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Studies of the Cellular Distribution of Superoxide Dismutases in Adult and Fetal Rat Tissues

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Activities of three types of superoxide dismutase in tissue fractions were significantly lower in fetal and adult brain and fetal limb preparations than in fetal and adult heart preparations. An exception was the cytoplasmic fraction of adult brain that had levels of Cu, Zn-superoxide dismutase activity comparable to those in cytoplasmic fractions of heart. In addition, Mn superoxide dismutase activity appeared to be very low in all fetal mitochondrial matrix fractions and cytoplasmic fractions as well as in adult brain. Finally, the results of these studies emphasize the importance of two antioxidant defense systems in the tissues studied, one associated with the mitochondrial electron transport system and the other, the cytosolic Cu, Zn enzyme.

Keywords: Superoxide dismutase, ETP, cytoplasm, fetal tissues

INTRODUCTION

We previously reported studies[1] that examined the production of superoxide by mitochondrial electron transport particles (ETP) from adult and conceptual tissues, and characterized properties that could be responsible for the higher levels of superoxide production in fetal tissues sensitive to transient uteroplacental hypoperfusion and their adult counterparts (fetal limb and brain but not fetal or adult heart). Parameters investigated included superoxide dismutase (SOD) activities of homogenates of adult and fetal tissues. These studies demonstrated markedly less SOD activity in homogenates prepared from sensitive conceptual and adult tissues compared with insensitive ones, suggesting that the presence of heightened concentrations of superoxide anion radical may have resulted from these decreased activities. In the current studies, we examined whether lowered levels of SOD activity in sensitive tissues reflected a general decrease in SOD activity or a specific lack of one or more of the SOD enzymes.

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MATERIALS AND METHODS

Embryo Explanation

Time-mated, primigravid Sprague-Dawley rats were obtained from a commercial vendor (B & K Laboratories, Freemont, Ca). The day on which copulation plugs were observed was designated day 0 of pregnancy. On gestational day 16, gravidas were sacrificed and uteri removed to dishes of cold Hank's balanced salt solution where they were slit along their antimesometrial border to remove individual implantation sites. The latter were rinsed free of blood and placed in cold 5% sucrose for dissection of fetal limbs, hearts and forebrains. When these were obtained, they were placed in a cold solution of 0.25M sucrose, 10 mM Tris HCl and 1mM EDTA, pH 7.4 (STE) for preparation of the cellular fractions. Heart and cerebral tissue were similarly removed from adult male rats (approx. 300g) and placed in cold STE.

Preparation of Cellular Fractions

Tissues were minced with scissors in iced STE before homogenization for 30 seconds with a motor driven blender (Tekmar) prior to homogenization. Mitochondria were prepared from all tissue homogenates by gentle disruption in cold STE using a power driven, teflon homogenizer immersed in ice. All subsequent procedures were carried out at 5°C. Tissue suspensions were centrifuged for 30 min. at 1500×g and the cloudy supernatant suspension carefully removed and recentrifuged at 12,000×g. The turbid supernatant representing the cytoplasmic fraction was saved for further studies. The packed residue was gently suspended in STE and centrifuged at 12,000×g. The final pellet (mitochondria) was suspended in STE and then sonicated for 3 min., packed in ice, at 120 watts in order to disrupt mitochondria. The suspension was then centrifuged for 45 min. at 140,000×g and the residue resuspended in STE. This residue, representing the mitochondrial electron transport particles (ETP) and the supernatant, representing the matrix fraction were saved for further studies.

Assay of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was determined spectrophotometrically at 37° as described by Crapo *et al.*[2] with results displayed on a strip chart recorder. The assay mixture consisted of water, 0.54 ml; 0.2 M KPO_4 (pH 7.4), 0.20 ml; 0.1 M sodium azide, 0.1 ml; 1% cytochrome *c*, 0.1 ml; xanthine oxidase, 0.03 ml; 10 mM xanthine, 0.01 ml; and 0.02 ml of tissue preparation making a total volume of 1.00 ml. The blank, read at 550 nm, was established by adding all components except xanthine (added to start the reaction) and the tissue homogenate. The rate of generation of superoxide anion radical was established after addition of xanthine by determination of the rate of cytochrome *c* reduction. Tissue homogenate was then added and the new rate of reduction of cytochrome *c* determined. The difference in rates represented SOD activity in the tissue sample. Finally, in order to distinguish between Cu, Zn-SOD (CN sensitive) and Mn-SOD (CN insensitive) activities, KCN (0.1 ml of 10 mM CN) was added to the initial assay mixture and the assay repeated. The extinction coefficient used for calculation of cytochrome *c* reduction was 19.1.[3]

Superoxide dismutase activity was also determined as previously described[1] with the oxygen polarograph at 37°C. In this assay, xanthine and xanthine oxidase were placed in the polarograph cell generating superoxide anion radical with a concomitant disappearance of oxygen. When a linear rate of oxygen disappearance was achieved, the preparation to be assayed was added to the cell and the new rate of oxygen disappearance determined. If the preparation contained SOD activity it converted superoxide produced by the xanthine-xanthine oxidase reaction to O_2 and hydrogen peroxide thus decreasing the observed rate of oxygen disappearance. The SOD activity of the preparation was calculated directly.[1] The

assay contained 0.3 ml of 0.2 M KPO_4, pH 7.4; 0.05 ml of 1:10 xanthine oxidase; 0.02 ml of 10 mM xanthine; tissue sample and sufficient water to give a final volume of 1.8 ml.

The various cellular fractions were assayed for SOD activity as follows: the mitochondrial particulate ETP fraction was assayed in the oxygen polarograph without added azide because the ETP SOD is strongly inhibited by azide, while the mitochondrial matrix fraction and the cytoplasmic fraction were assayed spectrophotometrically with and without CN to determine the Cu, Zn-SOD and Mn-SOD separately as described above. When fractions containing SOD activity not inhibited by azide were also assayed in the oxygen polarograph, results were in good agreement ($\pm 10\%$) with those obtained by the spectrophotometric method but the former method was less sensitive.

Statistical analyses were performed using the student t-test. Protein was determined by the method of Lowry *et al.*[4] NADH, type 7 horse heart cytochrome *c*, xanthine, xanthine oxidase and SOD were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Studies were performed to determine whether lowered levels of SOD activity reported previously[1] in sensitive tissues (fetal brain, limb) were due to a lack of a specific type of SOD or to a general lowering of activity of all the SOD's in the tissue. As shown in Table I, the particulate mitochondrial ETP fractions from all the tissues exhibited SOD activity in accord with the previously published work of Markossian and Nabanion,[5] and Markossian *et al.*[6] Activities for ETP SOD of adult and fetal heart preparations were much greater ($p < 0.01$) than activities of adult and fetal brain and limb preparations. Similarly, Cu, Zn-SOD and Mn-SOD activities in mitochondrial matrix fractions of adult and fetal heart were significantly higher ($p < 0.01$) than those from adult and fetal brain and limb with the exception of Mn-SOD which was undetectable in fetal heart. SOD activities in the cytoplasmic fraction showed a similar pattern with significantly lower activities ($p < 0.01$) for both Mn-SOD and Cu, Zn-SOD in fetal brain and limb and Mn-SOD activity in adult brain. However, the activities of Cu, Zn-SOD of cytoplasmic fractions from adult brain were high and not significantly different from activities of adult and fetal heart preparations. In summary, the activities of the three types of SOD in the tissue fractions were all significantly lower in fetal and adult brain and in limb preparations than in adult and fetal heart with the exception of high levels of Cu, Zn activity in the cytoplasmic fraction of adult brain tissue. In addition Mn-SOD activity appeared to be very low in all fetal mitochondrial matrix fractions and in fetal and adult brain cytoplasmic fractions. Total activities of the three types of SOD were calculated from the data shown in table I and the volumes of the various fractions. The distribution of the enzymes in the various tissues studied is shown in Figure 1. This figure emphasizes the possible importance of the ETP SOD in mitochondria and of Cu, Zn-SOD in the cytoplasm of the tissues studied.

DISCUSSION

Studies of the location and activities of the SOD's in teratogenically sensitive and insensitive tissues demonstrate that all of the SOD activities are markedly reduced in all sensitive tissue fractions (adult and fetal brain and limb) compared with insensitive heart. Cytoplasmic Cu, Zn-SOD is the only exception, showing comparable activity in adult brain and adult and fetal heart. It is of special interest that the SOD activity associated with preparations of ETP was approximately 6–10 fold greater than the activities of the Cu-Zn and Mn-SOD's in all the tissues studied and, therefore, may play a prominent role in the tissue metabolism of superoxide radical. It is of interest

TABLE I SOD Activities* of cellular fractions from adult and fetal tissues

Preparations†	Mitochondrial Fractions					Cytoplasmic Fractions		
	ETP			Matrix				
	Protein mg/ml	SOD activity	Cu, Zn-SOD activity	Mn-SOD activity	Protein mg/ml	Cu, Zn-SOD activity	Mn-SOD activity	Protein mg/ml
Heart, adult	0.41±0.01	570±23	53.1±15.4	59.2±16.0	0.16±0.02	24.2±4.0	19.3±2.6	0.87±0.07
Brain, adult	2.16±0.22	87.1±2.6	18.3±4.0	0.34±0.3	1.26±0.21	25.4±3.8	0.14±0.1	0.69±0.05
Heart, fetal d16	0.09±0.02	535±98	39.5±13.9	0.0	0.20±0.01	30.5±3.0	11.5±5.8	0.27±0.04
Brain, fetal d16	0.94±0.06	93.6±5.7	6.4±0.7	0.0	1.24±0.02	4.8±2.2	0.0	1.40±0.12
Limb, fetal d16	0.31±0.02	182±11	1.60±0.6	6.9±2.0	0.84±0.08	3.6±1.6	8.4±3.0	0.69±0.08

* SOD activity is expressed as nmoles of superoxide dismutated per mg protein per min.
† All values are the average±standard error of the mean for 5 experiments for adult preparations and 4 experiments for fetal preparations.

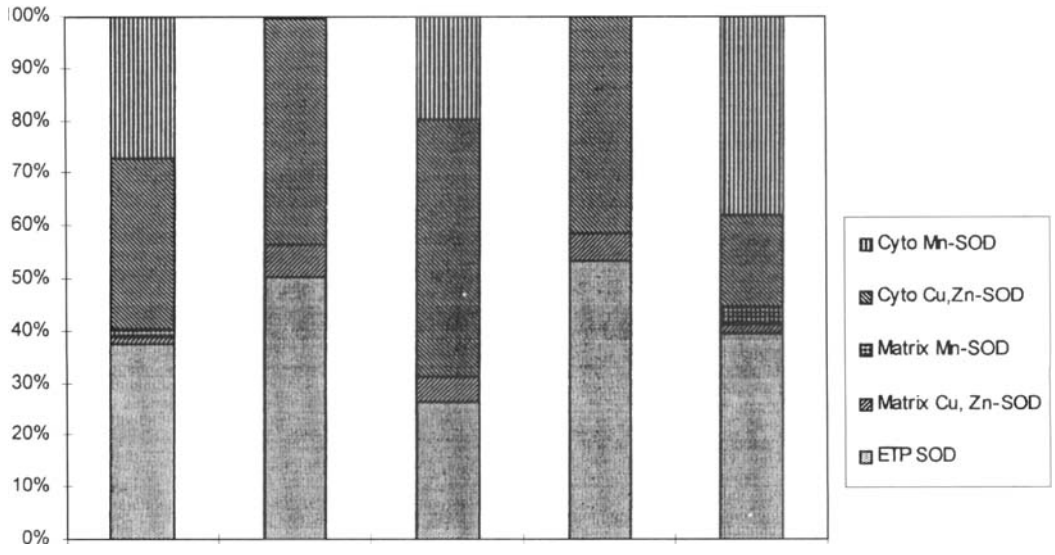

FIGURE 1 Composition of superoxide dismutases in fetal and adult tissues.

to speculate that the Zn and/or the third atom of Cu present in purified preparations of cytochrome oxidase (the terminal part of the ETP molecule)[7] may be necessary components of the particulate ETP SOD.

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