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Superoxide Radical Formation and Associated Biochemical Alterations in the Plasma Membrane of Brain, Heart, and Liver During the Lifetime of the Rat

Masaaki Sawada, Ulrike Sester, and John C. Carlson

Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Abstract Plasma membrane samples from rat brain, heart, and liver were examined for biochemical changes with age. A rise in superoxide radical (SOR) levels was followed by increases in thiobarbituric acid reactive substances and decreases in membrane fluidity with age. The earliest rise in SOR formation appeared in the plasma membrane from the brain. With age, protein synthesis also decreased significantly in tissue homogenates from brain and heart but was unchanged in the liver. Exposure of plasma membrane samples to in vitro–elevated SOR levels stimulated formation of lipid peroxides, as indicated by the thiobarbituric acid test, and resulted in a decrease in membrane fluidity in each tissue and in a decline in protein synthesis in brain and heart. Changes in brain lipid peroxidation and in membrane fluidity in brain and heart as a result of SOR supplementation were further enhanced due to age. In addition, the mechanism of SOR formation was examined in plasma membrane samples from the brain. SOR generation was Ca^{2+}-sensitive, blocked by superoxide dismutase or vitamin E and inhibited by both indomethacin, a cyclooxygenase inhibitor, and bromophenacyl bromide, a phospholipase A_2 inhibitor. These results show significant increases in SOR formation and biochemical alterations in plasma membranes from brain, heart, and liver in aging rats. SOR formation appears to be enzyme-mediated and elevated levels of this oxygen radical could be involved in membrane breakdown in older rats.

Key words: superoxide radical, lipid peroxidation, membrane fluidity, protein synthesis, calcium, aging

Free radicals appear to be an important factor in cellular degeneration involved in aging (Slater, 1972). Accumulation of potentially harmful oxygen radicals increases with age in a number of species (Harman, 1981), and can cause cellular changes that could result in the loss of homeostatic control and organ function. The culmination of such disorders also may explain the association of free radicals with a variety of illnesses, such as inflammatory diseases (McCord, 1985; Pryor, 1984) or cancer (Floyd, 1980).

We have previously used different tissues of the rat to investigate the effect of superoxide radicals (SOR) during aging (Sawada and Carlson, 1987). Brain mitochondria produced more SOR and revealed a higher level of lipid peroxides than the heart and liver. In the aging process, the brain seems to be of particular importance since this organ is responsible for the integration of biochemical and physiological activity, and age-related changes in the central nervous system are known to alter homeostatic processes of the whole organism (Meites et al., 1987). The nervous system contains a large amount of easily peroxidizable fatty acids (DeLeo et al., 1986) and it seems to be highly vulnerable to oxidative damage (Sawada and Carlson, 1987; Carney et al., 1991).

The purpose of the present study was to investigate whether the brain is an early site of age-related changes. Levels of SOR were determined in the plasma membrane from brain and compared with those from heart and liver of aging rats. To further assess the extent of age-associated changes in these tissues, alterations in protein synthesis activity, plasma membrane fluidity, lipid peroxidation, and sensitivity to calcium were also determined. In addition, the effects of exposure to increased SOR levels, generated in vitro using the xanthine/xanthine oxidase SOR generating system (Beauchamp and Fridovich, 1971; Fridovich, 1978), were examined.

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Address reprint requests to John C. Carlson, Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

MATERIALS AND METHODS

Animals

Male Wistar rats, raised in the departmental breeding colony (University of Waterloo, Waterloo, Ontario), were fed Purina rat chow and water ad libitum and kept at a light/dark cycle of 14/10 (h/h) at 25°C. The animals were killed by cervical dislocation at 1.5 to 105 weeks of age and whole homogenates or plasma membranes from brain, heart, and liver were isolated. In order to minimize oxidation, dissections were carried out in a nitrogen chamber, and samples were kept under nitrogen at 4°C until use.

Plasma Membrane Preparation

Plasma membranes from brain, heart, and liver were isolated essentially as described previously using a modified dextran-polyethylene glycol, two-phase separation method (Brunette and Till, 1971; Sawada and Carlson, 1989, 1990). In a nitrogen chamber, organs were cut into small pieces and homogenized at 4°C in 30 mM N-(2-hydroxyethyl) piperazine-N'3-propanesulfonic acid, pH 7.8 (EPPS-buffer) with six strokes of a motor-driven Teflon-glass homogenizer. The homogenate was brought to a final concentration of 0.1 μM $ZnCl_2$ and centrifuged at 700*g* for 10 min to remove large cell debris. Exactly 20 ml each of polyethylene glycol (PEG) and dextran were added to the supernatant, mixed thoroughly, and centrifuged for 10 min at 9,000*g* (4°C). The plasma membrane, located at the PEG/dextran interface, was removed and mixed with fresh PEG and dextran (20 ml each), and recentrifuged for 8 min at 9,000*g* (4°C). The plasma membrane, at the interface, was removed again, diluted with five volumes of 30 mM EPPS buffer, and centrifuged at 12,000*g* (4°C) for 10 min to wash and pellet the sample. During membrane preparation, a stream of nitrogen was added to the tubes for 3 min to reduce oxygen content and to minimize oxidation of the samples. In addition, the tubes were sealed with a screw cap to prevent the escape of nitrogen.

Marker Enzymes

Plasma membrane samples from brain, heart, and liver were assayed for marker enzyme activity (Table I), as described previously (Sawada and Carlson, 1989), to determine enrichment by measuring ouabain-sensitive Na^+/K^+ ATPase activity, a marker enzyme for the plasma membrane (Cole and Waddell, 1976). Possible contamination of membrane samples was determined by assaying the activities of NADH cytochrome C reductase, a marker enzyme for the endoplasmic reticulum (Sottocasa et al., 1967), and of succinate dehydrogenase, a marker enzyme for mitochondria (Pennington, 1961).

Protein contents were determined using the bicinchoninic acid reagent as described by Sorensen and Brodbeck (1986). Total phosphate was measured as described by Dittmer and Wells (1969).

Electron Spin Resonance

SOR levels were determined on a Varian E-12 electron spin resonance spectrometer (ESR) using 150 μg of membrane protein and Tiron

TABLE I. Marker Enzyme Activities of Rat Membrane Samples*

	Brain	Heart	Liver
Na^+/K^+ ATPase			
Specific activity	2.32 ± 0.11	1.32 ± 0.10	2.11 ± 0.16
Enrichment	11.02 ± 0.53	9.39 ± 0.70	10.05 ± 0.74
Succinate dehydrogenase			
Specific activity	0.06 ± 0.01	0.04 ± 0.01	0.14 ± 0.02
Enrichment	0.50 ± 0.08	0.29 ± 0.07	0.59 ± 0.07
Cytochrome C reductase			
Specific activity	1.24 ± 0.07	0.89 ± 0.18	1.10 ± 0.07
Enrichment	0.98 ± 0.05	0.87 ± 0.18	1.00 ± 0.07

*The specific activity of Na^+/K^+ ATPase (plasma membrane marker) is expressed as μg of PO_4 released/mg protein/h. The specific activity of succinate dehydrogenase (mitochondrial marker) is expressed as the μmol of p-iodonitrotetrazolium violet reduced/mg protein/h. The specific activity of cytochrome C reductase (endoplasmic reticulum marker) refers to the μmol of cytochrome C reduced/mg protein/h. Enrichment equals specific activity of the membrane sample/specific activity of homogenate. Each value represents four measurements.

(1,2-dihydroxybenzene-3,5-disulphonic acid) as a probe. Tiron reacts with SOR and produces a four-peak spectrum which reaches a steady state equilibrium after 5 min (Leshem et al., 1984). The level of SOR was determined by measuring the height of the ESR signal peak. The procedure follows that used previously in our laboratory (Sawada and Carlson, 1989, 1990). The spectrometer was calibrated using the xanthine/xanthine oxidase SOR generating system (Beauchamp and Fridovich, 1971; Fridovich, 1978).

Thiobarbituric Acid Test

The thiobarbituric acid (TBA) test (Uchiyama and Mihara, 1978) was used to assess lipid peroxidation (LP) in plasma membrane samples from brain, heart, and liver. It is employed frequently as an indicator of oxidative damage to polyunsaturated fatty acid and measures such breakdown products as malonaldehyde (Slater, 1984; Gutteridge and Halliwell, 1990). The results are expressed as the difference in the optical density at 535 and 520 nm.

Plasma Membrane Fluidity

Plasma membrane fluidity was measured by steady-state fluorescence polarization using a SLM model 8000 spectrofluorimeter with *trans* -parinaric acid as a probe. The procedure follows that described previously for membrane fluidity measurements (Sawada and Carlson, 1991).

Protein Synthesis Activity

Protein synthesis activity of tissue homogenates was obtained by measuring the activity of elongation factor 1α (EF-1α) as described by Crechet et al. (1986). The samples (200 μg of tissue extract) were incubated for 30 min at 37°C with saturating amounts of EF-2 and EF-1β (1–2 μg). Radioactivity was counted by liquid scintillation and is expressed as disintegration per minute (DPM).

In Vitro Incubation

In order to determine the effects of different agents on SOR formation, plasma membrane samples (200 μg of protein) were incubated in 30 mM EPPS buffer (pH 7.8) for 40 min at 39°C, under nitrogen, either with 20 μg of superoxide dismutase, 30 μg of vitamin E (d-α tocopherol), 15 μg of catalase, 50 μl (15 mM) indomethacin, 40 μl (20 μM) of bromophenacyl bromide, or different concentrations of calcium (0.5–16.0 mM). In one experiment the nitrogen was replaced with 100% oxygen to determine its effect on SOR production. The final volume of each sample for incubation was adjusted to 300 μl.

In brain plasma membrane samples, the xanthine/xanthine oxidase SOR generating system was used to determine whether experimentally augmented SOR levels would alter membrane fluidity, TBA reactants, or protein synthesis activity. This study was performed in two age groups, namely 4- and 105-week-old rats. Plasma membrane samples (200 μg of protein) for fluorescence polarization and the TBA test, or tissue homogenates (200 μg of protein) for protein synthesis activity measurements were mixed with 0.09 units of xanthine oxidase and 4 mM of xanthine. The mixture was incubated, as indicated above, and assayed using procedures outlined previously.

Statistical Analyses

The results of experiments involving a single variable were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc tests. Samples within a group were compared using the post-hoc Duncan's multiple range test. The post-hoc Tukey test served to compare among various groups. In experiments which involved SOR augmentation by xanthine/xanthine oxidase, data were analyzed by two-way ANOVA. Each data point represents the mean and standard error of three to five measurements, each using a single rat.

RESULTS

Marker Enzymes

Measurement of marker enzyme activities indicated a high enrichment (ninefold to 11-fold) of the plasma membrane marker, ouabain-sensitive Na^+/K^+ ATPase (Table I). The low activities of succinate dehydrogenase (mitochondrial marker) and NADPH cytochrome C oxidase (endoplasmic reticulum membrane marker) indicate that plasma membrane preparations were essentially free from such contaminants.

Age-Dependant SOR Formation

Levels of SOR in plasma membrane samples prepared from brain, heart, and liver are similar until 52 weeks of age (Fig. 1), when brain levels rose significantly ($P < 0.05$). Increases in this radical in heart and liver occurred at 65 ($P < 0.05$) and 78 weeks of age ($P < 0.05$), respectively.

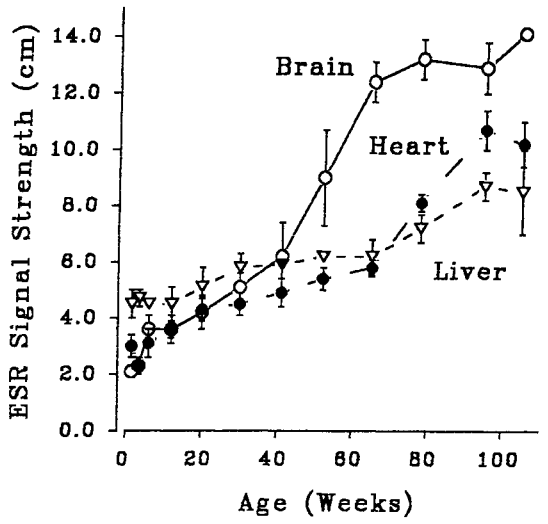

Fig. 1. Changes in SOR formation in plasma membrane samples from brain, heart, and liver during the lifetime of rats. The level of SOR was determined by measuring the height of the ESR signal. A significant rise in SOR levels occurred first in the brain (52 weeks) and was followed by the heart (65 weeks) and the liver (78 weeks). Each data point represents five measurements.

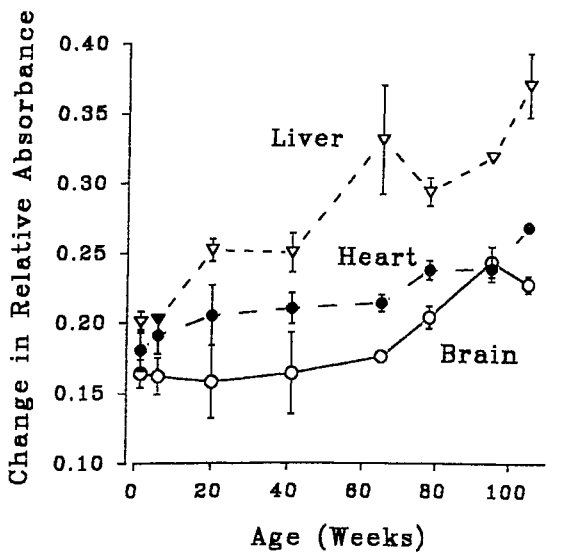

Fig. 2. Rate of LP as assessed by the TBA test in plasma membrane samples from brain, heart, and liver during the lifetime of rats. The data is presented as the change in relative absorbance between 535 and 520 nm. A significant increase occurred at 65, 95, and 105 weeks of age for the liver, brain, and heart respectively. Each data point represents four measurements.

TBA Test

Using the TBA test as an indicator of LP to assess membrane damage, there was an increase in TBA-reactive substances in plasma membrane samples from each of the three tissues examined (Fig. 2). A significant rise occurred from 95 weeks of age in the brain ($P < 0.05$), from 65 weeks of age in the liver ($P < 0.05$), and at 105 weeks in the heart ($P < 0.05$). It is interesting to note that the level of TBA reactants in brain and heart increased after the rise in their respective SOR levels, while that in liver occurred before SOR levels rose.

Plasma Membrane Fluidity

Fluorescence polarization was used to examine changes in plasma membrane fluidity. Using the probe *trans*-parinaric acid, membrane fluidity decreased significantly, as indicated by an elevation in the polarization ratio, in each sample (Fig. 3). However, the decrease in fluidity occurred earlier in plasma membrane samples from the brain (78 weeks, $P < 0.05$) than in samples from heart and liver (105 weeks, $P < 0.05$).

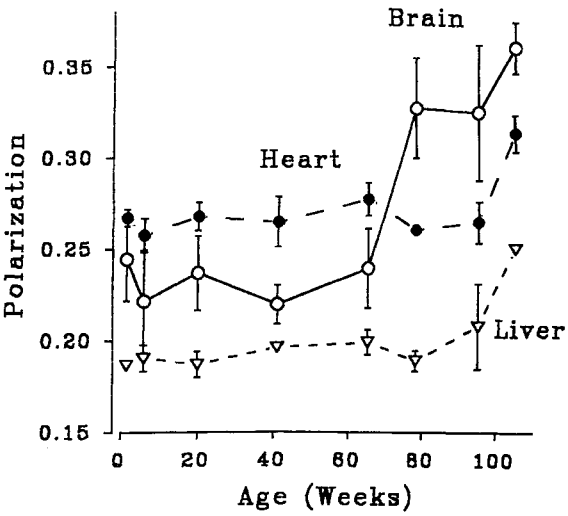

Fig. 3. Decrease in fluidity, as indicated by the increase in the fluorescence polarization ratio, in plasma membrane samples from brain, heart, and liver during the lifetime of rats. Samples prepared from the brain showed a significant decline at 78 weeks of age while membrane fluidity in samples from heart and liver did not decrease until 105 weeks of age. Each data point represents three measurements.

Protein Synthesis Activity

Protein synthesis activity in tissue homogenates was determined using an EF-1α assay (Fig. 4). A significant decline in EF-1α activity occurred in the heart at 95 weeks of age ($P < 0.05$) and in the brain at 105 weeks of age ($P < 0.05$). Interestingly, no age-dependent change in protein synthesis activity was detectable in the liver.

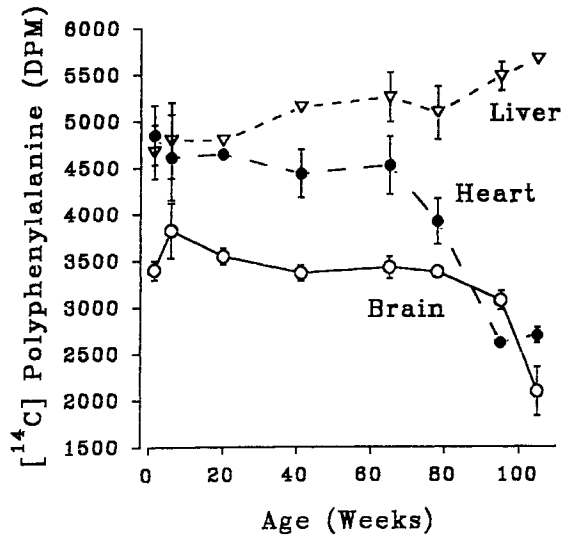

Fig. 4. Changes in EF-1α activity in tissue homogenates from brain, heart, and liver during the lifetime of rats. The data, expressed in DPM values, shows synthesis of [^{14}C]-labelled polyphenylalanine. A significant decline in protein synthesis activity was seen for the heart and brain at 95 and 105 weeks of age, respectively. Each data point represents three measurements.

Effect of Ca^{2+} on Brain SOR

Changes in SOR levels after addition of various concentrations of Ca^{2+} to plasma membrane samples from the brain are shown in Table II. In 4-week-old rats, 4 mM and higher concentrations of Ca^{2+} resulted in a significant increase in SOR levels ($P < 0.05$), while in 105-week-old animals a significant rise in SOR formation was already apparent starting at 2 mM Ca^{2+} ($P < 0.05$). In addition, two-way ANOVA revealed that the older brain is more susceptible to Ca^{2+}-dependent SOR formation. For both the 4- and the 105-week-old rats, the SOR levels tapered off at higher Ca^{2+} concentrations, indicating that the Ca^{2+}-induced rise in SOR levels was limited.

Alterations in SOR Levels by Inhibitors

Plasma membranes from the brain of 4- and 105-week-old rats were incubated with various inhibitors to elucidate the mechanism of SOR formation (Table III). In both age groups, superoxide dismutase, a specific SOR scavenging enzyme (Fridovich, 1978), and vitamin E, a general free radical scavenger (Burton and Ingold, 1981), significantly lowered SOR levels ($P < 0.05$). Addition of catalase, which converts H_2O_2 to O_2 and H_2O, did not alter the amount of this oxygen radical. Boiling significantly reduced SOR levels. Indomethacin, an inhibitor of cyclooxygenase (Vane, 1971), and bromophenacyl bromide, an inhibitor of phospholipase A_2 (Roberts et al., 1977), both resulted in a significant reduction in SOR ($P < 0.05$). SOR levels after indomethacin or bromophenacyl bromide treatment, however, were still higher than in superoxide dismutase or vitamine E treated groups. In plasma membrane samples where nitrogen was replaced with 100% oxygen, the SOR signal was higher than in any other group ($P < 0.05$).

TABLE II. Effect of Addition of Ca^{2+} on SOR Formation In Vitro in Plasma Membrane Samples From the Brain of 4- and 105-Week-Old Rats*

	SOR signal strength (cm)	
Ca^{2+} (mM)	4 weeks	105 weeks
0.0	2.4 ± 0.4	12.5 ± 1.4
0.5	2.0 ± 0.3	13.8 ± 0.5
1.0	2.4 ± 0.1	16.0 ± 0.9
2.0	3.2 ± 0.2	16.7 ± 0.4
4.0	3.6 ± 0.1	16.9 ± 0.7
8.0	3.4 ± 0.4	17.9 ± 0.7
16.0	3.9 ± 0.2	18.8 ± 0.4

*A significant rise in SOR occurred starting from 4 mM in 4-week-old and from 2 mM of Ca^{2+} in 105-week-old rats. Two-way ANOVA revealed that the older group was more sensitive to Ca^{2+}. SOR levels are represented by the height of the ESR signal in cm. Each value represents four measurements.

TABLE III. Effect of Inhibitors on SOR Formation In Vitro in Plasma Membrane Samples From Brain of 4- and 105-Week-Old Rats*

	SOR signal strength (cm)	
Treatment	4 weeks	105 weeks
Control	2.5 ± 0.4	13.0 ± 0.2
SOD	0.6 ± 0.3	0.5 ± 0.3
Vitamin E	0.5 ± 0.3	0.5 ± 0.3
Catalase	2.6 ± 0.3	11.8 ± 1.2
Boiling	0.2 ± 0.2	0.3 ± 0.3
Indomethacin	1.6 ± 0.2	10.3 ± 0.5
Bromophenacyl bromide	1.5 ± 0.3	10.4 ± 0.4
100% Oxygen	4.4 ± 0.3	16.7 ± 0.8

*Addition of superoxide dismutase (SOD), vitamin E, indomethacin, or bromophenacyl bromide resulted in a significant decline in SOR levels. Boiling also inhibited SOR formation. In addition, catalase had no effect while incubation with 100% oxygen greatly enhanced SOR levels. SOR levels are represented by the height of the ESR signal in cm. Each value represents four measurements.

Effect of Augmenting SOR Levels In Vitro

The effect of exposure of plasma membrane samples to elevated SOR levels generated by xanthine/xanthine oxidase treatment was examined in 4- and 105-week-old rats. A significant rise in formation of TBA reactive substances was observed in the brain ($P < 0.001$), the heart ($P < 0.001$), and the liver ($P < 0.02$) due to xanthine/xanthine oxidase treatment (Fig. 5). Furthermore, there was a significant interaction between age and treatment in the brain sample ($P < 0.001$), indicating that the brain plasma membrane from older rats is more susceptible to SOR damage. No such age-related susceptibility to in vitro augmented SOR was observed for the heart or for the liver.

Xanthine/xanthine oxidase treatment also resulted in a significant decrease in plasma membrane fluidity (Fig. 6) in each of the tissues examined ($P < 0.004$ for the brain, $P < 0.002$ for the heart, and $P < 0.004$ for the liver) and in a significant decline in protein synthesis activity (Fig. 7) in the brain ($P < 0.02$) and the liver ($P < 0.03$). In addition, a similar interaction between age and treatment was seen for the decline in membrane fluidity in the brain ($P < 0.02$) and in the heart ($P < 0.03$). However, for protein synthesis activity, no such age-dependent susceptibility to experimentally raised SOR levels was observed in any of the tissues examined.

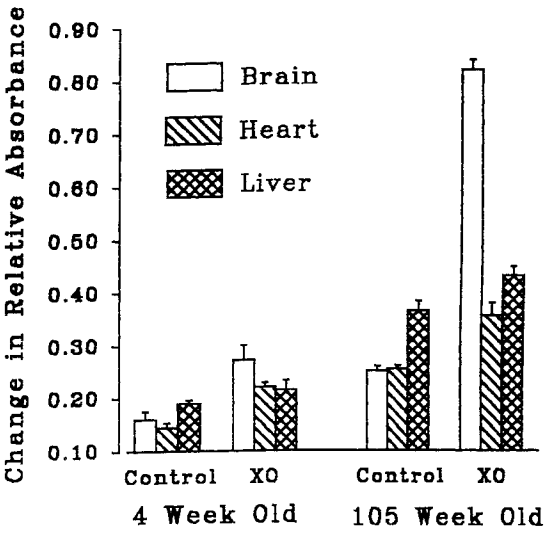

Fig. 5. The effect of augmentation of SOR levels in vitro by xanthine/xanthine oxidase treatment on TBA reactants in plasma membrane samples in brain, heart, and liver of 4- and 105-week-old rats. Each organ showed a significant increase in TBA reactants as a result of treatment. Also samples prepared from 105-week-old brain were more susceptible to peroxidation damage than the younger brain. Each data point represents three measurements.

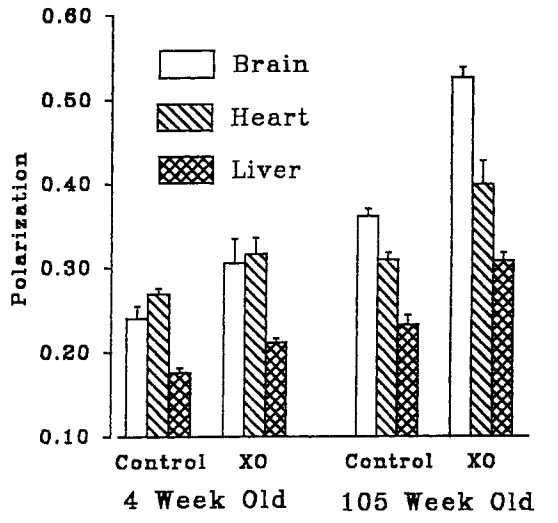

Fig. 6. Alteration in plasma membrane fluidity of brain, heart, and liver of 4- and 105-week-old rats. Samples were exposed in vitro to xanthine/xanthine oxidase to generate SOR. In each organ membrane fluidity decreased as a result of treatment. In addition, plasma membrane samples from the older brain and the older heart were more susceptible to fluidity changes. Each data point represents three measurements.

DISCUSSION

The results of this study show a clear-cut increase in SOR levels in plasma membrane samples in the brain, the heart, and the liver of the aging rat. The earliest rise in SOR levels, which occurred in the brain, corresponds to the time when death first appears in male rats in our colony (Sawada and Carlson, 1987). The production of toxic free radical species may be a major factor responsible for the accumulation of degenerative changes that occur with age (Harman, 1981; Sawada and Carlson, 1987, 1990). In the present study, the rise in TBA reactants, the fall in plasma membrane fluidity, and the decline in protein synthesis activity provide evidence that cellular breakdown follows the elevation in SOR generation. This idea is also supported by our finding that in vitro generation of SOR by xanthine/xanthine oxidase treatment consistently induced lipid peroxidation or other changes detected by the TBA test and a decrease in membrane fluidity in each of the tissues examined. These results clearly show that SOR is capable of causing plasma membrane degeneration. The plasma membrane is a potential site of SOR attack because of its hydro-

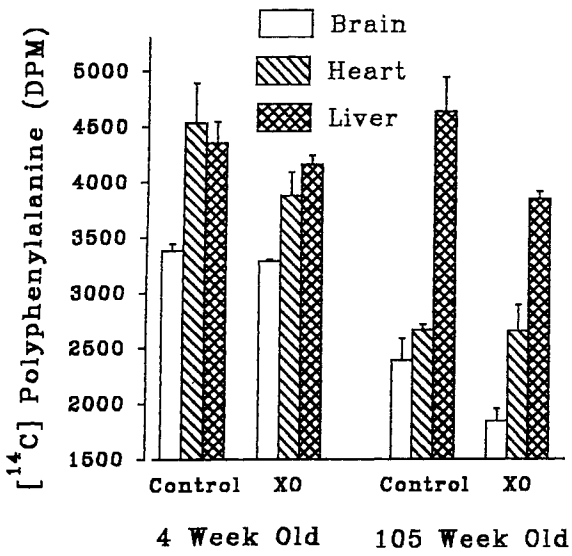

Fig. 7. Changes in protein synthesis activity after exposure of brain, heart, and liver homogenates from 4- and 105-week old rats to the xanthine/xanthine oxidase SOR generating system in vitro. The brain and liver showed a significant decline in protein synthesis activity due to treatment. Each data point represents three measurements.

phobic environment and the abundance of polyunsaturated fatty acids (Halliwell and Gutteridge, 1984). SOR working directly or indirectly such as by conversion to OH^- by the Haber-Weiss reaction causes lipid peroxidation (Fridovich, 1986) and cross-linking of proteins, lipids, and nucleic acids (Leibovitz and Siegel, 1980).

Although the mechanism of SOR generation in the plasma membrane under the current conditions is not completely understood, the observation of temperature sensitivity suggests enzyme mediation. The decrease in SOR formation by bromophenacyl bromide or its elevation by Ca^{2+} indicates the involvement of phospholipases, such as phospholipase A_2, which is a Ca^{2+}-dependent enzyme that is inhibited by bromophenacyl bromide (Roberts et al., 1977), and free fatty acid metabolites. Inhibition of the cyclooxygenase pathway, by indomethacin (Vane, 1971), also suggests that fatty acid products of this pathway may induce production of SOR. However, since the SOR increase could not be completely removed by these two inhibitors, other enzymes such as NADPH oxidase or xanthine oxidase may also play a role. The former is a multicomponent enzyme system, which has been extensively studied in neutrophils (Clark et al., 1987; Nauseef et al., 1991). It produces a burst of SOR from the plasma membrane upon stimulation by various agents including arachidonic acid. The possible involvement of NADPH in SOR formation is also strengthened by our finding that indomethacin, an inhibitor of the arachidonic acid metabolism via the cyclooxygenase pathway (Vane, 1971), was able to partly block the production of SOR. Activation of xanthine oxidase, an enzyme which is involved in purine breakdown and known to produce SOR (Fridovich, 1978), is another source. Either pathway may be considered, since such enzymes are widely distributed in the body (McCord, 1985), and they are activated under physiological conditions.

Age-related changes in the plasma membrane were accompanied by alterations in protein synthesis. EF-1α, a soluble intracellular protein found in eukaryotic cells, binds to transfer-RNA and is attached to ribosomes during protein synthesis (Crechet et al., 1986). Loss in EF-1α activity indicates a decrease in protein synthesis, which has been observed previously in many different aging organisms (Reff, 1985). In the present study, a decline in EF-1α activity occurred after the appearance of membrane breakdown as evidenced by increases in TBA reactive material and SOR formation, indicating that disruption of protein synthesis may be a later event in the aging process.

It is uncertain how changes in protein synthesis are coupled to membrane modification. Causality is inferred because of the sequential relationship and since our in vitro experiments showed that exposure to the SOR generating system resulted in a loss in protein synthesis activity. However, SOR does not readily leave the hydrophobic environment of the plasma membrane. Thus it seems likely that its effects could be indirect, for example through conversion of SOR to H_2O_2, which is cytotoxic, or to other reactive oxygen species (Halliwell and Gutteridge, 1986), which then could affect intracellular processes. A sustained high level of SOR in older tissues could drive these reactions to the point of overwhelming protective mechanisms. Formation of cytotoxic aldehydes as a result of LP (Esterbauer, 1982) represents another mechanism by which membrane damage affects cellular function. In addition, it is also possible that general membrane deterioration, which is known to occur with age (Packer et al., 1967; Sawada and Carlson, 1990), may be involved. Alterations in the intracellular ionic environment as a result of membrane rigidification during degen-

eration have been linked to a decrease in protein synthesis in older cells (Nagy, 1978).

An interesting finding was that the brain was the first organ to exhibit a significant increase in SOR levels. A similar observation was made in mitochondrial samples prepared from the same organs (Sawada and Carlson, 1987). The comparatively early rise in brain SOR indicates that the central nervous system may represent an early target for free radical attack. However, the mechanism responsible for this SOR increase is unknown. It may be related to biochemical characteristics of the brain that affect susceptibility of this tissue to free radicals. For example, the brain has high levels of lipids including polyunsaturated fatty acids (Crawford and Wells, 1979) and low levels of protective antioxidant enzymes and other antioxidant compounds (Floyd et al., 1984; Halliwell and Gutteridge, 1985; Semsei et al., 1991). The sensitivity of the brain to SOR was also evident in the present study since exposure to high concentrations of oxygen stimulated SOR formation in brain samples and generation of SOR by xanthine/xanthine oxidase stimulated an increase in appearance of TBA-reactive substance in the brain to a greater extent than in heart and liver. In addition, in vitro generation of SOR affected plasma membrane fluidity and protein syn-thesis. These changes observed in the brain are likely to alter the function of the nervous system.

Changes in brain function have effects beyond the central nervous system. Such modifications influence directly or indirectly all other organ systems in the body, since the brain is the control center of the organism, and is responsible for integrating sensory input and motor responses. The hypothalamus is a particularly important site, since it controls the neuroendocrine system and regulates homeostatic mechanisms. Recent studies also indicate that this part of the brain affects the immune system (Labeur et al., 1991). If the changes observed in the brain are indicative of hypothalamic alterations, then it is apparent that degeneration of this important site will affect the ability of the organism to adjust to various internal and external stimuli (Meites et al., 1987). A decrease in the ability to regulate compensatory responses is an important feature of the aging process (Shock, 1977). The comparatively early degenerative changes in the brain may play a role in the disruption of cellular homeostasis in the aging rat.

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