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Original Contribution

MDA, OXYPURINES, AND NUCLEOSIDES RELATE TO REPERFUSION IN SHORT-TERM INCOMPLETE CEREBRAL ISCHEMIA IN THE RAT

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Abstract—Short-term incomplete cerebral ischemia (5 min) was induced in the rat by the bilateral clamping of the common carotid arteries. Reperfusion was obtained by removing carotid clamping and was carried out for the following 10 min. Animals were sacrificed either at the end of ischemia or reperfusion. Controls were represented by a group of sham-operated rats. Peripheral venous blood samples were withdrawn from the femoral vein from rats subjected to cerebral reperfusion 5 min before ischemia, at the end of ischemia, and 10 min after reperfusion. Neutralized perchloric acid extracts of brain tissue were analyzed by a highly sensitive high-performance liquid chromatography (HPLC) method for the direct determination of malondialdehyde, oxypurines, nucleosides, nicotinic coenzymes, and high-energy phosphates. In addition, plasma concentrations of malondialdehyde, hypoxanthine, xanthine, inosine, uric acid, and adenosine were determined by the same HPLC technique. Incomplete cerebral ischemia induced the appearance of a significant amount (8.05 nmol/g w.w.; SD = 2.82) of cerebral malondialdehyde (which was undetectable in control animals) and a decrease of ascorbic acid. A further 6.6-fold increase of malondialdehyde (53.30 nmol/g w.w.; SD = 17.77) and a 18.5% decrease of ascorbic acid occurred after 10 min of reperfusion. Plasma malondialdehyde, which was present in minimal amount before ischemia (0.050 μmol/L; SD = 0.015), significantly increased after 5 min of ischemia (0.277 μmol/L; SD = 0.056) and was strikingly augmented after 10 min of reperfusion (0.682 μmol/L; SD = 0.094). A similar trend was observed for xanthine, uric acid, inosine, and adenosine, while hypoxanthine reached its maximal concentration after 5 min of incomplete ischemia, being significantly decreased after reperfusion. From the data obtained, it can be concluded that tissue concentrations of malondialdehyde and ascorbic acid, and plasma levels of malondialdehyde, oxypurines, and nucleosides, reflect both the oxygen radical-mediated tissue injury and the depression of energy metabolism, thus representing early biochemical markers of short-term incomplete brain ischemia and reperfusion in the rat. In particular, these results suggest the possibility of using the variation of malondialdehyde, oxypurines, and nucleosides in peripheral blood as a potential biochemical indicator of reperfusion damage occurring to postischemic tissues.

Keywords—MDA, Oxypurines, Nucleosides, Cerebral ischemia, Reperfusion, Peroxidative damage, Free radicals

INTRODUCTION

The rapid decrease of oxygen and of substrate availability to ischemic tissues induces the inhibition of oxidative metabolism, which is fundamental for the cellular energy requirement.[1,2] This is reflected by a sudden depletion of ATP, which is sequentially dephosphorylated to adenosine (partly deaminated to inosine) and up to the free purines hypoxanthine and xanthine, therefore leading to an accumulation of these compounds inside the tissue.[3,4] The subsequent oxidation of these purines to uric acid has been indicated to be catalyzed during reperfusion by xanthine oxidase (XO), which should originate by the proteolytic transformation of xanthine dehydrogenase.[5] In this oxidation reaction, molecular oxygen serves as an electron acceptor and is transformed into superoxide anion, which could be the starting point for the production of other more reactive oxygen radicals.[6–8] Since XO is differently distributed among the various animal species, and moreover, inside the same species among the different tissues, data concerning the transformation of xanthine dehydrogenase (XDH) into XO, particularly in the myocardial tissue, are controversial.[9–12] However, recent reports obtained in

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similar experimental conditions (i.e., the ischemic and reperfused isolated rat heart) seem to support its occurrence[13,14] and therefore its possible involvement in the generation of oxygen radicals, which could seriously damage macromolecules. In particular, peroxidation of phospholipids would seem the most relevant phenomenon which could produce a significant alteration of membrane structure.[13,15,16]

Various models of cerebral ischemia and reperfusion have shown that lipid peroxidation occurs to a significant extent to brain tissue,[17-19] which also produced a relevant amount of oxypurines after a prolonged period of ischemia.[20] At the present time, no information is available on the effects of short-term incomplete cerebral ischemia and reperfusion both on parameters directly reflecting an increased oxidative stress (such as malondialdehyde and ascorbic acid) and on parameters representative of energy metabolism (such as adenosine 5'-triphosphate [ATP], adenosine 5'-diphosphate [ADP], adenosine 5'-monophosphate [AMP], AMP, adenosine [Ado], inosine [Ino], hypoxanthine [Hyp], xanthine [Xan], uric acid). Furthermore, the concentrations of plasma malondialdehyde, oxypurines (Hyp, Xan, and uric acid), and nucleosides (Ado and Ino), as a function of the different conditions of cerebral perfusion, have not yet been evaluated in detail. We have recently developed a highly sensitive HPLC technique for the direct simultaneous separation of malondialdehyde (MDA), ascorbic acid, oxypurines, nucleosides, nicotinic coenzymes, and high-energy phosphates,[21] by which we were able to evaluate myocardial MDA variations (as well as the other aforementioned compounds) in control, ischemic, and reperfused isolated rat heart.[22]

The aim of the present study was to determine, by using this HPLC method, the modification of MDA and other metabolite levels in brain tissue of rats subjected to short-term cerebral ischemia and reperfusion, and to monitor the plasma concentrations of MDA, oxypurines, and nucleosides as a function of the various conditions of brain perfusion.

MATERIALS AND METHODS

Adult male Wistar rats of 350–400 g b.w. were used; they were fed with a standard diet and water ad libitum. Animals were anesthetized by dietylethere inhalation and then by propofol intraperitoneal (ip) injection (23 mg/kg b.w.). The right femoral artery and femoral vein were cannulated for continuous arterial pressure monitoring, intermittent blood gas sampling, and blood withdrawal. Body temperature was maintained at 37°C by means of a rectal thermometer and a heater system.

Mean arterial blood pressure (MABP) was monitored constantly throughout the duration of the experiments by a pressure transducer connected to a polygraph. Short-term incomplete ischemia (5 min) was induced in the brain by reversibly occluding the common carotid arteries with a temporary vascular clip. By removing carotid occlusions, brain reperfusion was carried out for 10 min. Sham-operated animals served as the control group. Treated rats were sacrificed by decapitation either at the end of ischemia or of reperfusion, while controls were killed after 30 min of anesthesia. The brain was removed from dead animals within 30 s from decapitation, immersed in liquid nitrogen, and, after weight determination, homogenized at 24,000 rpm in ice-cold 0.6 M $HClO_4$ by an Ultra-Turrax (Janke and Kunkel GmbH & Co. KG, IKA-Werk, Stanten, FRG) according to Lazzarino et al.[23]

Heparinized blood samples were withdrawn from the femoral vein at three different times, corresponding to 5 min before ischemia (zero time), 5 min after induction of ischemia, and 10 min after reperfusion only from rats subjected to brain reperfusion. Samples were quickly centrifuged at 4760 $g \times 10$ min at 4°C. Plasma was immediately deproteinized by 0.6 M ice-cold $HClO_4$ (1:2; v:v) and neutralized by the addition of 5 M K_2CO_3. Two hundred microliters of both tissue and plasma acid extracts, after filtration through a 0.45 HV Millipore filter, were then used for the HPLC analysis of MDA, ascorbic acid, oxypurines, nucleosides, nicotinic coenzymes, and high-energy phosphates. Separation of the various compounds was obtained by a slight modification of the method of Lazzarino et al.[21] by a Jasco HPLC apparatus (Tokyo, Japan) equipped with a Supelcosil LC-18T 3 μm particle size 15 cm $\times$ 4.6 mm column (Supelco, Bellefonte, PA). Briefly, separation was obtained using a step gradient formed by the following buffers: (a) 10 mM tetrabutyl ammonium hydroxide (Nova Chimica, Milan, Italy), 10 mM KH_2PO_4 with a final concentration of 0.25% methanol; (b) 2.8 mM tetrabutylammonium hydroxide, 100 mM KH_2PO_4 with a final concentration of 30% methanol.

Statistical comparisons were performed either by the one-way analysis of variance (comparison of tissue metabolites) or by the two-way analysis of variance (comparison of plasma metabolites).

RESULTS

The induction of incomplete cerebral ischemia by clamping carotid arteries produced an increase of blood pressure from 150 ± 10 to 200 ± 15 mm Hg (p

$< .001$) within 30 s, which remained unaltered throughout the duration of ischemia. The removal of vascular occlusion reversed MABP to preischemic value (145 ± 5 mm Hg) in about 30 s (n.s. with respect to preischemia value).

Figure 1 reports a HPLC analysis of 200 μL of cerebral tissue extracts of a control (Panel A), an ischemic (Panel B), and a reperfused rat (Panel C). The parameter which was most strikingly affected by ischemia and reperfusion is MDA. In effect, MDA was undetectable in control animals, while 8.05 nmol/g w.w. (SD = 2.82) of this compound were found in brain tissue of rats subjected to 5 min of incomplete cerebral ischemia, and 53.30 nmol/g w.w. (SD = 17.77) were determined in brain extracts of rats subjected to 5 min of incomplete cerebral ischemia + 10 min of reperfusion. Although the comparison of the retention time of standard MDA with MDA in the samples allowed the assignment of the peak without any uncertainty, as previously demonstrated by the cochromatograms reported elsewhere,[21] we performed a further step to show that the peak in the samples contained only MDA. Therefore, we collected both the peak corresponding to MDA of the sample from a rat subjected to 5 min of incomplete cerebral ischemia + 10 min of reperfusion and the peak of a standard MDA with a similar concentration than the sample. Figure 2 reports the two absorption spectra, both showing only a maximum of absorption at 266 nm (i.e., that of MDA).[24] It can be concluded that the concentration of MDA in our samples was correctly calculated (by comparing the area of the peak corresponding to MDA in the samples with that of the peak of standard MDA with the known concentration) and that the values reported in Tables 1 and 2 therefore represent the actual concentration of plasma and tissue MDA in the various experimental conditions. Data of tissue MDA variations as a function of cerebral ischemia and reperfusion certainly indicate that brain tissue is particularly susceptible to peroxidative damages, as also evidenced by the parallel decrease of cerebral ascorbic acid (Table 1). Variations of other metabolites as a function of incomplete brain ischemia and reperfusion are summarized in Table 1. It is worth mentioning the increase of Hyp, uric acid, Ino, Ado, guanosine diphosphate (GDP), and AMP and the concomitant decrease of inosine 5′-phosphate (IMP), guanosine 5′-triphosphate (GTP), and ATP that occurred after 5 min of incomplete cerebral ischemia. In contrast, reperfusion produced a significant increase of Xan, uric acid, and IMP and a tendency to increase GTP and ATP with respect to ischemia. Hyp, Ino, Ado, and IMP were normalized by removing carotid arteries' occlusion.

Figure 3 shows an HPLC separation obtained on 200 μL of deproteinized plasma from the same rat before incomplete cerebral ischemia (Panel A), after 5 min of incomplete cerebral ischemia (Panel B), and after 10 min of reperfusion (Panel C). MDA, Hyp, Xan, uric acid, Ino, and Ado could be always detected in plasma, but they were differently affected by incomplete cerebral ischemia and reperfusion. MDA, Xan, uric acid, Ino, and Ado reached their highest values after 10 min of reperfusion, while Hyp had its maximal concentration after 5 min of carotid arteries' occlusion and was significantly decreased after reperfusion. A summary of the data referring to the aforementioned parameters recorded in seven different rats is reported in Table 2. As can be observed, MDA increased 5 times at the end of ischemia and 13 times at the end of reperfusion. The most reasonable explanation for the increase of tissue and plasma MDA (needing oxygen to be produced) at the end of ischemia should be attributed to the particular experimental model adopted, which provides only an incomplete ischemia. In fact, although both carotid arteries were clamped, the residual cerebral perfusion, obtained by collateral flow, was certainly sufficient to allow a delivery of oxygen to the whole brain, thus also ensuring a partial washout of tissue catabolites during carotid clamping. The return of a complete oxygen availability to the brain (i.e., the starting point for reperfusion) produced an increase of peroxidation reactions, which could be revealed by the marked augmentation of both tissue and plasma MDA and by the decrease of tissue ascorbic acid.

The sum of oxypurines and nucleosides released in the bloodstream in the different perfusion conditions and the relative contribution of each compound are reported in Fig. 4 and in Table 3, respectively. The sum of oxypurines progressively increased in rat plasma as a consequence of the different conditions of cerebral perfusion. Interestingly, the relative contributions of Hyp and uric acid to the sum of oxypurines was affected by ischemia, particularly due to the marked increase of plasma Hyp.

Cerebral reperfusion induced oxidation of Hyp to Xan and uric acid, which significantly increased in plasma, thereby affecting their relative contribution to the sum of oxypurines. Similar to oxypurines, nucleosides increased both at the end of ischemia and of reperfusion. The relative contribution of Ino and Ado was markedly affected by reperfusion, which caused a 10-fold increase of Ino and a 1.7-fold increase of Ado with respect to preischemic values (see Table 1), thereby triplicating the percent of Ino in the sum of nucleosides.

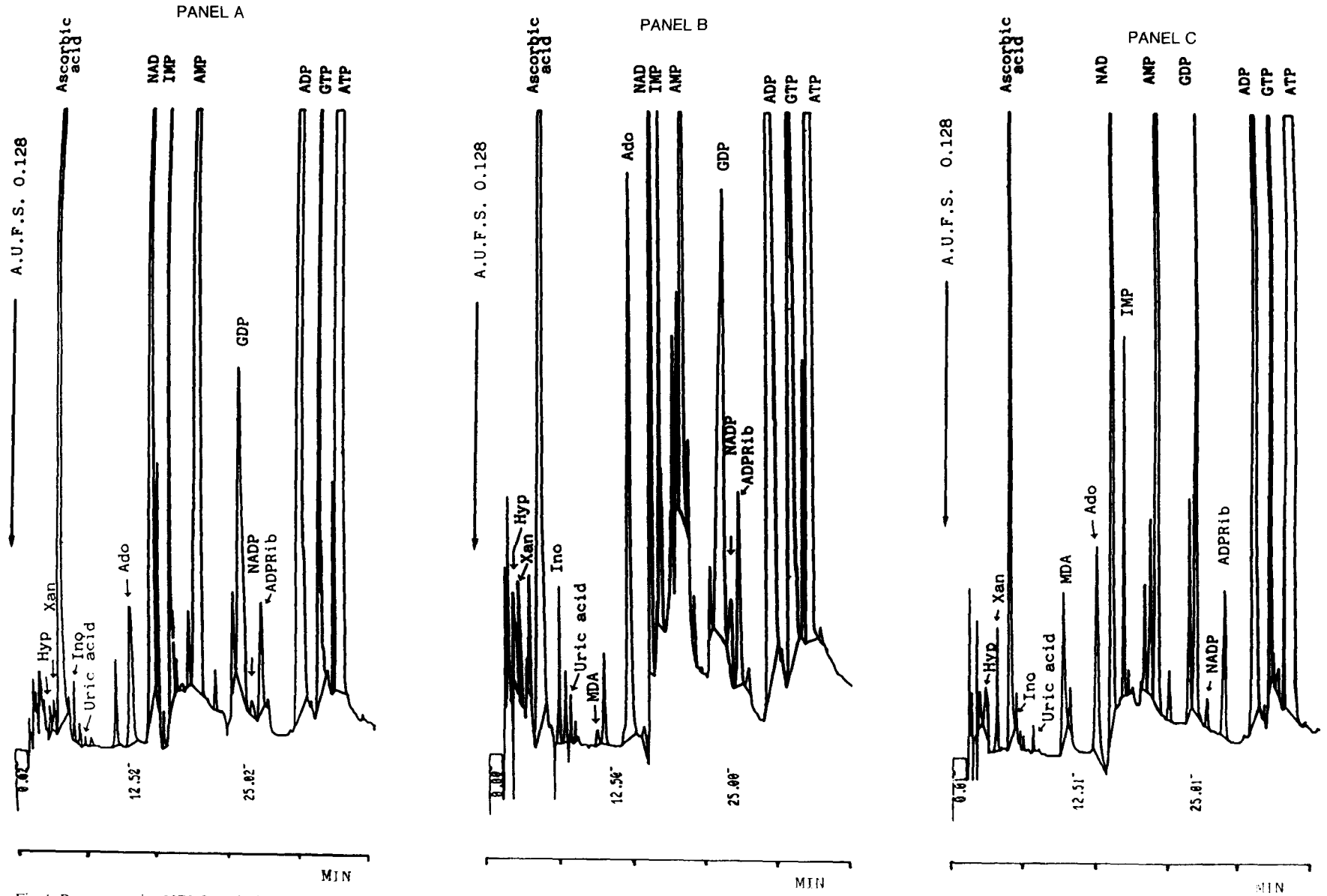

Fig. 1. Representative HPLC analysis of the simultaneous determination of MDA, oxypurines, nucleosides, high-energy phosphates, and nicotinic coenzymes of 200 μL of the $HClO_4$ extracts of cerebral tissue. Chromatograms refer to tissue samples analyzed from different rats before (Panel A), at the end of 5 min of incomplete cerebral ischemia (Panel B), and at the end of 10-min reperfusion (Panel C).

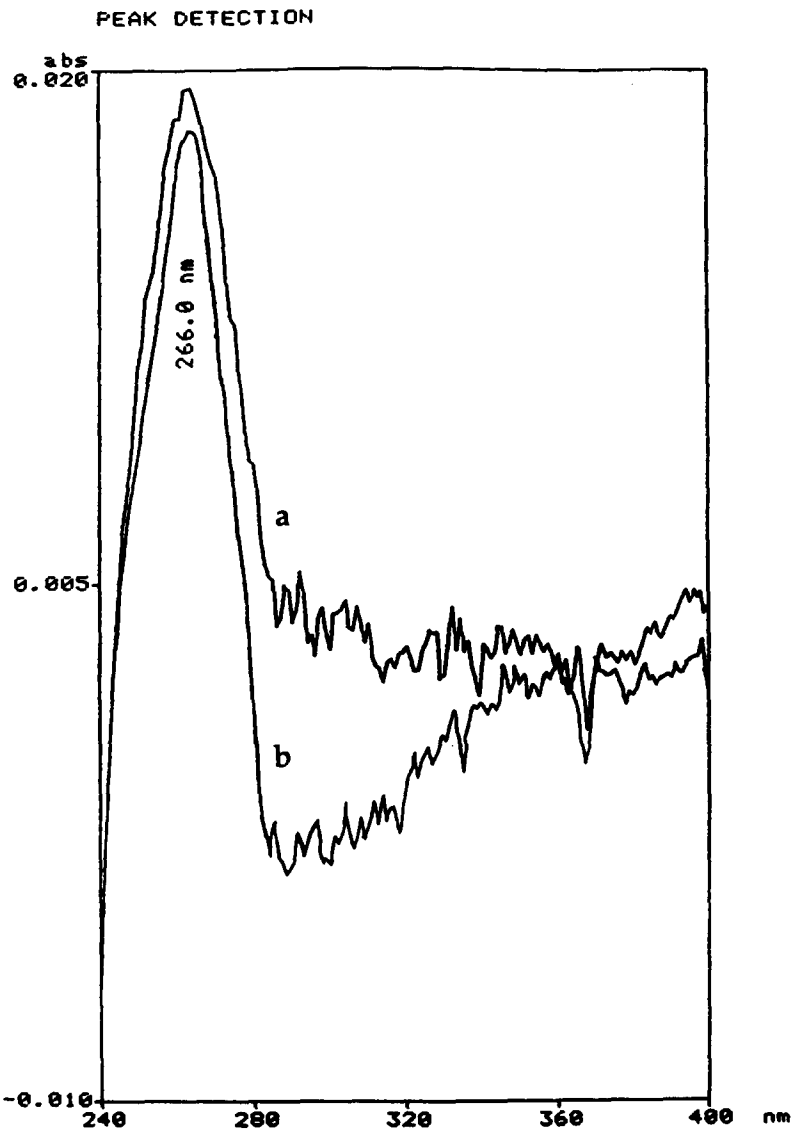

Fig. 2. Absorption spectra of the peak of MDA standard (A) and of the peak corresponding to MDA of a sample of cerebral extract from a rat subjected to 5 min of incomplete cerebral ischemia + 10 min of reperfusion (B). Both spectra were obtained against a blank represented by the buffer A used for the HPLC separation of MDA.

DISCUSSION

The determination of oxygen-derived free radical damage to ischemic brain has been generally carried out in several models of long-term cerebral ischemia in different animal species.[18,19] The change of lipid peroxidation, induced to brain tissue by oxygen deprivation, has been used often as a biochemical marker for the quantitation of tissue injury.[25,26] Apart the general concern about the poor specificity and reliability of the analytical technique for the evaluation of lipid peroxidation (such as the thiobarbituric acid [TBA] test or the assay of conjugated dienes),[27,28] the data available at the present time do not give information about the effect of the short-term incomplete cerebral ischemia and reperfusion on parameters representative of increased oxidative stress, such as MDA and ascorbic acid. Moreover, the knowledge of the plasma concentrations of MDA, oxypurines, and nucleosides as a function of the different conditions of cerebral perfusion is totally missing.

This study provides clear indications on the role of MDA as an early biochemical marker of peroxidative damage occurring to rat brain subjected to short-term incomplete ischemia and reperfusion. In fact, it is worthwhile recalling that no detectable amount of this compound could be revealed in brain tissue of control sham-operated animals. In contrast, the presence of considerable levels of MDA was observed after 5 min of incomplete cerebral ischemia, whose

Table 1. Variations of Cerebral MDA, Ascorbic Acid, Oxypurines, Nucleosides, High-Energy Phosphates, and Nicotinic Coenzymes as a Function of Short-Term Cerebral Ischemia and Reperfusion

	Hyp	Xan	Ascorbic acid	Ino	Uric acid	MDA	Ado	NAD	IMP	AMP	GDP	NADP	ADP	GTP	ATP
CONTROLS	2.80	2.56	1226.56	3.37	5.27	N.D.	13.66	387.25	105.51	301.21	107.47	7.12	740.03	179.07	1802.34
	(0.78)	(0.81)	(132.98)	(1.32)	(0.61)		(2.89)	(64.69)	(5.48)	(17.90)	(10.12)	(1.82)	(26.41)	(5.42)	(190.16)
ISCHEMIC	9.05[a]	3.98	1089.08	18.72[a]	19.22[a]	8.05[a]	28.43[a]	316.14	65.52[a]	389.57[a]	182.37[a]	3.19[a]	658.30	118.56[a]	1064.80[a]
	(0.75)	(1.30)	(237.80)	(4.02)	(12.44)	(2.82)	(3.50)	(20.94)	(17.01)	(9.11)	(27.49)	(1.41)	(61.04)	(11.46)	(282.46)
REPERFUSED	2.28[b]	9.49[a,b]	887.93[a]	8.05[a,b]	28.34[a,b]	53.30[a,b]	21.22[a]	328.40	111.59[b]	321.93	170.08[a]	5.28[b]	663.47	131.00[a]	1386.53[a]
	(0.48)	(0.89)	(52.69)	(1.44)	(0.74)	(17.77)	(2.66)	(15.37)	(4.44)	42.54)	(22.95)	(1.17)	(56.67)	(8.98)	(124.19)

Determination was performed by HPLC on 200 μL of the neutralized perchloric acid extracts of brain tissue.
Values represent the mean (SD) of seven different brain samples and are expressed as nmol/g w.w.
[a] Significantly different from controls ($p < .01$).
[b] Significantly different from ischemic ($p < .01$).

value was triplicated after 10 min of reperfusion. At the same time, plasma MDA increased both after ischemia and reperfusion with respect to preischemia. Since we used an HPLC assay that allows a reliable determination of MDA directly in perchloric acid tissue extracts, without any additional manipulation of the samples, it can be affirmed that our results give, for the first time (to the best of our knowledge), correct values of the brain concentrations of this compound in the various experimental conditions. The absence of cerebral MDA in our control animals, when directly assayed by HPLC, in comparison with the about 0.12 ± 0.18 μmol/g w.w. previously reported to be detectable in control animals when assayed by the TBA test,[26,29] strongly confirms the lack of specificity of this latter analytical method, therefore suggesting that the phenomenon of lipid peroxidation in postischemic tissues should be reevaluated. This is also supported by the concentration of plasma MDA from preischemic rats detected in our experiments, which corresponds to 0.050 μmol/L (i.e., ~200-fold lower than the value determined in another study in serum from control animals).[26]

It seems that, despite the short-term incomplete ischemia induced by carotid occlusion, molecular events leading to oxygen radical production occur to ischemic brain, which is therefore a highly sensitive tissue to peroxidative injury. In this respect, we may point out that the concentration of MDA detectable in isolated rat heart subjected to 30 min of global normothermic ischemia followed by 30 min of reperfusion is 0.118 μmol/g d.w.[22] (corresponding to about 12 nmol/g w.w.)—that is, about four times lower than the value recorded in reperfused brain (Table 1). This difference might be explained by the fourfold higher amount of total phospholipids in the brain with respect to the myocardium, which could render cerebral tissue more susceptible to peroxidation reaction chain. In this light, several studies have evidenced that the irreversible conversion of XDH into XO occurs in ischemic cerebral tissue, thus supporting the possibility that this enzymatic molecule is one of the major sources of oxygen free radicals in the brain.[30–32] If this mechanism were true, from the data reported in Table 1 and transforming the data in Table 2 into total nanomoles released/g w.w., assuming a total conversion of XDH into XO, the amount of O_2^- produced in the formation of Xan and uric acid from Hyp during 5 min of ischemia could be calculated as follows: 2 [(tissue uric $acid_{ischemia}$ − tissue uric $acid_{control}$) + (plasma uric $acid_{ischemia}$ − plasma uric $acid_{control}$)] + (tissue $Hyp_{ischemia}$ − tissue $Hyp_{control}$) + (plasma

Table 2. Concentrations of MDA, Oxypurines, and Nucleosides Determined by HPLC on 200 μL of Deproteinized Plasma Samples of Rats Subjected to Short-Term Cerebral Ischemia and Reperfusion

	MDA	Hyp	Xan	Uric acid	Ino	Ado
PREISCHEMIA	0.050	0.547	0.360	30.11	0.245	7.64
	(0.015)	(0.062)	(0.116)	(6.18)	(0.043)	(1.45)
ISCHEMIA	0.277[a]	17.95[a]	0.667[a]	40.47[a]	0.946[a]	9.24[a]
	(0.056)	(4.19)	(0.070)	(7.14)	(0.176)	(1.44)
REPERFUSION	0.682[a,b]	10.18[a,b]	2.12[a,b]	66.20[a,b]	2.89[a,b]	12.71[a,b]
	(0.094)	(2.86)	(0.43)	(9.38)	(0.51)	(2.29)

Values represent the mean (SD) of seven plasma samples and are expressed as μmol/L plasma.
[a] Significantly different from preischemia ($p < .001$).
[b] Significantly different from ischemia ($p < .001$).

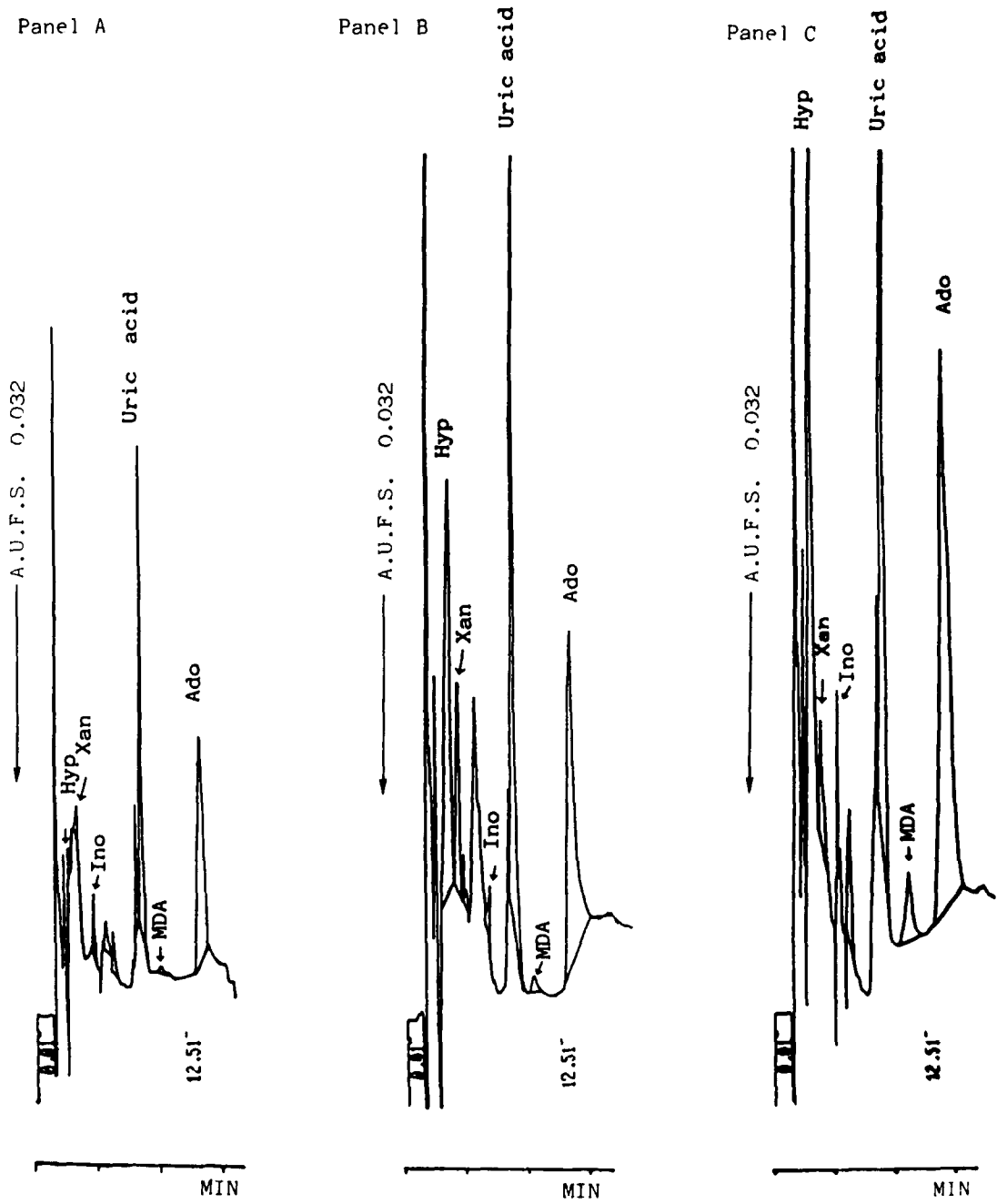

Fig. 3. Representative HPLC analysis of the simultaneous determination of MDA, oxypurines, and nucleosides of 200 μL of the $HClO_4$ extracts of plasma from peripheral venous blood of rats subjected to short-term incomplete cerebral ischemia and reperfusion. Chromatograms refer to plasma samples analyzed from the same rat before (Panel A), at the end of 5 min of incomplete cerebral ischemia (Panel B), and at the end of 10 min of reperfusion (Panel C).

$Hyp_{ischemia}$ − plasma $Hyp_{control}$) = 2 [(19.22 − 5.27) + (289.07 − 215.07)] + (3.98 − 2.56) + (4.76 − 2.57) = 179.51 nmol/g w.w. The same can be applied to data referring to Xan and uric acid determined after 15 min reperfusion, giving a value of 582.64 nmol/g w.w. of O_2^- produced. However, it has been reported that the conversion of XDH into XO, induced by 30 min of global cerebral ischemia in the rat, is of about 36%.[32] Since we did not measure the XDH transformed into XO, we can assume that the maximal amount of O_2^- generated in our experimental conditions should not exceed the 36% of the previously calculated values (i.e., 64.62 and 209.75 nmol/g w.w. during ischemia and reperfusion, respectively). In addition, the short-term incomplete ischemia adopted in our experiments could not have reproduced the

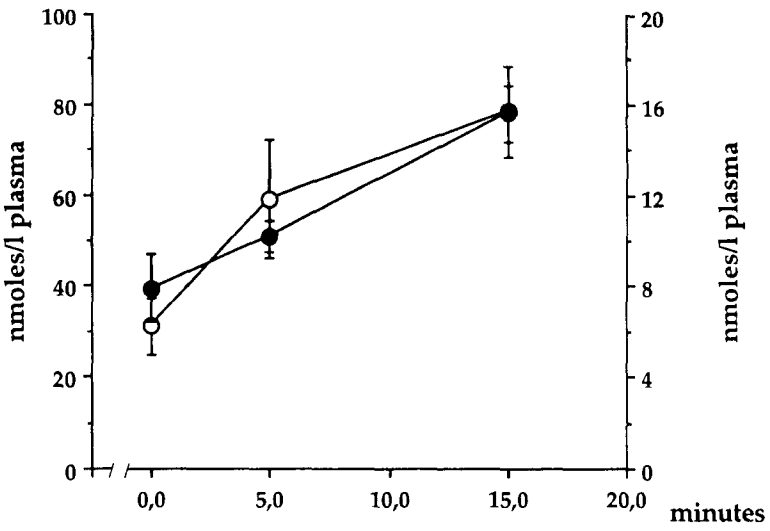

Fig. 4. Σ oxypurines (○) and Σ nucleosides (●) of plasma from peripheral blood of rats before incomplete cerebral ischemia, at the end of 5 min of carotid occlusion, and at the end of 10 min of reperfusion. Each point represents the mean of seven animals. Standard deviations are indicated by vertical bars.

same effects as 30 min of global ischemia. Therefore, the calculation of the O_2^- possibly produced in our experimental model by the brain tissue via the oxidation of Hyp and Xan to uric acid by XO is mainly indicative. However, the data reported in the present article certainly demonstrate the ability of the brain to produce such a relevant amount of Xan and uric acid to induce a dramatic increase of their tissue and plasma concentrations either at the end of ischemia or of reperfusion. This finding confirms previous reports[20,33] and indirectly demonstrates that an enzyme responsible for oxypurines production (Xan and uric acid) is activated by cerebral ischemia and reperfusion. However, independent of the mechanism of free radical production, the values of tissue and plasma MDA, especially those recorded after 10 min of reperfusion, clearly indicate the existence of a peroxidative damage occurring to membrane phospholipids as a consequence of short-term incomplete cerebral ischemia and reperfusion. The progressive decrease of tissue ascorbic acid supports the hypothesis of an increased oxidative stress that cannot be fully managed by the scavenging systems of the brain in order to avoid irreversible injuries to biomolecules.

Table 3. Change to Relative Contribution of Hyp, Xan, and Uric Acid to the Plasma Σ Oxypurines, and of Ino and Ado to the Plasma Σ Nucleosides, Released in the Bloodstream as a Function of Short-Term Cerebral Ischemia and Reperfusion in the Rat

	Relative Contribution (%) to the Σ Oxypurines			Relative Contribution (%) to the Σ Nucleosides	
	Hyp	Xan	Uric acid	Ino	Ado
Preischemia	1.76	1.16	97.08	3.11	96.89
Ischemia	30.38	1.13	68.49	9.28	90.72
Reperfusion	12.97	2.70	84.33	18.53	81.47

For the actual values of Σ oxypurine and Σ nucleosides, see Fig. 4.

Another consideration that can be drawn from these data is that, by utilizing MDA (assayed by proper analytical methods) as an early reliable biochemical marker of incomplete cerebral ischemia and reperfusion, a reconsideration on the sensitivity and spatial resolution of cerebral blood flow evaluation techniques (such as ^{14}C-butanol, I-antipirine, microspheres) should be necessary.[34-37] In fact, the reduction of cerebral blood flow obtained by occluding both carotid arteries, which is considered very far from the ischemic threshold, produced a marked increase of tissue and plasma MDA. Preliminary histopathologic studies (data not shown) also demonstrate that changes in tissue and plasma MDA could actually precede any visible neuronal damage. Furthermore, the relevant amount of both tissue and plasma MDA recorded after 5 min of incomplete cerebral ischemia indicates that the generation of oxygen radicals, able to promote the peroxidation of phospholipids and therefore the formation of MDA from their degradation, occurs during the reduction of oxygen availability. Although in contrast with a previous report,[38] this is in line with our recent finding that a significant concentration of MDA is detectable in the isolated perfused rat heart after 30 min of global normothermic ischemia.[22]

In conclusion, the present report suggests the exis-

tence of a close relationship between the changes of tissue MDA, oxypurines, and nucleosides and the reperfusion of ischemic brain. Moreover, the data referring to plasma variations of these metabolites offer an additional contribution to the goal of finding biochemical markers to be used as indexes for the evaluation of reperfusion damage occurring to postischemic tissues. Although potentially of great relevance, the prognostic value deserves further investigations.

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ABBREVIATIONS

Ado—adenosine
Hyp—hypoxanthine
Ino—inosine
MABP—mean arterial blood pressure
MDA—malondialdehyde
SD—standard deviation
TBA—thiobarbituric acid
Xan—xanthine
XDH—xanthine dehydrogenase
XO—xanthine oxidase