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Structural Alterations in Synaptosomal Membrane-Associated Proteins and Lipids by Transient Middle Cerebral Artery Occlusion in the Cat

N. C. Hall,[1,2] R. J. Dempsey,[4] J. M. Carney,[3] D. L. Donaldson,[4] and D. A. Butterfield,[1,2,5]

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We have previously reported that ischemia reperfusion injury results from free radical generation following transient global ischemia, and that this radical induced damage is evident in the synaptosomal membrane of the gerbil. [Hall et al, (1995) Neuroscience 64: 81–89] In the present study we have extended these observations to transient focal ischemia in the cat. We prepared synaptosomal membranes from frontal, parietal-temporal, and occipital regions of the cat cerebral cortex with reperfusion times of 1 and 3 hours following 1 hour right middle cerebral artery occlusion. The membranes were selectively labeled with protein and lipid specific paramagnetic spin labels and analyzed using electron paramagnetic resonance spectrometry. There were significant motional changes of both the protein and lipid specific spin labels in the parietal-temporal and occipital regions with 1 hour reperfusion; but, both parameters returned to control values by 3 hours reperfusion. No significant changes were observed in the normally perfused frontal pole at either reperfusion time. These results support the argument that free radicals play a critical role in cell damage at early reperfusion times following ischemia.

KEY WORDS: Spin label; electron paramagnetic resonance; somatosensory evoked potential; cerebral cortex.

INTRODUCTION

Cerebral ischemia/reperfusion injury results in multifactorial pathophysiological changes and leads to infarction (1,2). Oxygen radicals, generated at the onset of reperfusion, are implicated to play a pivotal role in initiating this tissue damage (3,4). Experiments conducted in our laboratory have demonstrated, through the use of protein- and lipid-specific spin labels and electron paramagnetic resonance spectrometry, that there is a time course of membrane alterations in gerbil synaptosomal membranes during reperfusion following 10 minute global ischemia (5). These membrane alterations are implicated to be a result of the initial burst of free radicals (5).

Free radicals, generated at the onset of reperfusion, can damage proteins by S-S cross-linking (6), generating protein hydroperoxides (7), and forming carbonyl groups on select amino acids (8,9). These oxidative protein modifications have been implicated to be markers for selective proteolysis (10). We have shown evidence that free radical mediated protein modification is responsible for the early alterations in the motion of the protein specific spin label MAL-6, observed in cortical synaptosomes using electron paramagnetic resonance, with standard free radical generating systems (11) and with transient global ischemia in the gerbil model (12).

[1] Department of Chemistry and
[2] Center of Membrane Sciences,
[3] Department of Pharmacology,
[4] Department of Neurosurgery, University of Kentucky, Lexington, Kentucky 40506.
[5] Address reprint requests to D. A. Butterfield, Department of Chemistry and Center of Membrane Sciences, Chemistry-Physics Building, University of Kentucky, Lexington, Kentucky 40506. Telephone: (606) 257-3184; Fax: (606) 257-5876.

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Another effect of ischemia reperfusion-induced free radical production is the peroxidation of lipids in the bilayer of the plasma membrane. Formation of lipid hydroperoxides is thought to occur as a result of OH· (13) and O_2· (14) attack on the polyunsaturated phospholipids which are abundant in the brain. Both the free radicals and the peroxides can activate phospholipases (15) and lead to further membrane structural alterations, altering membrane permeability, structure, and function (16). Support for this hypothesis has been provided by our studies of the motional changes of the lipid bilayer specific spin label 5-NS with reperfusion following ischemia (5).

Whether or not the synaptosomal membrane protein and lipid alterations observed in gerbils during global ischemia reperfusion injury are generalizable to other animal species has not been established. It is also yet to be determined if transient *focal* ischemia produces the same effects as transient global ischemia and other models of ischemic brain injury. To address these issues in the present study, we monitored the effects of 1 hour transient unilateral middle cerebral artery occlusion, followed by either 1 or 3 hours reperfusion, on cortical synaptosomal membrane proteins and lipids in multiple areas of the cat cerebral cortex. This research design allowed for internal control comparison with synaptosomal membranes from the cortical tissue on the non-ischemic side of the brain.

EXPERIMENTAL PROCEDURE

Materials. Ultra-pure sucrose, used for synaptosome isolation, was obtained from ICN Biochemicals. The protease inhibitors leupeptin, pepstatin A, and aprotinin were obtained from Calbiochem. The spin labels 2,2,6,6,-tetramethyl-4-maleimidopiperidine-1-oxyl (MAL-6) and 5-doxylstearic acid (5-NS) were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained from Sigma in the highest possible purity.

Animals. Healthy mongrel cats, of either sex, weighing 2–4 kg were fasted with free access to water for approximately 24 hours prior to the experiment. Cats were anesthetized with 30 mg/kg i.m. of ketamine, intubated and mechanically ventilated with a Harvard Apparatus large animal ventilator (Mills, MA). Xylazine (5 mg/Kg i.m.) was then administered followed by catheterization of the right femoral artery and both femoral veins with PE160 tubing. An additional 2.5 mg/kg i.m. of Xylazine was given just before eye surgery for additional analgesia and ketamine (3–6 mg/ml in lactated Ringers) was given intravenously to maintain anesthesia. The right eye was removed and the right middle cerebral artery (MCA) was isolated through a craniotomy (approximately 0.5 cm in diameter) drilled adjacent to the optical foramen. A snare (5-0 Prolene; Ethicon, Inc.; Somerville, New Jersey) was placed around the MCA and exited the orbit through double lumen vinyl tubing (Dural Plastics and Engineering; Dural, N.S.W., Australia). The base of the tube was treated with oxidized regenerated cellulose to prevent dental acrylic, poured into the orbit to stabilize the snare, from contacting the brain surface. A water jacket body temperature at 37–38 °C was maintained throughout the entire operation.

Heart rate and arterial pressure were monitored by connecting the arterial catheter to a pressure transducer (Gould P23XL; Gould, Inc., Oxnard CA) connected to an oscillograph (Grass Model 7D Polygraph; Grass Instruments Co.; Quincy, MA). A pH/blood gas analyzer (Instrumentation Laboratory System 1304 Blood Gas Analyzer; Instrumentation Laboratory Inc.; Lexington, MA) was used to measure arterial blood gases. Hematocrit was monitored using an IEC Clinical Centrifuge (Damon/IEC Division; Needham Hts., MA) and body temperature was continuously monitored using a telethermometer (Model 2100; Springs Instruments Co., Inc.; Yellow Springs, OH) and maintained at 37 °C throughout the study.

The surgical preparation was allowed to stabilize for 30 minutes prior to MCA occlusion. The proximal MCA was then occluded by pulling on both ends of the snare. Successful occlusion was confirmed by complete suppression of the cortical wave of the somatosensory evoked potential (SEP). The SEP's were monitored as described previously (17) and recorded at each Erb's point and at both cerebral hemispheres. Following 1 hour of MCA occlusion, the snare was released and blood allowed to reperfuse the brain for 1 or 3 hours. SEP recordings were obtained for each preparation before MCA occlusion, during occlusion, and just prior to sacrifice. The anesthetized cats were terminated at the appropriate times of reperfusion with close cardiac injection of a saturated KCl solution.

The brain was rapidly removed and dissected on a cold stage. The cortical mantle was dissected free, taking care to exclude the hippocampus and striatum. The telencephalon was separated from the underlying white matter. Slices of the cortex representing the frontal, parietal-temporal, and occipital regions, from both sides, were suspended in approximately 20 ml of ice-cold isolation buffer (0.32 M sucrose containing 4 μg/ml leupeptin, 4 μg/ml pepstatin A, 5 μg/ml aprotinin, 20 μg/ml type II-S soybean trypsin inhibitor, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM ethylenediaminetetraacidic acid (EDTA), 2 mM ethyleneglycoltetraacetic acid (EGTA), 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)) at pH 7.4 [Buffer A] and homogenized in a Wheaton 30 mL motor-driven Potter-type homogenizer with a teflon pestle.

Synaptosome Preparation. Synaptosomes were purified from homogenized cortices via ultracentrifugation across discontinuous sucrose gradients after a method previously described (18,19), and adapted in our laboratory (11,20,21). Crude homogenate (in isolation buffer as described above) was centrifuged at 4 °C, 1,500 *g* for 10 minutes, the supernatant carefully removed from the pellet and re-spun at 20,000 *g* for 10 minutes. The resulting pellet was resuspended in 4 mL of Buffer A (see above) at pH 8.0. This suspension was then layered on top of sucrose density gradients containing 11.7 ml each of 1.15 M (pH 8.5), 1.0 M (pH 8.0), and 0.85 M (pH 8.0) sucrose, each containing 2 mM EDTA, 2 mM EGTA, and 10 mM HEPES. Samples were then spun at 4 °C and 82,500g for 120 minutes in a SW28 swinging bucket rotor in a Beckman L2-65B refrigerated ultracentrifuge. Synaptosomes were removed from the 1.15 M/1.0 M sucrose interface and resuspended in approximately 20 ml of ice-cold lysing buffer, containing 10 mM HEPES, 2 mM EGTA and 2 mM EDTA, at pH 7.4. The resulting suspension was then spun down at 32,000 *g* for 10 minutes. The pellet was resuspended in ice-cold lysing buffer and spun down in the same manner two more times to rinse the synaptosomal membranes free of any residual cytoplasm. After the third wash the pellet was resuspended in 2.0 ml of the lysing buffer and assayed for total protein concentration by the method of Lowry et al (22). Synaptosomal suspensions from each independent pool were split into duplicates.

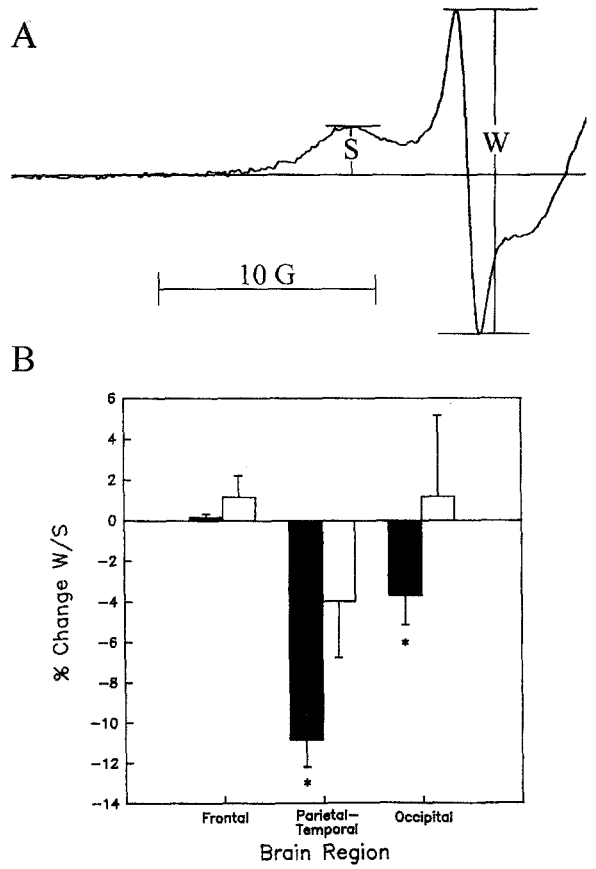

Fig. 1. Effects of 1 hour right unilateral MCAO followed by 1 or 3 hours reperfusion on W/S ratio in selected regions of the cat cerebral cortex. A) Example of W and S lines from MAL-6 labeled cortical synaptosomal membranes. B) Data are expressed as % change W/S from left hemisphere non-ischemic control ± standard deviation about the mean. Solid bars = 1 hour reperfusion. Open bars = 3 hours reperfusion. In all cases N = 3. * represents significant difference as compared to non-ischemic control ($p < 0.05$).

Spin Labeling. Synaptosomal membranes were labeled with the protein-specific spin label MAL-6, to a final concentration of 20 μg/mg protein as previously described (5,11,20,21). Ice-cold MAL-6 spin label (1 ml) was added to synaptosomal membranes containing 5.0 mg of protein. The resulting mixture was shaken by hand and then incubated at 4 °C for 16–18 hours. After incubation, samples were then centrifuged and washed 6 times in 10–15 volumes of lysing buffer to remove excess spin label. On the last wash the samples were brought to a total volume of ~400 μl in lysing buffer. Samples were allowed to equilibrate at room temperature for 30 minutes prior to EPR analysis. All EPR spectra were obtained on a Bruker 300 EPR instrument, equipped with computerized data acquisition and analysis capabilities, located in a climate-controlled room of constant temperature (20 ± 1 °C) and humidity. Instrumental parameters were: microwave frequency = 9.78 GHz, modulation amplitude = 0.32G at 100 kHz, and a time constant of 1.28 ms. The relevant spectral parameter (W/S ratio) was averaged for each duplicate pair of samples.

A separate aliquot of each of the synaptosomal membrane preparations was labeled with the lipid specific spin label 5-NS in a similar manner as erythrocyte membranes (23). The spin label was dissolved in chloroform to a concentration of 0.2 μM. In a test tube, 125 μl of this solution was evaporated under nitrogen gas, resulting in a thin layer of label on the test tube wall. The synaptosomal membranes were adjusted to a protein concentration of 2.5 mg/ml with lysing buffer and 360 μL added to each spin label tube. Duplicate labeling was performed for each sample. The samples were incubated at room temperature for 30 minutes prior to spectral acquisition. The instrumental parameters were the same as above. The relevant spectral parameter (HWHH) was averaged for each duplicate pair.

Data Analysis. Data was analyzed by appropriate Student's t-tests, one-way ANOVA, and post Hoc analyses. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

In order to determine whether the physical state of synaptosomal membrane-associated proteins was altered during transient focal ischemia in the cat, we utilized electron paramagnetic resonance (EPR) spectrometry to monitor motional changes of the protein-specific spin-label MAL-6. Our laboratory has previously shown that MAL-6 covalently binds primarily to membrane protein cysteine sulfhydryl groups when reacted with synaptosomes and synaptosomal membranes (11,20,24). MAL-6 is conceptualized as binding to two distinct populations of sites on these proteins. One site, the weakly immobilized site (W site), allows nearly free isotropic motion of the spin-label, while the motion of the spin-label at the other site, the strongly immobilized site (S site), is sterically hindered. The parameter used to monitor changes in the physical state of proteins is the ratio of the intensity of the $M_1 = +1$ low-field weakly immobilized EPR resonance line to the intensity of the $M_1 = +1$ low-field strongly immobilized EPR resonance line, or the W/S ratio (Fig. 1A). Our laboratory has demonstrated, both in vivo, via stroke (5,12), and in vitro, using an iron/H_2O_2 system (11), that oxidation of proteins causes a decrease in W/S ratio. We rationalize the decrease in the W/S ratio by assuming that W sites may be converted to S sites by protein S-S cross-linking (25,26,27), cross-linking of proteins by the lipid peroxidation product malondialdehyde (MDA) (28,29), or secondary and/or tertiary protein conformational changes as a result of carbonyl formation resulting from free radical attack on proteins (8,30,31,32,33).

At 1 hour of reperfusion following 1 hour unilateral middle cerebral artery occlusion (MCAO), there was a significant decrease ($p < 0.05$) in the W/S ratio of MAL-6 covalently bound to proteins in synaptosomal membranes from both the parietal-temporal and occipital regions of the right hemisphere of the cerebral cortex compared with the corresponding unaffected left hemispheric regions (Fig. 1B). The frontal region showed no

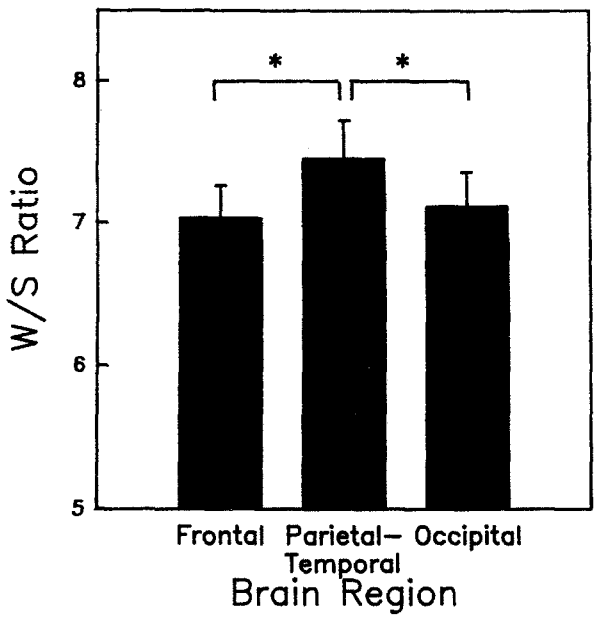

Fig. 2. Effects of 1 hour right unilateral middle cerebral artery occlusion with 1 and 3 hours reperfusion on W/S ratio in selected regions of the left cerebral cortex of the cat. Data are expressed as average W/S ratios combined from both 1 and 3 hours reperfusion ± standard deviation about the mean. N = 3. * represents a significant difference between the two groups indicated.

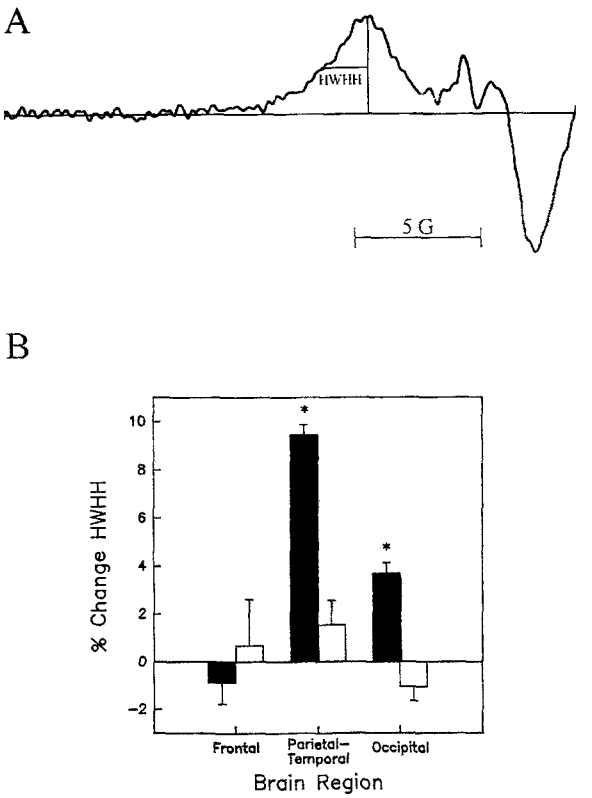

Fig. 3. A) A typical 5-NS labeled synaptosomal membrane EPR spectrum showing measurement of the HWHH of the $M_1 = +1$ low-field line. B) Effects of 1 hour right unilateral MCAO followed by 1 or 3 hours reperfusion on the HWHH parameter in selected regions of the cat cerebral cortex. Data are expressed as % change HWHH from left hemisphere non-ischemic control ± standard deviation about the mean. Solid bars = 1 hour reperfusion. Open bars = 3 hours reperfusion. In all cases N = 3. * represents significant difference as compared to non-ischemic control ($p < 0.05$).

significant effect when compared to the control side. The results were expressed as % change from corresponding non-ischemic left hemispheric regions. Cats sacrificed at 3 hours reperfusion exhibited no significant differences in the W/S ratio between the ischemia/reperfusion damaged right hemisphere and the unaffected left hemisphere in any of the three regions studied. It is interesting to note that the parietal-temporal region in the non-ischemic left hemisphere had an apparently higher baseline W/S ($p < 0.05$) than both the left frontal and left occipital regions at both reperfusion time points (Fig. 2).

The lipid specific spin label 5-NS was used to determine whether transient focal ischemia affected the motion and order of the phospholipids in synaptosomal membranes. 5-NS is an amphipathic molecule which intercalates into the lipid bilayer with its hydrophobic tail imbedded deeply in the hydrophobic center of the membrane and its polar head group at the hydrophilic surface of the bilayer (23,34). This orientation is responsible for the anisotropic motion of the fatty acyl chain of 5-NS inside the membrane. The EPR active nitroxide group is covalently attached to the alkyl side chain of the spin label. Therefore, the motion of the nitroxide group represents the intramembrane order and motion of the lipids (23,34). For this reason, 5-NS can be effectively used to study changes in the local environment of the hydrophobic phospholipid side chains near the surface of the lipid bilayer. The relevant parameter for determining the motional changes of the 5-NS spin label is the half-width at half-height (HWHH) of the $M_1 = +1$ low-field line of the EPR spectrum (Fig. 3A). Analogous to effects seen in chemical exchange phenomena, increased lipid motion, due to decreased lipid order, leads to increased line broadening resulting in increased values of HWHH (23,34). The largest HWHH changes from control values we have observed were on the order of 10–15% for various membrane perturbations (5,23,34).

Reperfusion of 1 hour following 1 hour unilateral right MCAO resulted in a significant increase in HWHH ($p < 0.005$) in both the parietal-temporal and occipital regions of the right hemisphere of the cortex compared to corresponding regions of the non-ischemic left hemisphere (Fig. 3B). At 3 hours reperfusion, the HWHH

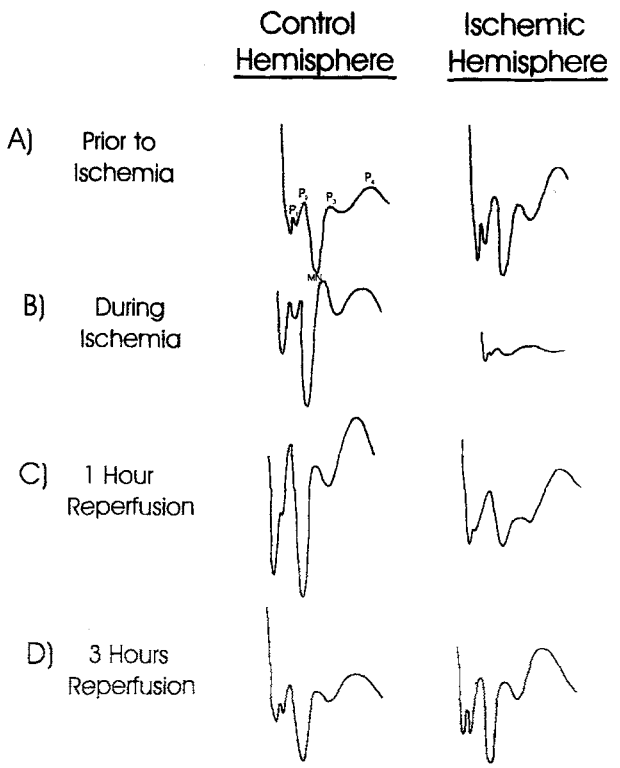

Fig. 4. Somatosensory evoked potentials (SEP's) recorded at the primary sensory cortex after median nerve stimulation for control vs. ischemic hemispheres of the cat A) prior to ischemia, B) during ischemia, C) with 1 hour reperfusion and D) with 3 hours reperfusion. The ratio of ischemic/control peak-to-peak amplitudes of the major positive-negative voltage complex (P_2-MN) was used as a measure of cortical functional recovery. The 1 and 3 hour reperfusion SEP's were recorded just prior to sacrifice of the animals. The SEP was lost during ischemia, followed by a partial recovery at 1 hour reperfusion and return to control by 3 hours reperfusion.

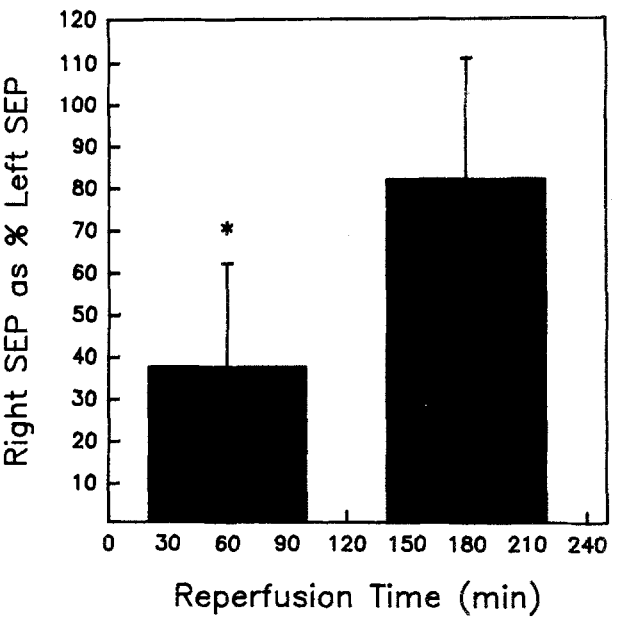

Fig. 5. A plot of the ratio of the amplitudes of the ischemia reperfusion damaged right SEP to the unaffected left SEP for reperfusion times of 1 and 3 hours following 1 hour transient middle cerebral artery occlusion. Data for each time point were collected just prior to death and compared to control values measured prior to ischemia. * represents significant difference from non-ischemic control ($p < 0.05$). N = 3–4.

values in both of these regions had returned to control values with no significant differences between corresponding regions of the two hemispheres. The frontal region of the right hemisphere was unaffected with respect to the HWHH parameter, compared to the nonischemic left hemisphere, at both reperfusion time points.

Fig. 4 presents typical somatosensory evoked potentials (SEP's) for both control and ischemic hemispheres of the cat brain at different time points during ischemia and reperfusion. These recordings include the four major reproducible positive voltage deflections (P_{1-4}) and the major negative voltage deflection (MN). The amplitude of the cortical potential was determined from the peak of the positive deflection, P_2, to the trough of the major negative deflection, MN. Prior to occlusion of the MCA, there was no significant difference between hemispheres in the P_2-MN interpeak amplitude. This was determined by comparing the right P_2-MN amplitude to the left P_2-MN amplitude. As seen in Fig. 4, the left cortical potential diminished during MCA occlusion, demonstrated a partial recovery at 1 hour reperfusion and returned to approximately control values by three hours reperfusion. Fig. 5 presents the mean recovery of SEP's with reperfusion times of 1 and 3 hours. To determine the extent of damage of the ischemia/reperfusion side of the brain the SEP amplitude of the damaged right cortical side was taken as a percent of that of the undamaged left cortical side just prior to death. The right hemisphere had not yet fully recovered its SEP at 1 hour reperfusion. The SEP recovery seemed to be nearly complete by 3 hours reperfusion with no significant difference from control at this later time point. Physiologic values including arterial blood gas determinations (pH, pCO2, and pO2), body temperature (Temp), mean arterial pressure (MAP) and heart rate (HR) are given in Table I.

DISCUSSION

To determine whether ischemia reperfusion-induced membrane alterations observed in gerbils can be

Table I. Physiologic Data*

Parameter		Pre Ischemia	End MCA Occlusion	1 Hour Reperfusion	2 Hours Reperfusion	3 Hours Reperfusion
pH						
	A	7.33 (0.04)	7.32 (0.03)	7.32 (0.03)		
	B	7.32 (0.04)	7.31 (0.03)	7.31 (0.02)	7.31 (0.04)	7.32 (0.01)
pCO2						
	A	27.57 (4.22)	27.43 (2.90)	28.37 (3.11)		
	B	28.47 (4.80)	25.87 (1.04)	25.27 (1.25)	25.83 (1.55)	28.4 (2.79)
pO2						
	A	87.13 (4.28)	89.00 (2.76)	88.87 (2.11)		
	B	89.13 (9.59)	88.97 (7.64)	89.40 (7.56)	93.17 (4.32)	92.3 (7.1)
Temp						
	A	37.23 (0.06)	37.23 (0.06)			
	B	37.07 (0.35)	37.20 (0.10)		37.17 (0.15)	37.1 (0.1)
MAP						
	A	92.67 (11.55)	84.67 (3.06)			
	B	77.33 (3.06)	80.00 (15.62)		74.00 (2.00)	72.67 (4.16)
HR						
	A	129 (9)	126 (2)			
	B	123 (14)	127 (19)		123 (9)	121 (8)

*Values are means ± (Standard deviation). A) represents data from cats subjected to 1 hour ischemia followed by 1 hour reperfusion. B) represents data from cats subjected to 1 hour ischemia followed by 3 hour reperfusion. MAP = mean arterial pressure; HR = heart rate.

generalized to other animal models and to determine if changes reported in global ischemia reperfusion injury also occur in focal ischemia reperfusion injury, we subjected cats to one hour of focal ischemia by unilateral middle cerebral artery occlusion followed by 1 or 3 hours of reperfusion. The results show significant alterations in the physical state of synaptosomal membrane proteins and bilayer lipids, as compared to the non-ischemic contra-lateral control tissue of parietal-temporal and occipital regions at 1 hour reperfusion (Fig. 1,3). The frontal pole, which is not ischemic in the preparation, was not different from the contra-lateral side. After 3 hours of reperfusion there is a lack of significant differences between any region and its corresponding non-ischemic control. Similar trends were observed in gerbil cortical tissue at 1 hour reperfusion compared to 3 hours (5). However, the protein alterations only partially returned to control values by three hours of reperfusion in the gerbil compared to a near complete recovery at this time point in the cat (5).

Ischemia/reperfusion injury, produced by middle cerebral artery occlusion, has been shown to bring about many deleterious effects including microvascular damage (35), loss of blood brain barrier regulation (36), altered calcium homeostasis (37,38), and loss of somatosensory evoked potential (17). It is well established that reactive oxygen species are capable of inducing tissue injury in many systems, including the central nervous system (4,5,39,40). The role of reactive oxygen species in ischemia reperfusion injury is supported by experimental evidence of increased hydrogen peroxide production in gerbils subjected to brain ischemia (41) and the beneficial effects of superoxide dismutase in the early stages of reperfusion following ischemia in newborn pigs (42). Our laboratory has demonstrated changes in the physical state of synaptosomal membrane associated proteins and lipids with transient global ischemia followed by various reperfusion times in the gerbil (5). We hypothesized that these alterations in the physical state of synaptosomal membrane proteins and lipids, observed at early times of reperfusion, are a direct consequence of reactive oxygen species production (5). Further, we suggested that the early free radical-mediated membrane damage triggers events which alter the physical state of membrane lipids at later reperfusion times (12). We have proposed a cascade of biochemical interconnections, occurring during the first 24 hours of reperfusion following ischemia, which may lead to the ultimate death of neurons. These events include phospholipase activation (15,43,44) and altered polyamine metabolism (45,46,47,48).

In support of this hypothesis, Nelson et al (49). have demonstrated that there is a significant increase in superoxide radical production for at least one hour during reperfusion following ischemia in the cat. It is in this time frame that we observed changes in the physical

state of neocortical synaptosomal membrane proteins and lipids (Fig. 1,3). The superoxide production ceases by two hours of reperfusion. We examined three hours reperfusion and found no changes in the physical state of proteins and lipids in cat neocortical synaptosomal membranes. These results are consistent with the time of initial membrane alterations, attributed to reactive oxygen species production, in gerbils we described previously (5). One of the conclusions reached from these previous studies was that there may be a window of opportunity for therapeutic intervention to avoid neuronal damage in the first several hours following transient ischemia (5,12). The applicability of these findings to humans requires the assumption that the mechanisms of global ischemia reperfusion-induced membrane alterations in the gerbil model can be generalized to include focal ischemia as well as other animal models.

The electroencephalogram (EEG) of the somatosensory evoked potential (SEP) has been shown to be an excellent indicator of neurophysiological function and cerebral ischemia (17,50,51). Similar to the present study, in the cat Hossmann (1988) has shown that a majority of animals, subjected to 1 hour transient global ischemia, regain EEG activity within the first three hours of reperfusion. Our current SEP results for transient focal ischemia show a recovery similar to those observed in the model of transient global ischemia. Likewise, the MAL-6 and 5-NS results show a recovery which parallels the recovery of SEP. In our study, at three hours reperfusion following 1 hour right middle cerebral artery occlusion there is no significant difference in the motion of either protein or lipid spin labels between the affected and unaffected cortical synaptosomal membranes in any of the regions studied. In Hossmann's study, the reappearance of continuous background EEG activity occurred typically between 1.5 and 3 hours reperfusion. Between the onset and 1.5 hours of reperfusion there is not a total recovery of EEG activity (52). This also correlates with changes we observed in the physical state of synaptosomal membrane proteins and lipids observed at 1 hour reperfusion between control and ischemic cortical tissue. The combination of these studies suggests that deficits in EEG and SEP activity at early reperfusion times may be associated with free radical-mediated membrane damage that occurs at the onset of reperfusion.

In addition to affecting EEG activity, early reperfusion membrane damage may be responsible for edema (36) and disruption of calcium homeostasis (53). Consistent with the free radical hypothesis, we have provided evidence that ischemia reperfusion injury induced membrane changes in gerbils can be prevented with preischemic administration of the free radical scavenger N-*tert*-butyl-α-phenylnitrone (PBN) and that lipid motional changes at later reperfusion time points (12–14 hours) can also be prevented in this way (12). These later time point alterations in synaptosomal membranes may be secondary consequences of polyamine metabolism (45,46,47,48) and/or phospholipase activation, induced by the initial ischemia/reperfusion-associated free radical production (12,15,43,44). PBN has been reported to be effective in preventing or reducing infarct volume following both transient and permanent MCAO in rats (54,55) and to prevent free radical production and damage following focal ischemia in the gerbil (12,56,57).

The current study provides evidence that the mechanisms of ischemia reperfusion-induced synaptosomal membrane damage may be similar between the cat and gerbil models as well as between focal and global ischemia. However, there is an obvious discrepancy between the time course of membrane structural recovery following the initial insult at the onset of reperfusion. With 10 minute transient global ischemia in the gerbil model, return of the 5-NS lipid label motion to control values occurred at around 6 hours reperfusion while the motion of the protein label MAL-6 did not return to control values at even 24 hours reperfusion. The current study shows that, with transient focal ischemia in the cat, both of these parameters return to control values by 3 hours reperfusion. The reasons for these differences possibly could be attributed to anatomical differences in the vasculature between the cat and the gerbil brain. In the focal ischemia model, the infarct area is surrounded by viable cells which conceivably could help process the plethora of deleterious byproducts formed during the ischemia reperfusion process. This could allow the plasma membranes of the neurons to recover to their "control" states at a more rapid rate following reperfusion than the global model of ischemia allows. The cells which are in the penumbra and have not reached the threshold of damage beyond which they are incapable of recovering can then regain all of their normal metabolic functions. We have theorized that the cells which are beyond repair ironically bring about their own demise during their repair efforts (5). We suggested that these secondary repair processes can save or kill the cell depending on the extent of initial damage (5). Other scenarios are possible, e.g., up-regulation of protein synthesis to replace oxidatively-damaged membrane proteins and lipids. An understanding of the biochemical mechanisms which lead to the stimulation of these damaging secondary effects could lead to effective therapeutic interventions.

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