Differential changes in Cu, Zn and Mn superoxide dismutase activity in developing rat brain and liver

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Summary. The aim of our study was to assess the pattern of copper and zinc-containing superoxide dismutase (Cu, ZnSOD) and manganese-containing superoxide dismutase (MnSOD) activity from embryonic life to senescence in rat brain and liver. The two isoenzymes showed different profiles in the two organs examined. In particular, the cerebral MnSOD activity profile suggests a primary role during differentiation of this enzymatic form.
Key words. Prenatal and postnatal development; Cu, Zn and Mn superoxide dismutase; free radicals; aging; differentiation.

At present the mechanisms by which selected genes become repressed or derepressed during development and are responsible for functional decline of differentiated cells have not been completely clarified. Recent unified hypotheses have suggested that the partially reduced forms of oxygen, and the antioxidant systems, may be involved not only in aging[2], but also in development and differentiation[3], and in the pathogenesis of various diseases[4-6]. During aerobic metabolism, O_2 is reduced to water via oxidative phosphorilation in the mitochondria; dangerous reactive intermediates may be produced when molecular oxygen is partially reduced. In particular, cell differentiation is accompanied by a strong increase in oxygen utilization[7] which, together with univalent reduction, is responsible for the increased production of oxygen free radicals[8] as superoxide anion (O_2^-), a highly reactive radical. These events are particularly frequent in the brain because of its high oxygen uptake, during aging, and in several degenerative neurological diseases such as Alzheimer's dementia[9].

One of the key enzymes in antiradical protection is superoxide dismutase[10] (SOD) which catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide via the reaction: $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. Mammalian cells contain two metalloprotein enzymes, a copper and zinc-containing superoxide dismutase (Cu, ZnSOD) in the cytoplasm and a manganese-containing superoxide dismutase (MnSOD) in the mitochondrial matrix. Their main role is to protect aerobic cells from potential damages due to superoxide anions. Another important function is the stable maintenance of the cellular redox state and the ionic balance, both of which are important for the configuration of the chromatin and gene expression[11]. Therefore, measurement of Cu, Zn and MnSOD activity during the life-span is important in the study of differentiation, aging and several pathological processes.

In the present study, we have determined a developmental profile of Cu, Zn and MnSOD, starting from day 14 of embryonic life and continuing to senescence (24 months after birth) in rat brain and liver, whose maturation occurs at different times.

Materials and methods

Wistar rats of various postnatal ages (1, 7, 16, 30, 60, 90, 180, 240, 360, 630 and 720 days) and prenatal rats of 14, 15, 17 and 20 days of gestational age, obtained by Caesarean section, were sacrificed by decapitation under ether anesthesia. Brain hemispheres and livers were excised, homogenized in 50 mM phosphate buffer (pH 7.8), sonicated in an ice bath for 4 min in 30-s bursts and centrifuged.

SOD activity was determined according to the method of Beauchamp and Fridovich[12] modified by Oberley and Spitz[13].

The assay is based on the inhibition of the conversion of nitro-blue tetrazolium (NBT) to blue formazan by SOD, mediated by superoxide radicals generated by the xanthine oxidase system. The reduction of NBT was followed at 560 nm with a Perkin-Elmer spectrophotometer. The data are expressed as units of SOD per mg of protein; 1 unit is equal to the amount of protein which gives half-maximum inhibition. MnSOD was determined by incubating samples with cyanide (5 mM), which inhibits Cu, ZnSOD. The activity of the latter enzyme was calculated as the difference between the total SOD and MnSOD activities. The proteins were evaluated by Lowry's method as reported by Scopes[14].

The data reported in figures 1, 2, 3 and 4 represent the mean ± SE of 3–7 samples. Tissue samples of brains and livers from prenatal and 1-day-old rats were pooled from 7–11 animals.

Results and discussion

The results of our study show differential changes in SOD activity that might influence gene expression during development (fig. 1). The possibility of distinguishing between the two isoenzymes allows for the following pattern to be suggested. During intrauterine development, in the brain, only Cu, ZnSOD with reduced but constant activity is present until day 16 after birth. Subsequently, the other enzymatic form, MnSOD, is detectable, and the first considerable increase of both enzymes is observed (fig. 2). This could be related to the increasing oxidative metabolism and the ongoing differentiation processes. In

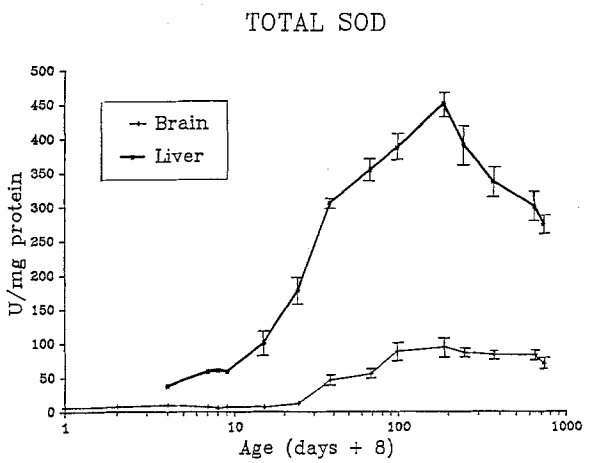

Figure 1. Age-dependent changes in total SOD activity in the rat brain and liver. Values represent the mean ± SE, of 3–7 samples. The abscissa, on a logarithmic scale, indicate each age increased by 8 days; consequently, birth takes place on the 8th day.

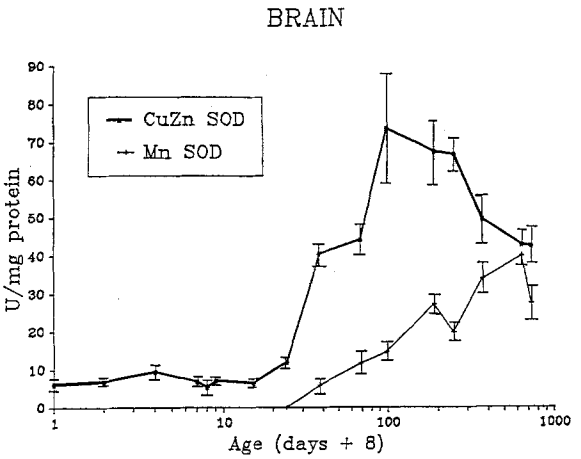

Figure 2. Age-dependent changes in Cu, ZnSOD and MnSOD activity in the rat brain. Other details as in figure 1.

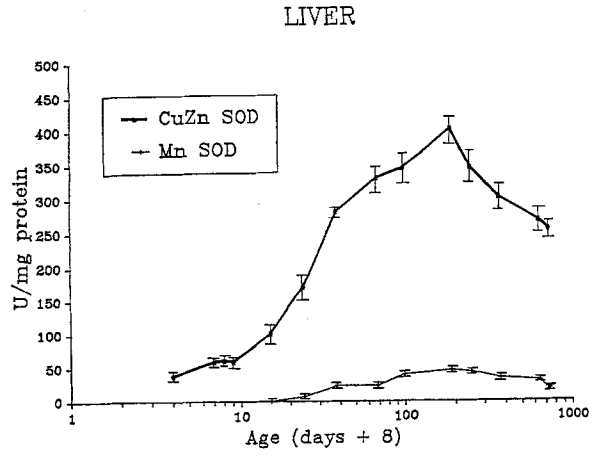

Figure 3. Age-dependent changes in Cu, ZnSOD and MnSOD activity in the rat liver. Other details as in figure 1.

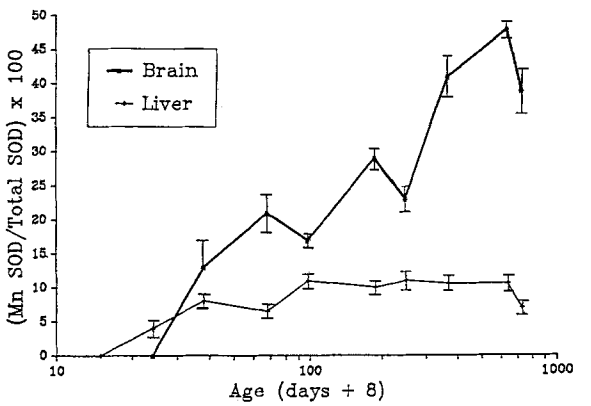

Figure 4. Changes of MnSOD activity as a percentage of total SOD activity with age in the rat brain and liver. Other details as in figure 1.

fact, at birth the rat brain is immature, and maturation takes place subsequently, as in humans. With development differences in the profiles of the two cerebral enzymes are seen. From the moment at which Cu, ZnSOD begins to decrease (about the 3rd month), MnSOD continues to increase considerably until it reaches its maximum at 21 months, and then it decreases. This is probably because of the major role that MnSOD seems to play in differentiation [15], a process which leads to increased mitochondrial respiration and an increased oxygen free radical production. It is possible that these radicals remove a repressor from the regulatory site of the MnSOD gene, thus increasing its activity until the aging process begins.

Since aging is characterized by a progressive loss of differentiation and a decrease of regulation control, the regulatory site of the MnSOD gene might be preferentially damaged. This would cause a decrease of enzyme activity in both organs, as we observed (figs 2 and 3). It is important to note that the function of MnSOD is to interact with the superoxide radicals, which derive from the electron transfer chain, which are particularly abundant in the brain. The isoenzyme Cu, ZnSOD, on the other hand, has a more general antioxidant function, which could explain the similar trend observed in the brain and the liver for this enzyme. The MnSOD/total SOD cerebral ratio is always higher than the hepatic ratio (fig. 4). Altogether, our data seem to emphasize a major role of MnSOD in cell differentiation, particularly in the brain.

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2 Harman, D., Proc. natl Acad. Sci. *78* (1981) 7124.
3 Sohal, R. S., and Allen, R. G., Adv. Free Radical Biol. Med. *2* (1986) 117.

4 Hall, E. D., and Braughler, J. M., Cent. nerv. Syst. Trauma *3* (1986) 281.
5 Saunders, R. D., Dugan, L., Demedink, P., Means, E. C., Harrocks, L. A., and Anderson, D. K., J. Neurochem. *49* (1987) 24.
6 Slivka, A., and Cohen, G., J. biol. Chem. *260* (1985) 15466.
7 Sun, A. S., Aggrawal, B. B., and Packer, L., Archs Biochem. Biophys. *170* (1975) 1.
8 Miquel, J., and Fleming, J. E., Exp. Geront. *19* (1984) 31.
9 Mizuno, Y., and Ohta, K., J. Neurochem. *46* (1986) 1344.
10 McCord, J. M., and Fridovich, I., J. biol. Chem. *244* (1969) 6049.
11 Sohal, R. S., and Allen, R. G., in: The Molecular Basis of Aging, p. 75. Plenum Press, New York 1985.
12 Beauchamp, C., and Fridovich, I., Analyt. Biochem. *44* (1971) 276.
13 Oberley, L. W., and Spitz, D. R., Meth. Enzymol. *105* (1984) 457.
14 Scopes, R., in: Protein Purification, p. 265. Springer, New York 1982.
15 Oberley, L. W., and Oberley, T. D., J. theor. Biol. *106* (1984) 403.

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Depolarization of natural skylight disrupts orientation of an avian nocturnal migrant

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Summary. Given a view of clear evening skies, migratory blackcaps (Aves: *Sylvia atricapilla*) orient appropriately in the absence of meaningful information from the geomagnetic field. When the intensity of natural skylight polarization patterns was reduced with pseudodepolarizers by over 90%, test birds were disoriented, and their migratory restlessness in autumn was drastically reduced.
Key words. Orientation; skylight polarization; bird migration; pseudodepolarizer; *Sylvia atricapilla.*

Nocturnal avian migrants often take off for migratory flights during the twilight period after sunset but before total darkness. At that time of day skylight polarization reaches its maximal intensity and, with a band of maximal polarization running perpendicular to the sun azimuth through the zenith, it provides a prominent potential compass cue for organisms able to perceive it [1-3]. In addition to the geomagnetic field, visual, sun-related information has been demonstrated to provide an important directional reference for nocturnal migrants during take-off [4, 5]. Despite some early failures to demonstrate the ability of birds to perceive the plane of polarization of linearly polarized light [6], it has now been firmly established that birds are able to differentiate e-vector directions [7-9]. It was possible to influence predictably the directional choices of migratory birds in funnel-shaped orientation cages [10] by covering them with polarizers; the birds tended to orient parallel to the artificial e-vector, no matter which way it was turned [9, 11-14]. These findings indicate that birds may use e-vector information as a compass for migratory orientation.

Two points, however, remain to be resolved: a) Does sun-related visual information used by nocturnal migrants consist of the azimuth of the sunset point, the polarization patterns, or both? b) What role do natural polarization patterns (as opposed to artificially imposed ones) play in the orientation system? Polarizers produce a highly artificial visual stimulus [9], and the birds oriented parallel to the e-vector rather than keeping an angle as they did (in control tests) with respect to the natural band of maximal polarization [13]. Such experiments do not prove that natural skylight polarization patterns have any significance in bird orientation. This can only be demonstrated by depolarizing the skylight as seen by the birds, and at the same time preventing orientation by the geomagnetic field. I here report results of the first such experiment, which provide evidence that birds can indeed use *natural* skylight polarization patterns for orientation, independently of the horizon glow of the setting sun.

Material and methods

Experiments were conducted with the blackcap (*Sylvia atricapilla*, Sylviidae), a widespread Palearctic nocturnal migrant which was previously shown to respond to e-vector manipulations during sunset orientation tests [13]. Thirty birds (27 juveniles, 3 adults) were caught prior to migration during July to mid-September and were housed in individual cages in Frankfurt am Main (FRG). Cages were placed in an outdoor aviary until late September, and again ten days prior to the spring tests and from then on, so that the birds could see parts of the natural sky. Orientation tests were performed during 15 September to 30 November 1986, and 8 April to 18 May 1987, with birds chosen at random from a pool of 29 in autumn and 14 in spring. Individuals used in spring had wintered indoors at Frankfurt in a photoperiod corresponding to the natural one of their winter range.

Aluminium orientation funnels (top diameter 35 cm, height 15 cm) lined with typewriter correction paper (Tipp-Ex) were covered either with clear plexiglass (controls) or with plexiglass plus a double-layer of a commercially available pseudodepolarizer (Hostaphan = polyethylene terephthalate, Hoechst AG, Wiesbaden). Two sheets of Hostaphan (each 0.18 mm thick) were taped