping into 2–3 mL of PBS using a rubber policeman. Cell viability was determined to be >95% by exclusion of trypan blue. At least 92% of peritoneal exudate cells were macrophages, as determined by differential counts (data not shown). The cells were then homogenized for determination of the enzyme activities or cultured in the presence of the hormones.

Culture procedure

Macrophages from control rats were left to adhere to the plates during 4 hr as described above. After this period, the adhered cells were cultured in MEM supplemented with 10% FBS and 20 μg/mL penicillin-streptomycin for 48 hr at 37°, in an artificially humidified atmosphere of 5% CO_2 in air under sterile conditions. Insulin (200 mU/mL), T_3/T_4 (120 and 30 μg/mL, respectively), and dexamethasone (2 mg/mL) were added to the culture medium for the assessment of their effects. The cultures were maintained in a LAB-LINE Microprocessor CO_2 incubator (U.S.A.). After 24 hr of culture, more than 98% of the macrophages were viable, as measured by trypan blue exclusion.

Enzyme assays

The extraction medium for the measurements of SOD, catalase, and GPX activities was 0.10 M sodium phosphate buffer at pH 7.0. For the SOD assay [20], 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 [12]. The procedures used for catalase and GPX assays were similar to those reported by Beutler [21] and Maral *et al.* [22], respectively. Catalase activity was determined by measuring the decomposition of hydrogen peroxide at 230 nm. The activity of GPX 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 in the medium. The enzyme assays were performed in a Zeiss DMR-10 spectrophotometer at 25°. Enzyme activities are expressed as micromoles per minute per 10^{10} cells, except for GPX, which is expressed by the formation of NADPH in nanomoles per minute per 10^{10} cells.

Hydrogen peroxide production

The production of hydrogen peroxide was measured by the method described by Pick and Mizel [23] and modified by Costa Rosa *et al.* [14], which uses phenol red and peroxidase.

Statistical analysis

Results are presented as means ± SEM. The paired *t*-test was employed to compare the effect of hormonal treatments against the control group, and the level of significance was set at $P < 0.01$.

RESULTS

The effects of the hormonal dysfunctions on CuZn-SOD, Mn-SOD, catalase, and GPX activities in macrophages are shown in Table 1. Adrenal demedullation of the rats reduced the activities of Mn-SOD (54%) and GPX (38%), whereas the administration of dexamethasone decreased the activity of Mn-SOD (27%) and increased that of catalase (34%) and GPX (26%).

Hypothyroidism decreased Mn-SOD (38%), catalase (34%), and GPX (29%) activities in resident macrophages. Thyroid hormones given in excess markedly increased Mn-SOD activity by 210% but reduced that of GPX (68%).

The alloxan-diabetic state provoked a marked reduction in CuZn-SOD (61%), catalase (76%), and GPX (24%) activities, whereas a very high increase was observed for Mn-SOD (130%). Starvation for 12 hr (which caused the glycemia of diabetic rats to normalize) did not modify the changes caused by the diabetic state on antioxidant enzyme activities of macrophages. Administration of insulin (2 U/kg body weight) fully reversed the enzymatic changes caused by diabetes.

The production of hydrogen peroxide by resident macrophages was reduced markedly due to the hormonal dysfunctions. As compared with control rats, the reduction was: 50% for adrenal demedullated, hypo- and hyperthyroidic rats, 90% for the dexamethasone-treated and diabetic groups, and 34% for insulin-treated diabetic rats.

The results of the experiments with cultured macrophages are shown in Table 2. Addition of insulin to the culture medium raised the activities of CuZn-SOD (45%), catalase (35%), and GPX (21%) but that of Mn-SOD was decreased by 46%. Thyroid hormones raised CuZn-SOD (58%) and Mn-SOD (56%) activities and decreased that of GPX (48%). Dexamethasone added to the culture medium reduced the activities of Mn-SOD and GPX by 59 and 47%, respectively. Hydrogen peroxide production by cultured macrophages was enhanced (17%) by the addition of insulin to the medium and reduced due to thyroid hormones (29%) and dexa-

Table 1. Effects of various hormonal dysfunctions on antioxidant enzyme activities and hydrogen peroxide production in resident macrophages

Groups	CuZn-SOD	Mn-SOD	Catalase	GPX	Hydrogen peroxide
Control	329 ± 20	3.7 ± 0.4	5.0 ± 0.5	357 ± 10	6.1 ± 0.4
ADM	355 ± 19	1.7 ± 0.2*	5.1 ± 0.5	223 ± 6*	3.0 ± 0.7*
Sham-ADM	330 ± 2.5	3.5 ± 0.2	4.9 ± 0.3	343 ± 17	5.4 ± 0.3
DEXA	335 ± 19	2.7 ± 0.2*	6.7 ± 0.5*	453 ± 30*	0.7 ± 0.1*
Saline-inj-I	320 ± 12	3.6 ± 0.3	5.2 ± 0.4	329 ± 28	5.9 ± 0.2
HIPOT	320 ± 17	2.3 ± 2.0*	3.3 ± 0.2*	254 ± 5*	3.1 ± 0.3*
Sham-HIPOT	342 ± 15	4.0 ± 0.5	4.8 ± 0.2	340 ± 29	6.0 ± 0.7
HIPERT	327 ± 18	11.5 ± 0.6*	4.2 ± 0.3	114 ± 2*	2.9 ± 0.8*
Saline-inj-II	327 ± 16	3.8 ± 0.3	5.3 ± 0.4	341 ± 17	5.7 ± 0.4
Diabet.	127 ± 8.0*	8.5 ± 0.6*	1.2 ± 0.1*	273 ± 22*	0.5 ± 0.1*
Diabet-fasted	119 ± 8*	9.0 ± 0.6*	1.5 ± 0.1*	234 ± 10*	0.5 ± 0.1*
Diabet-insulin	345 ± 27	4.0 ± 0.3	5.0 ± 0.3	345 ± 11	4.0 ± 0.3*
Diab-saline injected	321 ± 32	3.6 ± 0.3	4.7 ± 0.4	362 ± 19	5.3 ± 0.3

Resident macrophages were obtained from the intraperitoneal cavity of rats subjected to treatments causing different hormonal disfunctions by lavage with cold saline. The cells were then cultured for 4 hrs to obtain a pure macrophage preparation. After this period, macrophages were removed from the plates and homogenized in the extraction medium for assay of the enzymes and hydrogen peroxide production. For details on the induction of hormonal dysfunctions, cell preparation, enzyme assays, and hydrogen peroxide measurements see Materials and Methods. The activities of Cu/Zn- and Mn-SOD and catalase are expressed as μmol/min per 10^{10} cells, of GPX as nmol/min per 10^{10} cells, and hydrogen peroxide production as nmol/hr per mg protein. Values are means ± SEM of 8 rats per group. Abbreviations: ADM, adrenal demedullated group; DEXA, dexamethansone-injected rats; HIPOT, hypothyroid rats; HIPERT, hyperthyroid group; Diabet, alloxan-diabetic rats; Diabet-fasted, diabetic rats submitted to 24 hr fasting (normoglycemia but low insulinemia); Diabet-insulin, diabetic rats injected daily with insulin (2 U/kg body weight); SOD, superoxide dismutase; and GPX, glutathione peroxidase. Sham-operated groups for adrenal demedullated and hypothyroidic were included and showed results similar to those of the controls. Saline-inj-I followed the protocol of dexamethasone injection and saline-inj-II followed the protocol of thyroid hormone injection; the results were not different from controls. Diab-saline-injected rats were submitted to the same protocol of injections as the diabet-insulin-injected rats.

* $P < 0.01$, for comparison between control and treated groups (*t*-test).

Table 2. Effects of insulin, thyroid hormones (T_3/T_4) and dexamethasone on antioxidant enzyme activities and hydrogen peroxide production in 24-hr cultured macrophages

Additions	CuZn-SOD	Mn-SOD	Catalase	GPX	Hydrogen peroxide
None	323 ± 22	3.9 ± 0.4	4.8 ± 0.4	349 ± 11	2.8 ± 0.2
Insulin (1 U/mL)	471 ± 27*	2.1 ± 0.4*	6.5 ± 0.7*	423 ± 12*	3.3 ± 0.2*
T_3/T_4 (120/30 μg/mL)	541 ± 29*	6.1 ± 0.2*	5.0 ± 0.4	183 ± 13*	2.0 ± 0.2*
Dexamethasone (2 mg/mL)	312 ± 17	1.6 ± 0.2*	4.7 ± 0.4	184 ± 12*	1.3 ± 0.1*

Macrophages obtained from the intraperitoneal cavity of the rats were cultured as described in Materials and Methods. The activities of Cu/Zn- and Mn-SOD and catalase are expressed as μmol/min per 10^{10} cells, of GPX as nmol/min per 10^{10} cells, and hydrogen peroxide production as nmol/hr per mg protein. Results are means ± SEM of 6 plates from 3 different cell preparations. Abbreviations: SOD, superoxide dismutase; and GPX, gluthathione peroxidase.

* $P < 0.01$, compared with no addition (none).

methasone (54%). Experiments with epinephrine added to the culture medium were not performed, since this hormone reacts directly with superoxide species. The evaluation of the effect of epinephrine on antioxidant enzyme activities in macrophages was then carried out *in vivo* by the removal of adrenal medulla as described above and shown in Table 1.

DISCUSSION

The hormonal dysfunctions did affect the antioxidant enzyme activities in resident macrophages. This fact might be important for macrophage function and consequently for inflammatory and immune responses. The bactericidal and tumoricidal activities of the macrophages were probably reduced due to demedullation of the adrenal, dexamethasone treatment, hypo- and hyperthyroidism and diabetes, as indicated by the low production of hydrogen peroxide.

Glucocorticoids are known to suppress immunological and inflammatory responses [8]. Additionally, administration of pharmacological doses of glucocorticoids may result in reactivation of latent infections such as tuberculosis [24]. This hormone, however, presents effects also on macrophages such as an inhibiting effect on the proliferative response of monocytes to colony-stimulating factor [25] and of its differentiation into macrophages [26]. They also inhibit the phagocytic and cytotoxic functions of macrophages [27]; this latter may be related to the reduced production of hydrogen peroxide (Tables 1 and 2) and Mn-SOD enzyme activity caused by dexamethasone (Tables 1 and 2). It is interesting to note that the results obtained *in vivo* were not fully reproduced in cultured macrophages (compare Tables 1 and 2). In fact, addition of dexamethasone to the culture medium caused a marked decrease of all enzyme activities in 48-hr cultured macrophages, whereas treatment of the rats with this drug led to an increase in catalase and GPX activities. This discrepancy possibly occurred due to the fact that administration of glucocorticoids *in vivo* also modifies the plasma levels of other hormones such as ACTH and corticotropin-releasing hormone. Further studies must be performed to examine the effects of these peptide hormones on antioxidant enzyme activities in macrophages.

Evidence has been presented that oxidative stress may influence the function of the immune system of humans and experimental animals [28]. Most of the investigations on this subject have proposed a central role played by the glucocorticoids in the impairment of immune function under a stress condition [29–31]. During stress, however, the sympathetic nervous system is stimulated and epinephrine is secreted from adrenal medulla [32]. This hormone might also participate in the regulation of immune function as indicated by the fact that immune cells display β-adrenoreceptors [33, 34]. Indeed, recent work in our laboratory has shown that epinephrine stimulates H_2O_2 production in incubated rat macrophages [35] and, as shown in Table 1, the removal of the adrenal medulla reduced its production by resident macrophages. The results presented in Table 1 support the proposition that adrenaline may physiologically regulate the activities of Mn-SOD and GPX in resident peritoneal macrophages. The significance of this fact for the functions played by macrophages *in vivo* remains to be elucidated.

Administration of thyroid hormones caused a marked increase of Mn-SOD activity in macrophages (Table 1), as was also observed previously in the lymphoid organs (spleen, thymus and mesenteric lymph nodes) and muscles [17]. The important role played by the thyroid hormones for the regulation of this enzyme activity is further supported by the fact that the removal of the thyroid gland provoked a significant reduction of Mn-SOD activity. Thyroid hormones seem to maintain the activities of other antioxidant enzymes at control levels. Indeed, the removal of the gland led to diminished catalase and GPX activities. Interestingly, there was an increment in CuZn-SOD activity when T_4 was added to cultured macrophages. This is a pharmacological condition that may determine a variety of changes in the cells not observed *in vivo*. It is noteworthy that the activity of GPX was inhibited by thyroid hormones both *in vivo* and *in vitro*. Similar findings were found in our previous study [17] in the thymus and skeletal muscles. Experiments performed by others indicate that the hypermetabolic state caused by hyperthyroidism accelerates free radical production in the mitochondria [36, 37]. However, the increase in the oxidative stress caused by thyroid hormones could also be related to changes in the activities of the antioxidant enzymes reported here.

Alterations in SOD, GPX, and catalase activities have been reported in experimental diabetes [38]. These findings are very often controversial and seem to vary with the tissue studied. For example, rats treated with streptozotocin or alloxan showed depressed activity of CuZn-

SOD in the liver, kidney, and erythrocytes [38]. The activity of GPX in the kidney was shown to increase but there was no change in the lung, liver, and erythrocytes of alloxan-diabetic rats [39, 40]. Catalase activity was shown to be low in the aorta of diabetic rats and was not modified in the kidney [39]. As shown in Table 1, diabetes decreased the activities of CuZn-SOD, catalase, and GPX and raised that of Mn-SOD in resident macrophages. These changes remained when glycemia was normalized by 24 hr fasting and were fully reversed by insulin administration. These observations may be related to the high incidence of infections and impaired inflammatory response that occur in diabetic patients [41]. It is noteworthy that hydrogen peroxide production was reduced markedly in macrophages from diabetic rats as compared with controls, and this effect was reversed by insulin administration. In fact, insulin stimulated hydrogen peroxide production by macrophages both *in vivo* and *in vitro* (Tables 1 and 2). The findings *in vivo* were basically reproduced in cultured macrophages (Table 2); the addition of insulin to culture medium increased the activities of CuZn-SOD, catalase, and GPX but caused a reduction of that of Mn-SOD.

In conclusion, glucocorticoids, epinephrine, thyroid hormones, and insulin do modulate the antioxidant enzyme activities and hydrogen peroxide production in macrophages. This fact may be important for the role played by these cells during immune and inflammatory responses.

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