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EFFECT OF ACIDOSIS AND ANOXIA ON IRON DELOCALIZATION FROM BRAIN HOMOGENATES

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Abstract—Cortical homogenates were prepared from rat brain in Krebs–Ringer phosphate media adjusted to pH 7, 6 or 5 and incubated for 1 hr under aerotic or anaerobic conditions in the presence of dipyridyl, an iron chelator. Low molecular weight species (LMWS) iron was measured spectrophotometrically after passing of the homogenates through a 10,000-M_r ultrafiltration membrane. Following aerobic incubation, LMWS iron reached 1.24 μg/g tissue at pH 7, and increased 1.7-fold at pH 6 and 3.1-fold at pH 5. Anoxia enhanced significantly the amount of ultrafiltrable iron at the three pH values, the LMWS iron level being increased by 190% at pH 7, by 113% at pH 6, and by 77% at pH 5. Addition of the ultrafiltrates to brain membranes caused significant rises in the production of lipid peroxides assessed by the thiobarbituric acid test, indicating that LMWS iron was in a form capable for catalysing oxygen-derived free radical-mediated lipid peroxidation. It was concluded that decompartmentalization of intracellular iron may be an important factor in the initiation of peroxidative damage to ischemic cells.

Among the changes which occur within ischemic cells, the lowering of pH may account for the development of irreversible brain damage [1]. The cytotoxicity of acidosis has been demonstrated in neuronal and glial cell cultures [2, 3], and in animals submitted to transient cerebral ischemia severe tissue acidosis aggravates cell damage and contributes to an impaired post-ischemic recovery [1, 4]. One possible effect of acidosis is to stimulate free radical generation. So, the production of lipid peroxides by brain homogenates is enhanced markedly by lowering the pH from 7 to 6 [5–9] and similar results have been reported recently [10] in cortical brain slices, a more physiological preparation which retains a major portion of whole tissue organization.

The role of iron in initiating lipid peroxidation is well known [11–13] and a possible effect of tissue acidosis is, as suggested by Bernheim [6], to dissociate protein-bound iron and to provide a source of iron in a form capable of catalysing free radical formation. It has been postulated that cells may contain low molecular weight iron complexes, but the ability of this loosely bound iron to participate in redox reactions and to promote lipid peroxidation is relatively unknown.

Our study was designed to answer two key questions. First, is there evidence that acidosis leads to iron delocalization in brain homogenates incubated in aerobic and anaerobic conditions? Second, is this delocalized iron capable of promoting lipid peroxidation?

MATERIALS AND METHODS

Experiments were performed on brain cortical tissue from fed male Wistar rats (body weight 200–400 g). The animals were killed by decapitation, the brain was removed and cortical tissue was isolated at 0°, rinsed with cold saline and homogenized by sonication in appropriate media. Trace amounts of iron being naturally present in buffer solutions, iron contamination was tested [14] spectrophotometrically after addition of ascorbic acid (250 μM) and 2,2′-dipyridyl (5 mM, Sigma Chemical Co., Poole, U.K.), an iron chelator which forms a stable ferrous complex absorbing at 520 nm. Under our experimental conditions, the iron concentration of the buffer solutions was less than 0.2 μM.

For measuring iron delocalization, cortical tissue was homogenized in 20 vol. of Krebs–Ringer phosphate medium (120 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 16 mM sodium phosphate pH 7.4, 10 mM glucose) adjusted to pH 7, 6 or 5 by addition of phosphoric acid. The homogenates were incubated in Nalgene vials in a shaker-water bath at 37°, under either air or anaerobic conditions. For anaerobic experiments, incubations were performed under nitrogen; all solutions were previously purged with nitrogen and glucose oxidase (Sigma) was added to the incubation mixture (200 U/g tissue) to scavenge any remaining oxygen).

The incubations were made in the presence or absence of dipyridyl. Following 1 hr of incubation, the homogenates were centrifuged at 0° at 15,000 *g* for 20 min. The supernatant was then passed through a 10,000-M_r ultrafiltration membrane (Grace Company, Amicon Division, Epernon, France). When dipyridyl (5 mM) was present during incubation of homogenates, the absorbance of the ultrafiltrate was read directly at 520 nm. When incubations were made without dipyridyl, the latter

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(5 mM) and ascorbic acid (250 μM, in order to reduce Fe^{3+} to Fe^{2+}) were added to ultrafiltrates before spectrophotometric measurement. The absorbances were converted to iron concentration using a standard curve of $Fe(NH_4)_2(SO_4)_2,6\ H_2O$. The results were expressed as μg of low molecular weight species (LMWS†) iron per g of fresh tissue.

In order to relate the amount of LMWS iron to total tissue iron content, the latter was determined using a modification of the method described by Riederer *et al.* [15]. About 100 mg of cortical tissue were homogenized in 10 vol. of saline adjusted to pH 2.5 by HCl and containing pepsin (10 mg, Sigma), ascorbic acid (20 mg) and 2,2'-dipyridyl (5 mM). After digestion for 24 hr at 37° and centrifugation, the absorbance was read at 520 nm against a blank containing all the reagents minus the tissue.

In another series of experiments, the peroxidant effect of LMWS iron was tested on brain tissue membranes in comparison with standard solutions of ferrous sulfate. Brain membranes were prepared as described by Harik *et al.* [16]. Cortical tissue was homogenized using a glass–teflon homogenizer in 20 vol. of 0.3 M sucrose containing 10 mM Tris–buffer pH 7.4. The homogenate was centrifuged at 1000 *g* for 10 min. The supernatant was then centrifuged at 48,000 *g* for 40 min. The pellet was homogenized in 10 mM Tris buffer, pH 7.4, and used as the source of brain membranes.

Cortical homogenates were incubated for 1 hr under air at 37° in Krebs–Ringer phosphate media without dipyridyl, adjusted to pH 7 or 6, and submitted to ultrafiltration as described. After addition of ascorbic acid (250 μM) in order to reduce Fe^{3+} to Fe^{2+}, the ultrafiltrates were adjusted to pH 7 and aliquots of 500 μL were added to 100 μL of brain membrane suspension (corresponding to about 0.5 mg of protein). The mixtures were incubated under air for 30 min at 37° and lipid peroxides were evaluated by measuring TBARS as described previously [17]. The amounts of TBARS were quantified using a standard curve of malondialdehyde prepared with malondialdehyde-bis-dimethylacetal (Aldrich Chimie, Strasbourg, France) and expressed as nmol malondialdehyde per mg of protein. Proteins were assayed according to the method of Lowry *et al.* [18], using bovine serum albumin as a standard.

The effect of ferrous sulfate on lipid peroxidation of brain membranes was assessed by measuring TBARS after the addition of various amounts of ferrous sulfate plus ascorbic acid. Brain membranes (about 0.5 mg of protein) were suspended in 500 μL of Krebs–Ringer solution pH 7. Ferrous sulfate (final concentration from 2 to 10 μM) and ascorbic acid (final concentration from 50 to 250 μM) were added and measurements of TBARS were performed following 30 min of incubation under air at 37°.

The data were given as means ± SEM and compared using two-way or one-way analysis of variance followed by Newman-Keuls test. The level of significance was set at $P < 0.05$.

† Abbreviations: LMWS, low molecular weight species; TBARS, thiobarbituric acid-reactive substances.

RESULTS

Iron delocalization from brain homogenates

When brain homogenates were incubated under aerobic conditions without dipyridyl, the level of LMWS iron in the ultrafiltrate reached 0.60 ± 0.05 μg/g tissue at pH 7 ($N = 9$). It increased significantly by lowering of the pH, reaching 1.14 ± 0.17 μg/g at pH 6 (N = 9) and 1.38 ± 0.08 μg/g at pH 5 (N = 6).

The presence of dipyridyl during incubation of homogenates enhanced the amount of delocalized iron. Under aerobic conditions, the level of LMWS iron reached 1.24 μg/g tissue at pH 7 and it increased significantly by 72% at pH 6 and by 210% at pH 5 (Table 1). Anoxia enhanced significantly the amount of delocalized iron at the three pH values. LMWS iron level was increased by 190% at pH 7, by 113% at pH 6 and by 77% at pH 5 (Table 1). When compared to the total tissue iron content, the LMWS iron represented from 5.7 (aerobic conditions, pH 7) to 31.2% (anaerobic conditions, pH 5) of the total iron (Table 1).

Effect of LMWS iron on lipid peroxidation of brain membranes

Production of TBARS by brain membranes incubated at pH 7 was very low (0.12 ± 0.02 nmol/mg protein/30 min). It was enhanced markedly, in a dose-dependent manner, by the addition of ferrous sulfate in the presence of ascorbic acid (Fig. 1). The addition of the ultrafiltrates obtained from brain homogenates resulted also in an enhancement of lipid peroxidation (Table 2). TBARS production reached 2.23 nmol/protein when ultrafiltrates were obtained from homogenates preincubated at pH 7 and 5.14 nmol/mg protein when ultrafiltrates were obtained from homogenates preincubated at pH 6.

DISCUSSION

The first point to be discussed concerns the methodology used for evaluating iron delocalization from brain homogenates. Homogenates incubated in Krebs–Ringer phosphate media were subjected to ultrafiltration in order to remote LMWS iron and the amount of delocalized iron was evaluated using dipyridyl, an iron chelator which forms a stable, coloured ferrous complex. Our results show that the amount of LMWS iron was 2–3-fold enhanced when dipyridyl was added during the incubation of brain homogenates and the possibility exists that dipyridyl itself promotes the release of iron from bound sites. This mechanism seems unlikely as nearly all tissue iron occurs in ferric form bound to ferritin and transferrin, and iron chelation by dipyridyl would need previous reduction of Fe^{3+} to Fe^{2+}. Another mechanism can explain the lowered levels of LMWS iron when brain homogenates were incubated without dipyridyl. Ferrous irons may autoxidize during incubation to form ferric ions which are not detectable by dipyridyl. Ferrous salts are extremely unstable in phosphate-buffered media and they autoxidize readily (19). Ferric salts are virtually insoluble at neutral pH and they are only partially reducible by ascorbic acid.

Table 1. Effect of acidosis and anoxia on LMWS iron level

pH value	Condition	LMWS iron level μg/g tissue	LMWS iron level % of total iron
7	Aerobic (N = 13)	1.24 ± 0.11*	5.7
	Anaerobic (N = 13)	3.60 ± 0.24†‡	16.6
6	Aerobic (N = 13)	2.14 ± 0.04*	9.8
	Anaerobic (N = 13)	4.56 ± 0.24†‡	21.0
5	Aerobic (N = 13)	3.84 ± 0.19*	17.6
	Anaerobic (N = 13)	6.79 ± 0.22†‡	31.2

LMWS iron was obtained by ultrafiltration of brain homogenates incubated under aerobic or anaerobic conditions for 1 hr in Krebs–Ringer phosphate buffer containing dipyridyl (5 mM) at pH 7, 6 or 5. Total iron was equal to 21.7 μg/g tissue.

Values are means ± SEM, N = number of experiments. Two-way analysis of variance.

* $P < 0.05$ between the different pH values under aerobic conditions.

† $P < 0.05$ between the different pH values under anaerobic conditions.

‡ $P < 0.05$ between aerobic and anaerobic conditions for the same pH value.

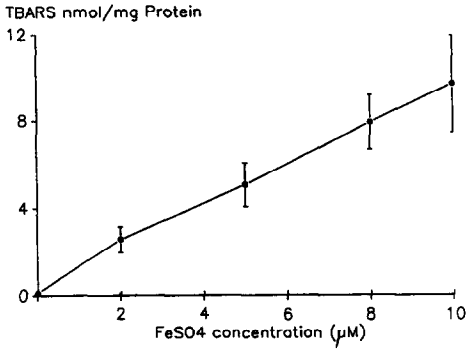

Fig. 1. Effect of ferrous sulfate (2–10 μM) plus ascorbic acid (50–250 μM) on lipid peroxidation of brain membranes incubated under air for 30 min at pH 7. Means ± SEM of values from 14 experiments.

Table 2. Effect of LMWS iron on lipid peroxidation of brain membranes

	TBARS (nmol/mg protein)
Membranes alone (N = 12)	0.12 ± 0.02
Membranes + ultrafiltrate from homogenate incubated at pH 7 (N = 12)	2.23 ± 0.08*
Membranes + ultrafiltrate from homogenate incubated at pH 6 (N = 12)	5.14 ± 0.23*†

Ultrafiltrates obtained from brain homogenates were added to brain membranes and TBARS were measured following 30 min of incubation at pH 7. Values are means ± SEM, N = number of experiments. One way analysis of variance followed by Newman–Keuls test.

* $P < 0.05$ vs membranes alone.

† $P < 0.05$ vs ultrafiltrate from homogenate incubated at pH 7.

Acidosis and accumulation of reducing agents are two characteristic features of ischemic cells. In the intact brain, intracellular pH has been reported to be 7.0–7.1 [20]. During complete ischemia, it has been estimated to fall to about 6.2 under normoglycemic conditions and below 6.0, accompanied by excessive accumulation of lactate, under hyperglycemic conditions [21]. Further studies have shown that during ischemia glial cells become more acidic that neurons [22, 23]. Under hyperglycemic conditions, direct measurements of intracellular pH have demonstrated [24] that astrocytic pH reached an average of 5.3 while neuronal cytoplasm equilibrated with extracellular pH [6.2].

Our results provide direct evidence that both anoxia and acidosis of a degree encountered during brain ischemia lead to increases in the level of LMWS iron of brain homogenates incubated at 37°. Our study made no attempt to identify the source of the delocalized iron. In tissues, nearly all iron occurs in biologically inactive form bound to ferritin and transferrin [25], but the relative abundance of these iron-containing proteins in the brain has not been quantified precisely [26].

In the cell, iron is stored mainly as ferric hydroxyde micelles within ferritin molecules. However, iron must be available for exchange with transferrin and for metabolic requirements. So, it has been proposed that a small "chelatable" or "transit" iron pool exists in the cytosol, which is thought to be bound to low molecular weight chelates such as nucleotides, organic acids, glycine and cysteine [27, 28], but neither the size nor the chemical nature of this "transit" pool has been clearly established. Accurate quantitation of this iron pool is difficult as it constitutes probably a very small fraction of the total iron. Our results indicate that ultrafiltrable iron obtained from brain homogenates incubated at pH 7 under aerobic conditions represents 5.7% of the total iron content of the tissue. However, the physiological significance of this value is questionable as it may be influenced by tissue homogenization and perhaps by proteolysis of iron proteins during incubation.

The mechanisms leading to iron mobilization from ferritin have been studied extensively [29–33]. Iron release requires reduction of the ferric ion stored in the molecule. Superoxide anion and other reducing agents can release iron from ferritin but the nature of the physiological reductant is unknown. Dihydroflavins have been considered as possible mediators [34] and reducing equivalents such as NADH or NADPH which accumulate during ischemia might interact with ferritin via flavin mononucleotides to release ferrous iron. Whether iron reduction by dihydroflavins involves access, via channels, to the interior of the ferritin molecule or direct reduction of iron bound at the outer surface of the protein shell remains to be established [35].

It has been demonstrated that the rate of mobilization of ferritin iron by various reducing agents, including flavin nucleotides, was accelerated by acidification [30]. This might explain the potentiating effect of acidosis on iron delocalization in brain homogenates incubated under anaerobic conditions (Table 1). Another possible source for LMWS iron in acidified brain homogenates is transferrin. Ferric ions seem to be bound to transferrin via a HCO_3^- or CO_3^{2-} bridge that can be disrupted by protonic attack [36]. Transferrin is present in brain cells and interstitial fluid [26, 37] and a supplementary source in brain homogenates may be contaminating blood. Assuming the plasma iron content equal to 1.2 ng/μL, the haematocrit 50%, and the blood content of cortical tissue 9 μL/g [38], the amount of contaminating transferrin-bound iron would not exceed 5.4 ng/g tissue, a negligible value compared to the level of LMWS iron present in brain homogenates (1.2 μg/g tissue for brain homogenates incubated at pH 7).

Some studies have shown previously an iron delocalization from organs submitted to ischemia and reperfusion. By measuring the amount of iron available for DFO chelation [39], Healing *et al.* [40] reported that in kidneys the amount of chelatable iron increased with the duration of ischemia but returned rapidly to preischemic level during aerobic reperfusion. By measuring LMWS iron by the passage of tissue homogenates through a 30,000-M_r membrane, Holt *et al.* [41] have shown that the LMWS iron level increased 60% in myocardial tissue 2 hr after ligation of the left coronary artery in dogs. In the canine cerebral cortex, LMWS iron isolated by ultrafiltration through a 30,000-M_r membrane was not altered significantly following 15 or 45 min of cardiac arrest but increased threefold after 2 hr of reperfusion [42–44]. It is worth noting that the level of ultrafiltrable iron reported by these authors in non-ischemic canine cerebral cortex was between 90 and 123 nmol/g tissue. These values are much higher than that found in the present study in rat cortical homogenates incubated at pH 7 (1.24 μg/g i.e. 22 nmol/g). This discrepancy may originate from differences in the animal species, in the condition of homogenization or in the size of the ultrafiltration membrane (30,000 vs 10,000 M_r in the present study).

Our results which demonstrate that LMWS iron is in a form capable of intiating the lipid peroxidation of brain membranes indicate that iron may be an important factor in the initiation of cell damage under ischemic conditions. A role of iron in the pathogenesis of ischemic and reperfusion injury has been shown by *in vivo* studies demonstrating the protective effect of iron chelating agents such as DFO [45, 46] or 21-aminosteroids [47] which are potent inhibitors of iron catalysed lipid peroxidation.

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REFERENCES

1. Siesjö BK, Acidosis and brain damage. *Neurochem Path* **9**: 31–88, 1988.
2. Goldman SA, Pulsinelli WA, Clarke WY, Kraig RP and Plum F, The effects of extracellular acidosis on neurons and glia *in vitro*. *J Cereb Blood Flow Metab* **9**: 471–477, 1989.
3. Walz W and Wuttke WA, Resistance of astrocyte electrical membrane properties to acidosis changes in the presence of lactate. *Brain Res* **504**: 82–86, 1989.
4. Marie C and Bralet J, Blood glucose level and morphological brain damage following cerebral ischemia. *Cerebrovasc Brain Metab Rev* **3**: 29–38, 1991.
5. Barber AA, Addendum: mechanisms of lipid peroxide formation in rat tissue homogenates. *Radiat Res* **3**(Suppl): 33–43, 1963.
6. Bernheim F, Biochemical implications of pro-oxidants and antioxidants. *Radiat Res* **3**(Suppl): 17–32, 1963.
7. Stocks J, Gutteridge JMC, Sharp RJ and Dormandy TL, Assay using brain homogenates for measuring the antioxidant activity of biological fluids. *Clin Sci Mol Med* **47**: 215–222, 1974.
8. Siesjö BK, Bendek G, Koide T, Westergerg E and Wieloch T, Influence of acidosis on lipid peroxidation in brain tissues *in vitro*. *J Cereb Blood Flow Metab* **5**: 253–258, 1985.
9. Rehncrona S, Hauge HN and Siesjö BK, Enhancement of iron-catalyzed free radical formation by acidosis in brain homogenates: difference in effect by lactic acid and CO_2. *J Cereb Blood Flow Metab* **9**: 65–70, 1989.
10. Bralet J, Bouvier C, Schreiber L and Boquillon M, Effect of acidosis on lipid peroxidation in brain slices. *Brain Res* **539**: 175–177, 1991.
11. Braughler JM, Duncan LA and Chase RL, The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ration in initiation. *J Biol Chem* **261**: 10282–10289, 1986.
12. Halliwell B and Gutteridge JMC, Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch Biochem Biophys* **246**: 501–514, 1986.
13. Dunford HB, Free radicals in iron-containing systems. *Free Rad Biol Med* **3**: 405–421, 1987.
14. Buettner GR, In the absence of catalytic metals ascorbate does not autoxidize at pH 7: ascorbate as a test for catalytic metals. *J Biochem Biophys Methods* **16**: 27–40, 1988.
15. Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K and Youdim MBH, Transition metals, ferritin, glutathione and ascorbic acid in parkinsonian brains. *J Neurochem* **52**: 515–520, 1989.
16. Harik SI, Doull GH and Dick APK, Specific ouabain binding to brain microvessels and choroid plexus. *J Cereb Blood Flow Metab* **5**: 156–160, 1985.
17. Bromont C, Marie C and Bralet J, Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. *Stroke* **20**: 918–924, 1989.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.

19. Tadolini B, Oxygen toxicity. The influence of adenine-nucleotides and phosphate on Fe^{2+} autoxidation. *Free Rad Res Commun* **5**: 237–243, 1989.
20. Mabe H, Blomquist P and Siesjö BK, Intracellular pH in the brain following transient ischemia. *J Cereb Blood Flow Metab* **3**: 109–114, 1983.
21. Siesjö BK, Acid-base homeostasis in the brain: physiology, chemistry, and neurochemical pathology. In: *Progress in Brain Research* (Eds. Kogure K, Hossmann KA, Siesjö BK and Welsh FA), Vol. 63, pp. 121–154. Elsevier, Amsterdam, 1985.
22. Kraig RP, Pulsinelli WA and Plum F, Heterogenous distribution of hydrogen and bicarbonate ions during complete brain ischemia. In: *Progress in Brain Research* (Eds. Kogure K, Hossmann KA, Siesjö BK and Welsh FA), Vol. 63, pp. 155–166. Elsevier, Amsterdam, 1985.
23. Kraig RP, Pulsinelli WA and Plum F, Carbonic acid buffer changes during complete brain ischemia. *Am J Physiol* **250**: R348–R357, 1986.
24. Kraig RP and Chesler M, Astrocytic acidosis in hyperglycemic and complete ischemia. *J Cereb Blood Flow Metab* **10**: 104–114, 1990.
25. Aisen P and Listowsky L, Iron transport and storage proteins. *Annu Rev Biochem* **49**: 357–393, 1980.
26. Mash DC, Pablo J, Flynn DD, Efange SMN and Weiner WJ, Characterization and distribution of transferrin receptors in the rat brain. *J Neurochem* **55**: 1972–1979, 1990.
27. Bakkeren DL, Jeu-Jaspars CMH, Van der Heul C and Van Eijk HG, Analysis of iron-binding components in the low molecular weight fraction of rat reticulocyte cytosol. *Int J Biochem* **17**: 925–930, 1985.
28. Mulligan M, Althaus B and Linder MC, Non-ferritin, non-heme iron pools in rat tissues. *Int J Biochem* **18**: 791–798, 1986.
29. Mazur A, Baez S and Shorr E, The mechanism of iron release from ferritin as related to its biological properties. *J Biol Chem* **213**: 147–160, 1955.
30. Funk F, Lenders JP, Crichton RR and Schneider W, Reductive mobilisation of ferritin iron. *Eur J Biochem* **152**: 167–172, 1985.
31. Thomas CE, Morehouse LA and Aust SD, Ferritin and superoxide-dependent lipid peroxidation. *J Biol Chem* **260**: 3275–3280, 1985.
32. Thomas CE and Aust D, Reductive release of iron from ferritin by cation free radicals of paraquat and other bipyridyls. *J Biol Chem* **261**: 13064–13070, 1986.
33. Monteiro HP, Vile GF and Winterbourn CC, An iron chelator is not required for reductive iron release from ferritin by radical generating systems. *Free Rad Res Commun* **7**: 33–35, 1989.
34. Jones T, Spencer R and Walsh C, Mechanisms and kinetics of iron release from ferritin by dihydroflavins and dihydroflavin analogues. *Biochemistry* **17**: 4011–4017, 1978.
35. Crichton RR, Iron uptake and utilization by mammalian cells. II. Intracellular iron utilization. *Trends Biochem Sci* **9**: 283–286, 1984.
36. Aisen P, Some physiochemical aspects of iron metabolism. In: *Iron Metabolism, CIBA Foundation Symposium*, No. 41, pp. 1–17. Elsevier, Amsterdam, 1979.
37. Taylor EM and Morgan EH, Developmental changes in transferrin and iron uptake by the brain in the rat. *Dev Brain Res* **55**: 35–42, 1990.
38. Cremer JE and Seville MP, Regional brain blood flow, blood volume, and haematocrit values in the adult rat. *J Cereb Blood Flow Metab* **3**: 254–256, 1983.
39. Gower J, Healing G and Green C, Measurement by HPLC of desferrioxamine-available iron in rabbit kidneys to assess the effect of ischaemia on the distribution of iron within the total pool. *Free Rad Res Commun* **5**: 291–299, 1989.
40. Healing G, Gower J, Fuller B and Green C, Intracellular iron redistribution. An important determinant of reperfusion damage to rabbit kidneys. *Biochem Pharmacol* **39**: 1239–1245, 1990.
41. Holt S, Gunderson M, Joyce K, Nayini NR, Eyster GF, Garitano AM, Zonia C, Krause GS, Aust SD and White BC, Myocardial tissue iron delocalization and evidence for lipid peroxidation after two hours of ischemia. *Ann Emerg Med* **15**: 1155–1159, 1986.
42. Nayini NR, White BC, Aust SD, Huang RR, Indrieri RJ, Evans AT, Bialek H, Jacobs WA and Komara J, Post resuscitation iron delocalization and malondialdehyde production in the brain following prolonged cardiac arrest. *J Free Rad Biol Med* **1**: 111–116, 1985.
43. Krause GS, Joyce KM, Nayini NR, Zonia CL, Garritano AM, Hoehner TJ, Evans AT, Indreri RJ, Huang RR, Aust SD and White BC, Cardiac arrest and resuscitation: brain iron delocalization during reperfusion. *Ann Emerg Med* **14**: 1037–1043, 1985.
44. Komara JS, Nayini NR, Bialick HA, Indrieri RJ, Evans AT, Garritano AM, Hoehner TJ, Jacobs WA, Huang RR, Krause GS, White BC and Aust SD, Brain iron delocalization and lipid peroxidation following cardiac arrest. *Ann Emerg Med* **15**: 384–389, 1986.
45. Kompala SD, Babbs CF and Blaho KE, Effects of deferoxamine on late deaths following CPR in rats. *Ann Emerg Med* **15**: 405–407, 1986.
46. Ikeda Y, Ikeda K and Long DM, Comparative study of different iron-chelating agents in cold-induced brain edema. *Neurosurgery* **24**: 820–824, 1989.
47. Hall ED and Braughler JM, Central nervous system trauma and stroke. II. Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation. *Free Rad Biol Med* **6**: 303–313, 1989.