Free Rad. Res. Comms., Vol. 1, No. 5, pp. 339-346
8755-0199/86/0105-0339 $25.00/0

Printed in Great Britain

COPPER-ZINC CONTAINING AND MANGANESE CONTAINING SUPEROXIDE DISMUTASE IN THE GROUND SQUIRREL/*CITELLUS CITELLUS*/ — THE EFFECT OF HIBERNATION

V.M. PETROVIĆ, BILJANA MILIĆ, M. SPASIĆ and ZORICA SAIČIĆ

Department of Endocrinology and Metabolism Institute for Biological Research and Faculty of Sciences University of Belgrade, 29 Novembra str. 142 Yugoslavia

(Received August 8, 1985; in final form August 27, 1985)

The distribution of Copper-Zinc containing and Manganese-containing superoxide dismutase in the liver, kidney, interscapular brown adipose tissue (IBAT) and brain of the ground squirrel, as well as the effect of hibernation, was studied. Activity of both forms of SOD was highest in the liver and lowest in the brain. Activity of the Mn SOD in relation to total SOD was higher in the liver and kidney of the ground squirrel as compared with results reported for other rodents. The highest activity of Mn SOD in relation to total SOD was found in the IBAT and brain (36% and 49%, respectively). Total SOD activity per mg proteins and per g wet mass in IBAT and brain of hibernating animals was increased: for IBAT, $p < 0,05$ and $p < 0,025$, respectively; for brain, $p < 0,01$ and $p < 0,025$, respectively. Protein content in hibernating ground squirrel was not significantly changed. In the hibernating ground squirrel CuZn SOD activity in IBAT and brain was higher as compared with the active animal ($p < 0,025$ and $p < 0,005$, respectively). In the liver and kidney CuZn SOD was not significantly changed during the hibernation. In the liver and brain of the hibernating animals a lower Mn SOD activity was found ($p < 0,005$ and $p < 0,05$, respectively).

INTRODUCTION

Copper-zinc containing superoxide dismutases (CuZn SOD) are found in all eucaryotic cells, such as yeasts, plants, and animals but not in procaryotic cells. Two exceptions to this are found in Photobacterium legionathi and Caulobacter crescentus, which also contain CuZn SOD[1,2,3]. The molecular weight of CuZn SOD in eucaryotic cells is around 32 000 and it contain two protein subunits, each of which bears an active site containing one copper ion and one zinc ion. The copper ions appear to function in the dismutation reaction by undergoing alternate oxidation and reduction and Zn appears to stabilize the enzyme[4].

Manganese containing superoxide dismutase (Mn SOD) has been found in several bacteria and also in extracts of plant, animal and human tissues. The activity of the Mn SOD in relation to CuZn SOD depends on the tissue and on the species[5]. Mammalian erythrocytes contain no Mn SOD, it was found to be about 10% of total SOD activity in rat liver but much more in human liver[6].

Subcellular fractionation studies upon the liver of rats have shown that most of the CuZn SOD is in the cytosol of the cell, with some activity being present in lysosomes and possibly between the inner and outer mitochondrial membranes. The Mn SOD is located in the mitochondrial matrix but it seems that in human and baboon liver there is some Mn SOD outside the mitochondria[7,4].

We have recently shown that a considerable activity of superoxide dismutase occured in the tissues of the ground squirrel except for the lung in which SOD was low. As compared with the rat the activity of total SOD in the ground squirrel expressed in U/mg protein was at about the same level in all tissues studied except for the liver and lung where the activity of this enzyme was lower then in the rat[8].

In the present experiment we study the relation between CuZn SOD and Mn SOD activity in the liver, kidney, brown adipose tissue and brain of the ground squirrel during the summer and winter-in the active control and hibernating animals.

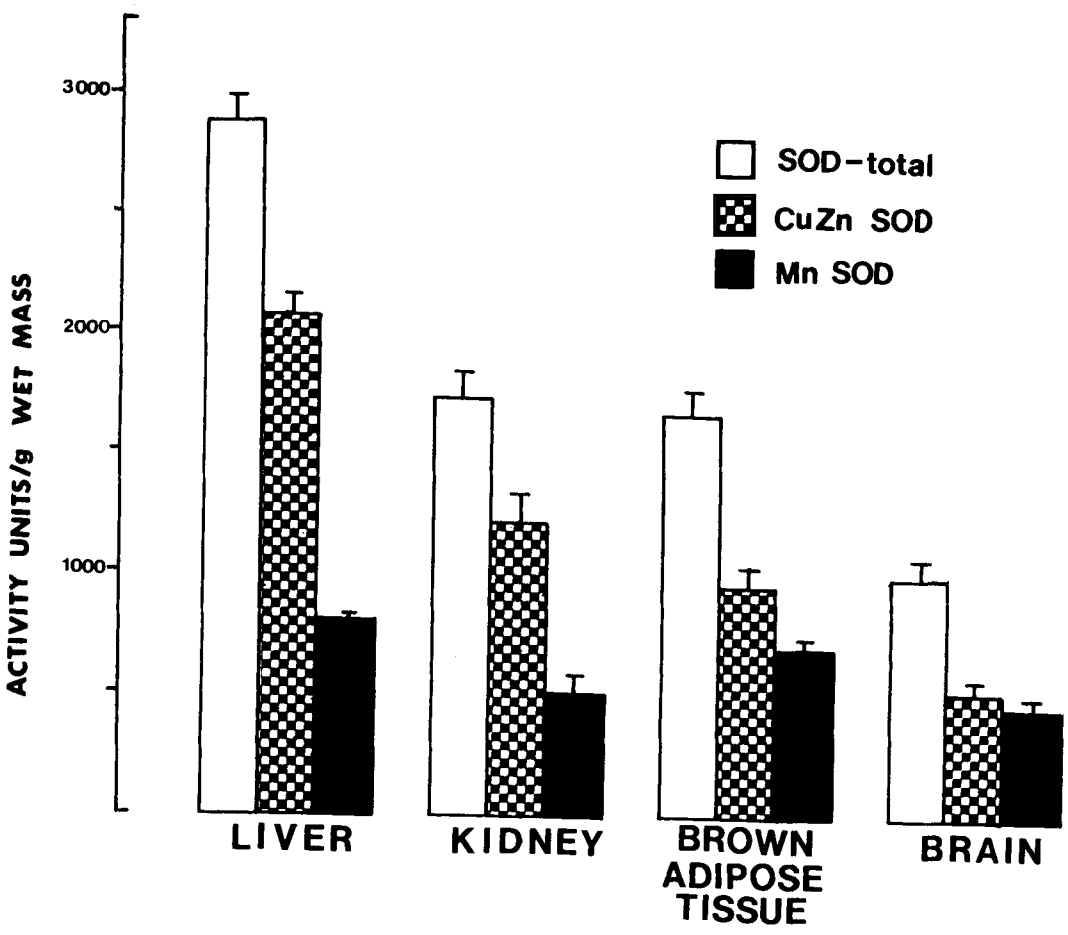
MATERIALS AND METHODS

Adult male ground squirrels (*Citellus citellus*) were housed in individual plastic cages with woodchip bedding in a room at 20–25°C during the summer and at 6–8°C during the winter. They were maintained on daily illumination and given a pelleted rat diet and water ad. lib. One group consisting of 6 animals was tested in summer and the second group of hibernating animals was tested in winter. Animals were killed by decapitation between 8–10 a.m. Tissues were removed within 3 min, the liver being perfused prior to the removal, and than minced and dispersed with a loosely fitted Potter-Elvehjem pestle in 4 vol of the buffer containing 0.25 M sucrose-Tris, pH 7.5. The extraction of SOD was performed by the method of Takada *et al.*[9]. The homogenate was sonicated for 30 seconds with 10 kHz on ice and than centrifuged for 30 min at 105 000 g. Total SOD activity was determined by the method described by Misra and Fridovich[10]. This method is based on the capacity of SOD to inhibit autooxidation of adrenaline into adrenochrome. One unit of SOD activity was defined as amount of protein causing 50% inhibition of autooxidation of adrenaline in volume of 3.2 ml reaction mixture containing 3×10^{-4} M adrenaline, 1×10^{-4} EDTA and 0.05 M Na_2CO_3 at pH 10.2. The procedure was performed at 26°C. For the determination of Mn SOD activity samples were diluted with equal volumes of 8 mM KCN. After the incubation of 20 min at the room temperature, Mn SOD was determined in the same reaction mixture as total SOD with final concentration of 4 mM KCN. Assays, in which the inhibition of autooxidation of adrenaline up to 45% was achieved, were used for the calculation of SOD activity. In this region the percentage inhibition was proportional to the volume of extract added. Protein was determined according to the method of Lowry *et al.*[11].

RESULTS

Distribution of CuZn SOD and Mn SOD activity in the ground squirrel

In the active ground squirrel studied in summer activity of CuZn SOD was highest in the liver, lower in the kidney and interscapular brown adipose tissue and lowest in the brain (Figure 1). Mn SOD was also highest in the liver than in other tissues studied but differences were smaller than for CuZn SOD activity. Activity of the Mn SOD in



RE 1 Distribution of CuZn SOD, Mn SOD and total SOD activity in the active ground squirrel (*Citellus citellus*) studied in summer. Data shown repres
an ± SE of 6 animals. Body temperature of animals was 36°C.

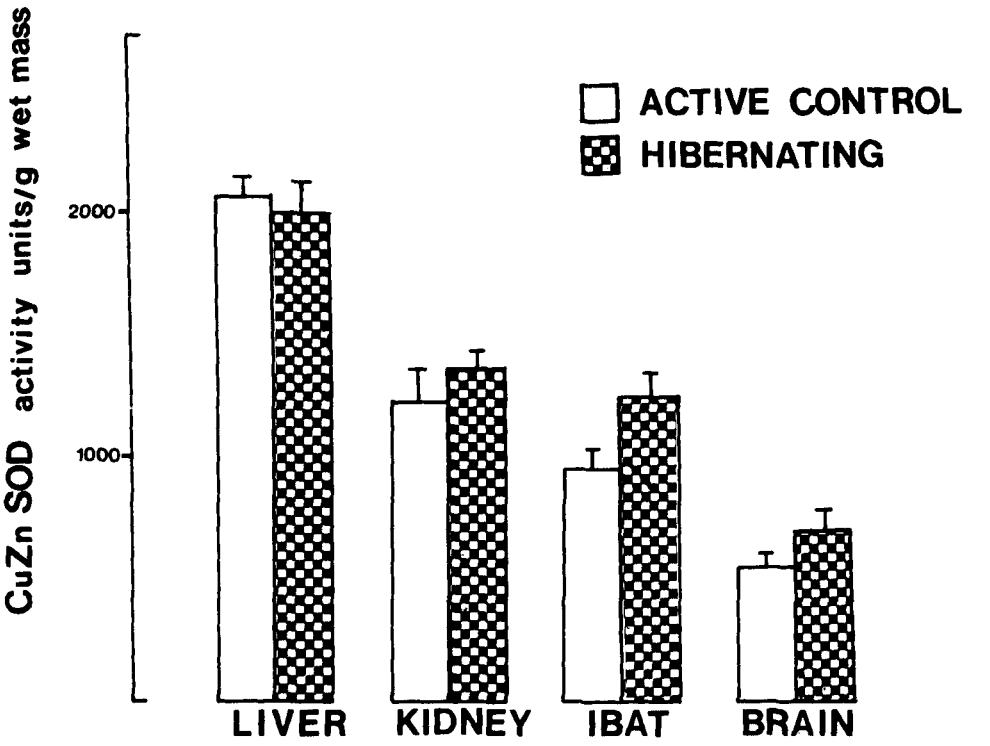


FIGURE 2 CuZn SOD activity in the hibernating ground squirrel in winter (body temperature of animals was 6–8°C), compared with the active normothermic animals studied in summer. For IBAT difference was significant ($p < 0{,}005$). Data shown represent the mean ± SE of 6 active control and 6 hibernating animals.

relation to total SOD was higher in the ground squirrel as compared with results reported for the rat and guinea pig. In the kidney and brain activity of Mn SOD was at about the same level. Two tissues studied: interscapular brown adipose tissue and brain showed an extremly high Mn SOD activity as compared with the total activity (36% and 49% respectively).

Effect of hibernation on SOD activity

Total SOD activity per mg protein and per g wet mass in IBAT and brain of hibernating animals was increased: for IBAT, $p < 0{,}05$ and $p < 0{,}025$, respectively; for brain, $p < 0{,}01$ and $p < 0{,}025$, respectively. Protein content in hibernating ground squirrel was not significantly changed in respect to control (Table I).

As shown in Figure 2 in the hibernating ground squirrel examined in winter (body temperature of animals was 6–8°C) CuZn SOD activity in IBAT and brain was higher as compared with the active animal studied in summer ($p < 0{,}025$ and $p < 0{,}005$, respectively). For IBAT the values are 1236 ± 112 and 960 ± 72 and for brain 840 ± 41 and 542 ± 77. In the liver and kidney CuZn SOD was not significantly changed during the hibernation. However as presented in Figure 3 in the liver and brain of the hibernating animals a lower Mn SOD activity was found ($p < 0{,}005$ and $p < 0{,}05$,

TABLE I

Superoxide dismutase activity and protein content in the liver, kidney, interscapular brown adipose tissue (IBAT) and brain of hibernating and active round squirrels. Mean ± SEM of 6 animals. IBAT and brain total SOD activity u/g wet mass for active control and hibernating G.S. $p < 0,025$; IBAT brain total SOD u/mg protein, active control and hibernating G.S. $p < 0,05$ and $p < 0,01$ respectively.

n	Liver		Kidney		IBAT		Brain	
nals	Active control	Hibernating	Active control	Hibernating	Active control	Hibernating	Active control	Hibernat
SOD vet mass	2860 ±119	2701 ±163	1744 ±102	1843 ±73	1677 ±83	2000* ±147	1078 ±80	1275* ±74
ein wet mass	58,00 ±1,59	54,98 ±1,23	30,97 ±2,65	33,44 ±1,87	21,51 ±1,02	23,40 ±1,51	16,74 ±1,07	16,23 ±1,25
SOD protein	49,48 ±2,50	49,05 ±2,42	57,40 ±4,36	55,99 ±4,45	78,03 ±2,08	85,52* ±4,05	61,84 ±4,31	79,43* ±5,63

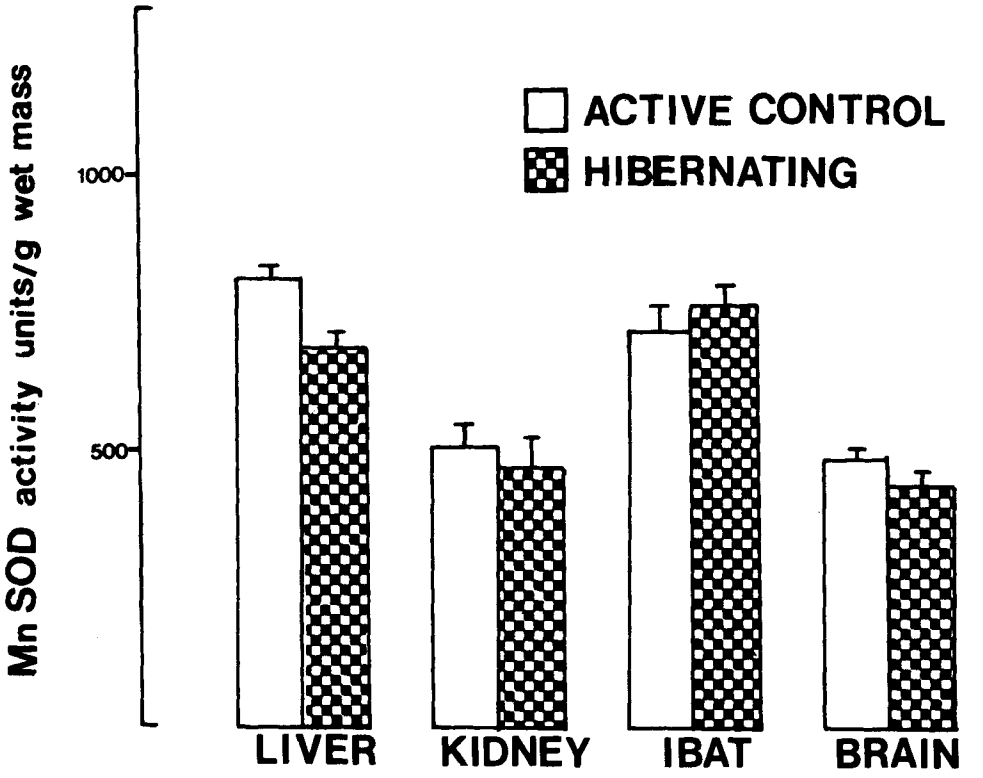


FIGURE 3 Mn SOD activity in the hibernating ground squirrel studied in winter (body temperature of animals was 6–8°C) compared with the active normothermic animals studied in summer. For liver difference was significant ($p < 0{,}005$). Data shown represent the mean ± SE of 6 active control and 6 hibernating animals.

respectively). For the liver the values are 694 ± 30 and 817 ± 19 and for the brain 488 ± 25 and 536 ± 16.

DISCUSSION

In the present experiments we have used the method described by Takada *et al.*[9] which includes the use of sonication for the extraction of total SOD and the use of cyanide for the separation of cyanide insensitive SOD which corresponds to manganese SOD in animal tissues. Cyanide-insensitive superoxide dismutase is mainly located in mitochondria[12].

From our present results it may be seen that in the active ground squirrel the highest CuZn SOD activity was found in the liver, being lower in the kidney and interscapular brown adipose tissue and the lowest in the brain. The same distribution of CuZn SOD for all tissues studied was found in our previous experiment[8], in which the method of Misra and Fridovich[10] for the extraction of enzymes was used. As far as the distribution of Mn SOD is concerned, in the present experiment this activity was highest in the liver compared with other tissues but differences between these organs were lower

than for CuZn SOD. In addition in the kidney and brain the activity was about the same level. Peeters-Joris *et al.*[13] reported for the rat that the activity of mitochondrial SOD (which corresponds to cyanide insensitive SOD in our experiment) in the liver was also significantly higher than in the kidney and brain. But if compared with our present results differences reported by these authors for rats tissues are markedly higher than differences between SOD in the tissues of the ground squirrel.

According to Mc. Cord *et al.*[14] and Salin *et al.*[15] in the human and chicken livers, Mn SOD accounts for about 50% but in the rat and bovine liver Mn SOD accounts not more than 10% of total SOD, being "very low" in guinea pig. From our present data we may see that in the liver and kidney of the active ground squirrel Mn SOD accounts in the total with 27% and 29% respectively which is significantly higher as compared with other rodents studied: rats and guinea pigs[16] (and our unpublished results). It is to be pointed out that Mn SOD in the interscapular brown adipose tissue and brain of the active ground squirrel accounts for 36% and 49% respectively of the total activity. It is interesting that the activity of both CuZn SOD and Mn SOD in the brain of the ground squirrel was at the same level, which differs from the results obtained in the human and other species studied[17,18].

Copper-zinc and manganese containing superoxide dismutase were studied under different physiological and pathological conditions in animals and humans and a changes in the interrelation was found[19,20,21]. Ground squirrel may be of use as a model for the study of free radicals and corresponding enzymes because of the animal spontaneous changes from active normothermic into the hibernating state, and vice versa, in which the level of O_2 consumption are different[22].

The increase in total and CuZn SOD activity in IBAT and brain of the hibernating ground squirrel is linked with the specific role of these tissues in hibernators. It was shown that during the autumn and winter the mass of interscapular brown adipose tissue was increased as well as the absolute amount of norepinephrine. High turnover rate of noradrenaline was observed in IBAT of hibernating ground squirrel, suggesting a constant release of norepinephrine from the sympathetic nerve endings of this tissue[23]. The hibernation is achieved by and controlled through changes in hypothalamus and other parts of the brain which are rich in catecholamines[24]. Thyroid hormones were shown to produce an increase in SOD activity in the brain[25]. These data should be taken into consideration at least in part for the explanation of the increase in SOD activity in brain during the hibernation. Decrease in Mn SOD activity in the liver and brain may be due to the changes in mitochondrial respiration during the hibernation as compared with the active state.

References

1. C.J. Steffens, J.V. Bannister, W.H. Bannister, L. Flohe, W.A. Guzler, S.A.M. Kim and F. Otting, *Hoppe-Seylers Z. Physiol. Chem.*, **364**, 675-690, (1983).
2. J.R. Martin and I. Fridovich, *J. Biol. Chem.*, **256**, 6080, (1981).
3. A.M. Michelson and K. Puget, *Acta Physiol. Scand. Suppl.*, **492**, 67-80, (1981).
4. B. Halliwell and J.M.C. Guteridge, *Free Radicals in Biology and Medicine* Oxford Sci. Publ., Clarendon Press, Oxford, 84-110, (1985).
5. A.P. Autor, *J. Biol. Chem.*, **257**, 2713-2718, and *Lipid Peroxides in Biology and Medicine* Academic Press, 131-147, (1982).
6. B.L. Geller and D.R. Winge, *Analitical. Biochem.*, **128**, 86-92, (1983).
7. I. Fridovich, *Soc. Coll. org. B. Halliwell, London, Biochem.*, Trans, 67-68, (1981).
8. V.M. Petrović, Z. Saičić, B. Milić, M. Spasić and R. Radojičić, *Comp. Biochem. Physiol. 75B*, **4**, 699-700, (1983).

9. Y. Takada, T. Noguchi, T. Okabe and M. Kajiyoma, *Cancer Research*, **42**, 4233–4235, (1982).
10. H.P. Misra and I. Fridovich, *J. Biol. Chem.*, **247**, 3170–3175, (1972).
11. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265–275, (1951).
12. L.F. Panchenko, O.S. Brusov, A.M. Gerasimov and T.D. Lotkaeva, *Febs Letters*, **55**, 84–85, (1975).
13. C. Peeters-Joris, A.M. Vandevoorde and P. Baudhuin, *Biochem J.*, **150**, 31–39, (1975).
14. J.M. Mc. Cord, J.A. Doyle, E.D. Day, L.J. Rizzolo and M.L. Salin, *Superoxide and superoxide dismutase* Academic press 129–138, (1977).
15. M.L. Salin, E.D. Day and J.D. Crapo, *Arch. Biochem. Biophys.*, **187**, 223–228, (1978).
16. I. Fridovich, *Pathology of Oxygen* Academic Press, New York, 1–17, (1982).
17. S. Marklund and G. Westman, *Oxygen and oxygen* radicals in *Biochemistry and Biology* Academic Press, 693–697, (1981).
18. R.A. Weisiger and I. Fridovich, *J. Biol. Chem.*, **248**, 3582, (1973).
19. S. Marklund, G. Westman, E. Lundgren and G. Roos, *Cancer Research*, **42**, 1955–1961, (1982).
20. N.G. Westman and S.L. Marklund, *Cancer Research*, **41**, 2962–2966, (1981).
21. A.M. Michelson, *Pathology of Oxygen* Academic Press, 277–303, (1982).
22. C.H. Kayser, *The Physiology of Natural Hibernation* Pergamon Press, Oxford, 241–247, (1961).
23. P.R. Draskoczy and C.D. Lyman, *The J. Pharmacol. and Experimental Therapeutics*, **155**, 101–112, (1967).
24. N. Mrosovsky, *Hibernation and the Hypothalamus*, ACC Meredith Corporation, 20–24, (1971).
25. V.M. Petrović, M. Spasić, Z. Saičić, B. Milić and R. Radojičić, *Experientia*, **38**, 1355–1356, (1982).

Accepted by Dr. B. Halliwell