COMPARISON OF CYTOPLASMIC SUPEROXIDE DISMUTASE IN LIVER, HEART AND BRAIN OF AGING RATS AND MICE.

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Received September 13,1976

SUMMARY Comparisons of the specific activity of cytoplasmic superoxide dismutase, in homogenates of liver, brain and heart demonstrate considerably reduced activity in livers of aging rats and mice, a very small reduction in specific activity in the heart and no reduction in the brain of aging animals.

Antisera elicited in rabbits against purified superoxide dismutase from liver of either young or old rats revealed complete cross-reactivity with the heart and brain enzymes. They also exhibited complete cross-reactivity with the mouse enzymes from all three organs. This finding has, for the first time, enabled a comparison of possible age-dependent alterations in the same enzyme antigen in different cell types.

Despite the differences in age-related changes in specific activity in homogenates of liver, heart and brain the enzyme shows a considerable decline in catalytic activity per antigenic unit in all three organs in both aging rats and mice.

Peroxidative damage has been implicated as one of the principal causes of age-related damage to cells (1,2). Such damage is at least partially associated with the free radical, superoxide O_2^- (3), which is formed by several enzyme systems (4-10). The primary defence against the toxic effects of O_2^- is its dismutation by superoxide dismutase (11,12,13).

In a previous report, we demonstrated that purified cytoplasmic superoxide dismutase from livers of old rats exhibited a reduction of about 60% in specific activity as compared to that of the enzyme from young animals (14). In addition we showed that the "old" enzyme possessed about twice as much antigenically cross-reacting material per unit of activity as did the "young" enzyme. The "old" enzyme also exhibited considerably greater sensitivity to elevated temperatures than did the "young" enzyme. No differences were detected in molecular weight, electrophoretic mobility, Ki for cyanide and antigenic

identity between "young" and "old" enzyme. The reduction in superoxide dismutase activity as a function of age could result in an impaired protection against the toxic effects of O_2^- and thus might lead to severe cellular damage. This assumption is plausible as it has been shown, for instance, that the application of external superoxide dismutase protects mice against radiation damage (15).

Different organs and cell types exhibit broad differences in the degree of age-dependent damage in structure and function. It was thus deemed important to compare the age-related alterations in function of an enzyme which appears in the same molecular form in various organs. Superoxide dismutase has been reported to appear in the same antigenic form in human liver, brain and erythrocytes (16). The present work confirms, in rats and mice, this finding of identical forms of superoxide dismutase in liver and brain and adds the heart enzyme to the list.

MATERIALS AND METHODS

Preparation of homogenates : Rat (WF) and mouse (C57Bl/6J) liver, heart and brain were homogenized in three volumes of double distilled water. The mixtures were centrifuged at 13,000 x g for 15 min and the supernatants were used for enzyme assay. "Young" and "old" homogenates were prepared from 8 and 32 months old rats and from 8 and 28 months old mice, respectively.

Enzyme assay : Superoxide dismutase activity was determined by the pyrogallol method (14). One unit of activity is defined as the amount of enzyme causing 50% inhibition of the autooxidation of 0.4 mM pyrogallol at 25°C.

Purification of rat liver superoxide dismutase from young (6 months) and old (30 months) animals, preparation of antiserum, immunoprecipitation and double diffusion in gel (Ouchterlony test) were done as previously described (14).

Protein was determined according to the method of Lowry et al.(17).

RESULTS

The specific activity of superoxide dismutase in homogenates of liver, heart and brain from rats and mice of various ages was determined. An age-dependent decrease in the specific activity of superoxide dismutase from rat and mouse livers was observed, while no such decrease occurred in the enzyme

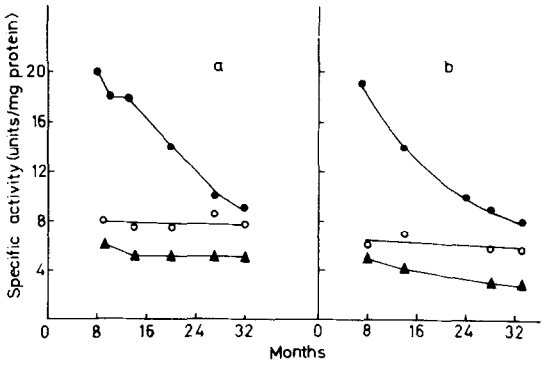

Fig. 1. The specific activity of superoxide dismutase in rat (a) and mouse (b) liver ●—●, brain ○—○ and heart ▲—▲ homogenates at various ages.

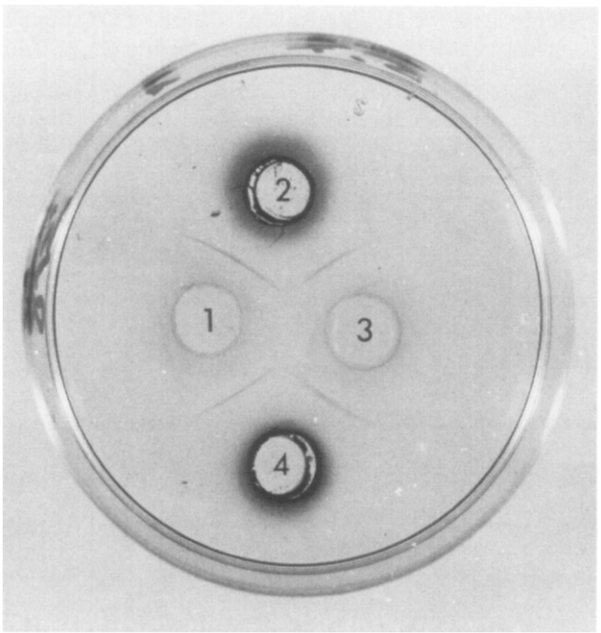

Fig. 2. Immunodiffusion of monospecific antisera prepared against purified "young" and "old" rat liver superoxide dismutase. 1 - anti "young" enzyme, 2 - "young" rat liver homogenate, 3 - anti "old" enzyme, 4 - "old" rat liver homogenate.

from brains. In the heart, the enzyme shows a small decline in specific activity in both the rat and the mouse (Fig. 1).

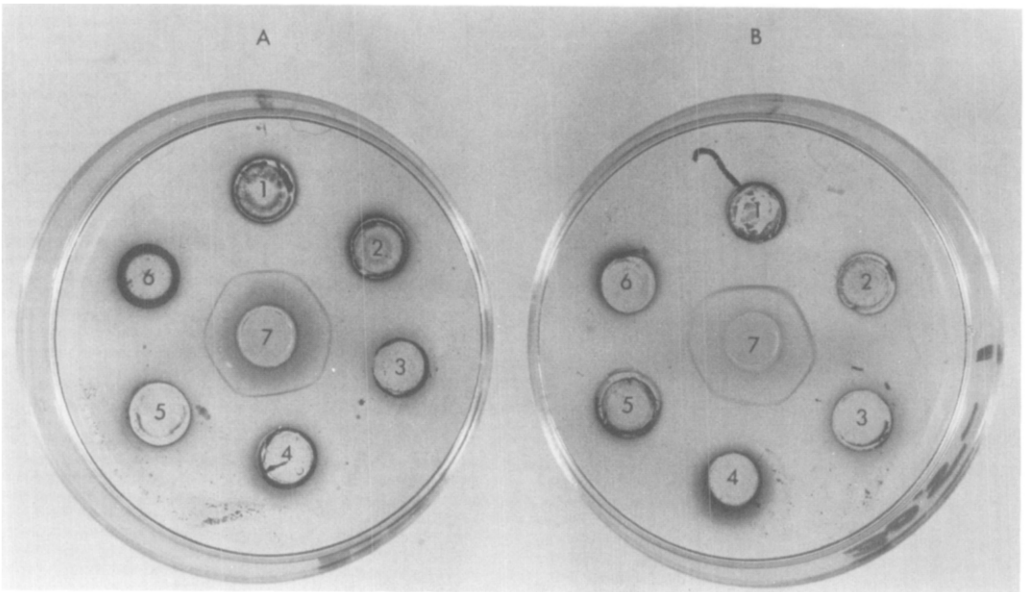

Fig. 3. Immunodiffusion of monospecific antiserum prepared against purified "young" rat liver superoxide dismutase. (A) 1 - "young" rat liver homogenate, 2 - "old" rat liver homogenate, 3 - "old" rat heart homogenate, 4 - "young" rat heart homogenate, 5 - "old" rat brain homogenate, 6 - "young" rat brain homogenate, 7 - antiserum.
(B) "young" homogenates of : 1 - rat liver, 2 - mouse liver, 3 - rat brain, 4 - mouse brain, 5 - rat heart, 6 - mouse heart, 7 - antiserum.

Fig. 2 and Fig. 3 show the immunological identity between superoxide dismutase from various organs of young and old rats and mice. Antisera prepared against purified "young" and "old" rat liver superoxide dismutase formed a single precipitin line with the enzyme present in liver homogenates from young and old rats (Fig. 2). Antiserum prepared against purified "young" rat liver superoxide dismutase formed a single precipitin line with "young" and "old" superoxide dismutases present in the homogenates of the various organs obtained from rat and mouse (Fig. 3).

The immunological identity of superoxide dismutase from liver, brain and heart of young and old rats and mice has allowed us to compare the presence of cross-reacting material in these tissues from old animals. Fig. 4 shows the immunoprecipitation of superoxide dismutase in liver (a), heart (b)

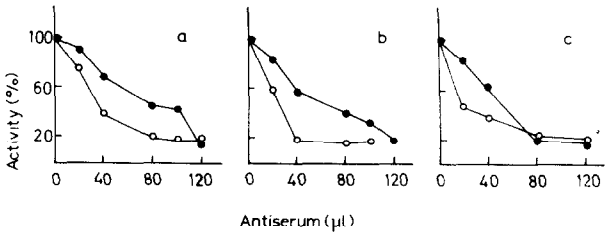

Fig. 4. Immunoprecipitation of superoxide dismutase in tissue homogenates from young (0——0) and old (●——●) rats. The initial enzyme activities of both homogenates were adjusted to the same level and increasing amounts of antisera prepared against "young" purified rat liver superoxide dismutase were added in total volume of 0.4 ml. After overnight incubation at 4°C and centrifugation the enzyme activity remaining in the supernatants was determined, a - liver, b - heart, c - brain.

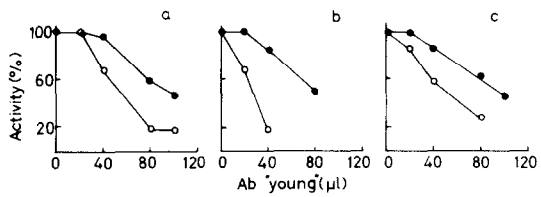

Fig. 5. Immunoprecipitation of superoxide dismutase in tissue homogenates from young (0——0) and old (●——●) mice. The reaction conditions were those of Fig. 4. a - liver, b - heart, c - brain.

and brain (c) homogenates from old and young rats by antiserum prepared against purified "young" rat liver superoxide dismutase. In each case, increasing amounts of the antisera were added to aliquots of enzyme from "young" or "old" homogenates which were adjusted to the same initial level of enzyme activity. After overnight incubation at 4°C and centrifugation the enzyme activity remaining in the supernatants was determined. It can be seen that considerably more antiserum is required to precipitate equal quantities of enzyme activity from "old" homogenates as compared to "young" homogenates. The ratio of the volume of antiserum required to precipitate 50% of the enzyme in "old" homogenate to that required to precipitate 50% of the enzyme in "young" homogenate is 2.5 - 2.6 for liver, heart and brain.

Rabbit antiserum prepared against rat liver superoxide dismutase recognizes the superoxide dismutase of mouse liver, heart and brain (Fig. 3b). Fig. 5 shows the immunoprecipitation of superoxide dismutase from mouse liver, heart and brain using antiserum to "young" rat liver superoxide dismutase. As in the case of the rat (Fig. 4) larger quantities of antiserum are required to precipitate equal amounts of enzyme activity from "old" homogenates as compared to "young" homogenates. Thus the presence of antigenically cross-reacting material is demonstrated in "old" liver, heart and brain homogenates of mice as well as rats. Similar results (not shown) were obtained in immune precipitation studies using antisera produced against "old" enzyme.

DISCUSSION

The accumulation of altered enzyme molecules in aging animals is a widespread phenomenon. To date twelve out of thirteen enzymes studied showed considerable proportions of faulty molecules in aging organisms. This phenomenon has been found in nematodes, mice, rats and man (for a review, see ref. 18). We have deemed it of great interest to study whether the same enzyme is altered to the same degree in different cell types as a function of age. In liver homogenates the specific activity of superoxide dismutase dropped considerably with age, while it declined only slightly, if at all, in heart and showed no decline in brain (Fig. 1). This is in contrast to a similar degree of decline in specific activity per antigenic unit (i.e. the appearance of cross-reacting material) in liver, heart and brain. These findings show for the first time that the accumulation of cross-reacting material with reduced catalytic activity in aging animals is not necessarily associated with a reduction in the specific activity of enzymes in tissue homogenates, as has been shown in previous work (19-25).

In light of these results an interesting possibility can be suggested. In as much as both brain and heart cells are either more sensitive to oxygen toxicity or are normally exposed to higher concentrations of oxygen in vivo than liver cells, a control mechanism may exist in these cells which maintains

a certain necessary level of superoxide dismutase activity for protection. Thus, the cells in old organisms synthesize more enzyme molecules in order to compensate for the partial loss of activity and thus maintain a constant level of superoxide dismutase activity. Whether this phenomenon is unique to superoxide dismutase in heart and brain or is common to other enzymes which are essential for cell viability is at present unknown. Our findings do, however, show that the same enzyme behaves differently in various mammalian tissues during aging.

ACKNOWLEDGEMENT

The expert technical assistance of Mrs. Lea Rozenfelder is gratefully acknowledged. The work reported here was supported by grants from Deutsche Forschungsgemeinschaft and NIH Grant No. 5 RO1 HD07740-02.

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