0031-3998/93/3304-0336$03.00/0
PEDIATRIC RESEARCH

Vol. 33, No. 4, 1993
Printed in U.S.A.

Prevention of Postasphyxial Increase in Lipid Peroxides and Retinal Function Deterioration in the Newborn Pig by Inhibition of Cyclooxygenase Activity and Free Radical Generation

SYLVAIN CHEMTOB, MARIE-SYLVIE ROY, DANIEL ABRAN, HENDRIKA FERNANDEZ, AND DAYA R. VARMA

Departments of Pediatrics, Pharmacology and Ophthalmology, Research Center of Hôpital Ste. Justine, and Department of Pharmacology and Experimental Therapeutics, McGill University, Montreal, Quebec, Canada

ABSTRACT. Free radicals have been implicated in the development of injury to the immature retina. Asphyxia increases free radicals as well as prostaglandins (PG) in neural tissues. We assessed whether in the retina the cyclooxygenase pathway contributes to free radical formation after oxidative insults such as asphyxia, which in turn disrupts retinal function. Newborn pigs were treated with either saline, ibuprofen (194 μmol/kg i.v.), or allopurinol (1 mmol/kg i.v.), and retinal malondialdehyde (MDA), hydroperoxides, PGE_2 and $PGF_{2\alpha}$ levels, and the amplitudes and implicit times of the a- and b-waves of the full-field electroretinogram were measured before and 1 h after a 5-min period of asphyxia. In saline-treated animals, asphyxia caused a marked increase ($p < 0.01$) in MDA, hydroperoxides, PGE_2, and $PGF_{2\alpha}$ concentrations in the retina. This was associated with a significant decrease ($p < 0.01$) in the b-wave amplitude measured under scotopic and photopic conditions and an increase in the b-wave implicit times. Ibuprofen and another cyclooxygenase inhibitor, indomethacin (28 μmol/kg i.v.), decreased PGE_2 and $PGF_{2\alpha}$ levels and prevented the increase in MDA and hydroperoxides after asphyxia. Allopurinol maintained low concentrations of MDA and hydroperoxides after asphyxia. Both ibuprofen and allopurinol prevented the postasphyxial changes in the b-wave amplitude and diminished the delay in implicit time observed after asphyxia in saline-treated pigs. Our findings suggest that in the retina after asphyxia free radicals appear to originate primarily from the cyclooxygenase pathway and contribute to the deterioration in retinal electrophysiologic function of the newborn animal. Cyclooxygenase inhibitors, like free radical scavengers, may protect retinal function from deteriorating after oxidative stresses. (*Pediatr Res* 33: 336–340, 1993)

Abbreviations

ERG, electroretinogram
MDA, malondialdehyde
PG, prostaglandin

Received August 24, 1992; accepted December 17, 1992.
Correspondence: Sylvain Chemtob, M.D., Ph.D., Research Center of Hôpital Ste. Justine, Departments of Pediatrics and Pharmacology, 3175 Côte Ste. Catherine, Montreal, Quebec, H3T-1C5 Canada.
Supported in part by grants from the Medical Research Council of Canada, the Fonds de la Recherche en Santé du Québec, and Biopedia Inc.

Free radicals and PG increase in the retina and other neural tissues after asphyxia (1, 2). However, in contrast to primary PG, which may even be cytoprotective (3, 4), free radicals have been clearly shown to cause tissue damage, including disruption of retinal cellular morphology (5, 6) and electrophysiologic function (7, 8).

Free radicals can arise from various metabolic sources including the cyclooxygenase pathway (9, 10). In some tissues after certain types of oxidative stresses, such as after an ischemic insult to the heart and brain, the cyclooxygenase pathway is a major source of free radicals (11–14).

There exists increasing evidence that lipid peroxides, formed from the oxidation of unsaturated fatty acids by superoxide and/or hydroxyl radicals, may be implicated in damage to the immature retina (15, 16). However, it is unknown whether in the retina the cyclooxygenase pathway contributes significantly to free radical formation after oxidative insults such as asphyxia and in turn causes disruption of retinal function. Because PG (1) and free radicals (2) increase in neural tissue after asphyxia, we speculated that the free radicals produced in the retina after asphyxia arise largely from the cyclooxygenase pathway and cause damage to this tissue. To test this hypothesis, newborn pigs were treated with the cyclooxygenase inhibitor ibuprofen and lipid peroxide levels and retinal electrophysiologic function, assessed before and after asphyxia, were compared with those of animals treated with the neuroprotective free radical scavenger allopurinol (14, 17).

MATERIALS AND METHODS

This study was approved by the Animal Care Committee of Hôpital Ste. Justine.

The newborn pig was selected as our model because its retina has characteristics similar to those of the human retina. The retina of the piglet contains both rods and cones (18), with the latter being centrally concentrated, it is holangiotic without a tapetum (19), and it has separate retinal and choroidal blood supplies (20).

Surgical Preparation. One- to 3-d-old pigs (1.4–2.0 kg) were anesthetized with halothane (0.5%). A femoral artery was catheterized with a polyethylene umbilical catheter (Argyle, 3½ French, Sherwood, St. Louis, MO) to measure blood pressure using a Statham pressure transducer (Gould Inc, Valley View, OH) connected to a multichannel recorder (TA240, Gould Inc) and to sample blood for measurement of pH, P_{O_2}, and P_{CO_2}. A small polyethylene catheter (Intramedic PE-50, Becton Dickinson & Co, Parsippany, NJ) was placed in the femoral vein for administration of drugs. Tracheostomy was performed and the animals were ventilated (small animal respirator, Harvard Apparatus Co., South Natick, MA) with air.

After the surgery, halothane was discontinued and the animals were injected i.v. with α-chloralose (161 μmol/kg followed by 32 μmol/kg/h; 50 mg/kg and 10 mg/kg/h, respectively) and paralyzed with pancuronium [0.14 μmol/kg (0.1 mg/kg), twice]. Animals were placed prone on a cloth sling and their body temperature was maintained at 38°C using a heating pad. The piglets were allowed to stabilize for approximately 1½ h before the experiments were started.

Experimental Protocol and ERG. Animals were randomly assigned to receive an i.v. injection of saline (1–2 mL/kg, $n = 7$), ibuprofen [194 μmol/kg (40 mg/kg), $n = 7$], or allopurinol [1 mmol/kg (140 mg/kg), $n = 6$] 45 min before the first ERG was recorded. The doses of drugs were selected because they have been previously shown to inhibit prostanoid synthesis and to reduce lipid peroxide levels in the CNS and retina or to exhibit neuroprotective properties (14, 17, 21). Although allopurinol is also a xanthine oxidase inhibitor at much lower doses, the doses used in this study also produce primarily free radical scavenging effects (17).

The pupils were maximally dilated with 1% cyclopentolate. ERG were recorded using corneal lens unipolar electrodes (ERG-Jet, Universo, La-Chaux-de-Fonds Switzerland) placed on each cornea humidified with hydroxypropylmethylcellulose. Reference and ground electrodes were placed above the eyelids and neck, respectively. The heads of the animals were positioned inside a Ganzfeld stimulator (LKB Technologies Inc., Gaithersburg, MD) of 44-cm diameter. The flash stimulus (Grass Instrument Co., Quincy, MA) was set at 3.65 cd·s/m^2.

ERG were obtained as we previously reported (22). The pigs were kept in a dark chamber for 30 min before a baseline scotopic (dark-adapted; combined rod/cone response) standardized full-field ERG was recorded. After 10 min of light adaptation at an illumination of 34.6 cd/m^2, the photopic (light-adapted; selective cone) responses were determined. The pigs were then asphyxiated by interrupting ventilation for exactly 5 min, using a model of apneic asphyxia previously described (1, 2). Thirty min after the asphyxial period, the animals were dark-adapted for another 30 min, after which scotopic ERG were recorded; 10 min after light adaptation, photopic responses were again determined. Thus, standardized protocol ERG were recorded before and 60 min after asphyxia; this latter time was selected in accordance with a deterioration in retinal hemodynamics that we observed in preliminary studies (23).

ERG signals were amplified using an Epic 2000 electrodiagnostic instrument (LKB Technologies Inc.) at a bandwidth of 0.3 to 500 Hz. To assure accuracy and reproducibility of the electrophysiologic signals, a minimum of five responses for each scotopic and photopic ERG were recorded and then stored on computer disk for subsequent analysis. For each averaged ERG, the amplitudes and implicit times of the a-wave (generated by the photoreceptors) and b-wave (generated largely by the Müller glial cells) were measured. The amplitude of the a-wave was calculated as the difference in voltage from baseline to the maximum negative deflection of the first portion of the ERG, and the amplitude of the b-wave was calculated from this negative deflection (of the a-wave) to the maximum positive deflection (see Fig. 1). The implicit times for both waves were calculated as the times from the flash onset to the peak of each wave.

The results are presented as the difference between preasphyxial and postasphyxial values for scotopic and photopic a- and b-wave amplitude and implicit time. Arterial blood gases were also measured at the same times and 10 s before the end of the asphyxial period.

Measurements of MDA, Hydroperoxides, $PGF_{2\alpha}$, and PGE_2. To measure in the retina the levels of the lipid peroxides, MDA and hydroperoxides, and of the major retinal PG, PGE_2 and $PGF_{2\alpha}$ (21), experiments were also performed on 21 separate animals (1.2–2.1 kg) treated as described above and killed [with pentobarbital 0.5 mmol/kg (120 mg/kg) i.v.] either before or 60 min after asphyxia ($n = 3$–4 per time period per treatment group). In addition, we tested whether the effects of ibuprofen on MDA levels could be reproduced by indomethacin, a structurally unrelated cyclooxygenase inhibitor. Seven pigs were treated with indomethacin [28 μmol/kg (10 mg/kg) i.v.], a dose that completely inhibits cyclooxygenase in the retina (21), and the animals were killed before or 60 min after asphyxia. Mean blood pressure, heart rate, and arterial blood gases and pH measured before, at the end of the period of asphyxia, and 1 h later were nearly identical to those of animals from which ERG were recorded (Table 1). Immediately after animals were killed, liquid N_2 was poured on each eye; the eyes were then removed and stored at −80°C until the assay was performed within 1 mo of storage; all products measured were stable over this time period.

The retinas were removed on ice and suspended in a cold buffer (pH 7.4) of the following composition: 5 mM Tris-HCl, 0.67 mM acetylsalicylic acid, 0.5 mM EGTA, and 100 μM butylated hydroxytoluene. The tissue was homogenized and centrifuged at 1000 × g for 10 min to remove undisrupted cells and nuclei. Protein (24), MDA, hydroperoxides, $PGF_{2\alpha}$, and PGE_2 were assayed on the supernatant. The protein content of each eye was not changed by the asphyxia (preasphyxia: 2.8 ± 0.2 mg; postasphyxia: 2.8 ± 0.1 mg).

MDA measurements. MDA was measured by the thiobarbituric acid reaction (25). Briefly, the samples were added to a 17 mM solution of thiobarbituric acid further acidified with acetic acid and heated to 90–100°C for 1 h. After cooling, 1-butanol was added and the samples were centrifuged at 1000 × g for 10 min. Absorbance of the upper phase was read at 532 nm (DU-64, Beckman spectrophotometer). Standard curves were obtained with malonaldehyde bisdimethyl acetal (26); the interassay variability was ≤2.5%.

Hydroperoxide measurements. Hydroperoxides were measured to obtain a desirable additional index of peroxidation. These were determined by oxidation of $FeCl_2$, 250 μM, under acidic conditions (H_2SO_4, pH = 2–3) in the presence of butylated hydroxytoluene, 4 mM, and xylenol orange, 100 μM, and the absorption was read at 560 nm (27). Standard curves were obtained with t-butyl-hydroperoxide; the interassay variability was <3.5%.

PG measurements. For determination of PG, the supernatant obtained after homogenization, as described above, was further centrifuged at 50 000 × g for 30 min at 4°C to remove membranes to enhance extraction of PG on octadecylsilyl silica columns (28). PG were extracted according to the method of Powell (28). The supernatant was dissolved in 15% ethanol and acidified to pH 3 with glacial acetic acid. The samples were applied to octadecylsilyl silica columns, which were then washed with 15% aqueous ethanol followed by petroleum ether, and the PG were subsequently eluted with methyl formate. The efficiency of recovery after extraction for all PG measured was >96%.

$PGF_{2\alpha}$ and PGE_2 were measured by RIA technique, as we have described (21). The interassay variability was <5%.

Drugs and Chemicals. Ibuprofen, indomethacin, allopurinol, acetylsalicylic acid, thiobarbituric acid, malonaldehyde bisdimethyl acetal, butylated hydroxytoluene, xylenol orange, and t-butyl-hydroperoxide were purchased from Sigma Chemical Co. (St. Louis, MO). RIA kits for $PGF_{2\alpha}$ and PGE_2 were obtained from Advanced Magnetics (Boston, MA). Ibuprofen and indomethacin were dissolved in NaCl 150 mM and NaOH 0.3 N titrated to pH 7.4 (21). Allopurinol was dissolved in NaCl 150 mM alkalinized with NaOH to pH 11 (14). All other chemicals were purchased from Fisher Laboratories (Montreal, Quebec, Canada).

Statistical Analysis. No significant differences in ERG parameters were found between the right and left eyes. Thus, for each pair of eyes, an average of the measures for each parameter was calculated.

Statistical analyses were performed by univariate analysis of variance for repeated measures and two-way analysis of variance,

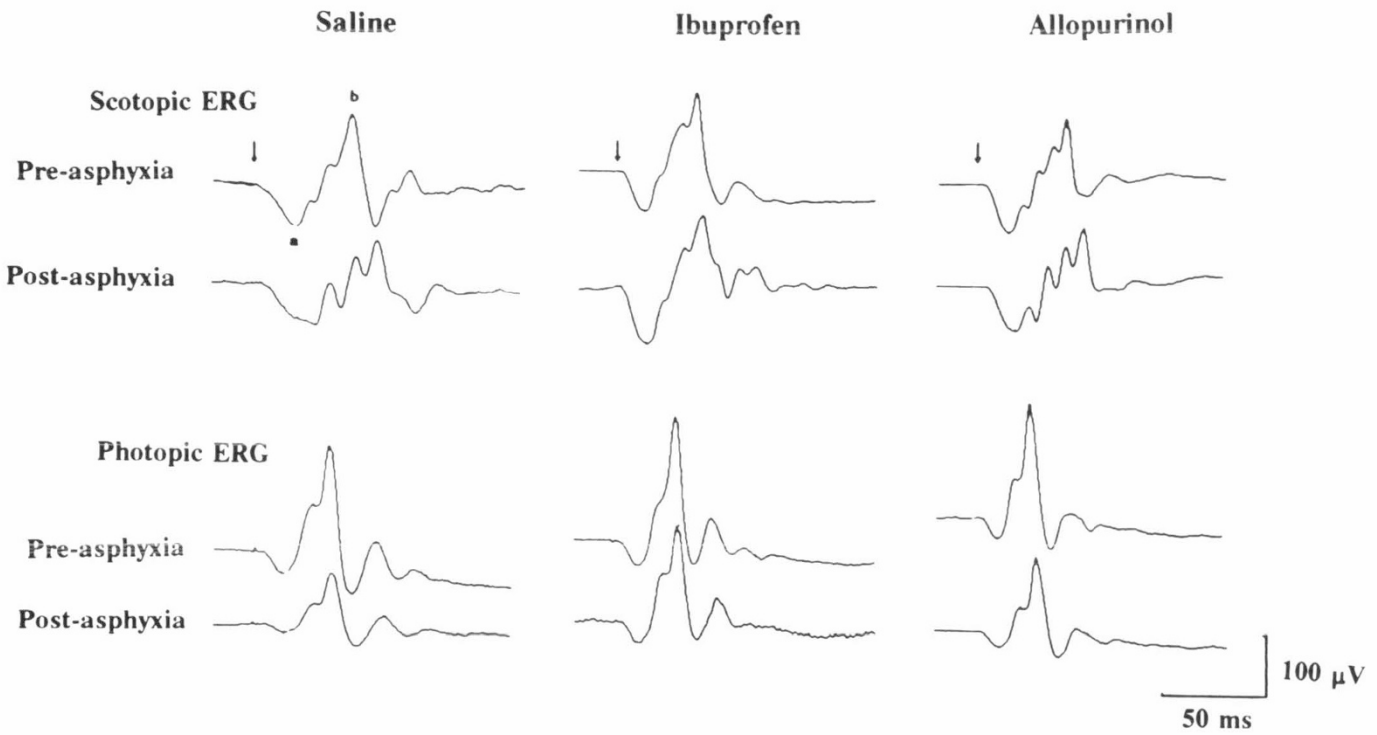

Fig. 1. Typical scotopic (dark-adapted; for combined rod/cone response) and photopic (light-adapted; for selective cone response) electroretinographic (*ERG*) tracings before and 1 h after a 5-min period of asphyxia after i.v. treatment with saline, ibuprofen (194 μmol/kg or 40 mg/kg), or allopurinol (1 mmol/kg or 140 mg/kg) in newborn pigs. The letters *a* and *b* (on the scotopic ERG after saline treatment) identify the a- and b-waves. The *arrows* refer to the flash stimuli.

Table 1. *Mean blood pressure, heart rate, blood gases, and pH before, during, and after 5-min period of asphyxia in saline-, ibuprofen-, and allopurinol-treated piglets**

	Preasphyxia	During asphyxia†	1 h Postasphyxia
Saline			
pH	7.363 ± 0.017	7.031 ± 0.033‡	7.393 ± 0.022
P_{CO_2} (kPa)	5.39 ± 0.26	11.17 ± 0.77‡	5.29 ± 0.44
P_{O_2} (kPa)	14.26 ± 0.72	1.79 ± 0.51‡	14.40 ± 0.86
MBP (mm Hg)	72 ± 4	55 ± 7‡	72 ± 4
HR (beats/min)	212 ± 12	90 ± 14‡	215 ± 16
Ibuprofen (194 μmol/kg)			
pH	7.390 ± 0.02	7.011 ± 0.026‡	7.379 ± 0.021
P_{CO_2} (kPa)	5.20 ± 0.53	11.57 ± 0.43‡	4.95 ± 0.32
P_{O_2} (kPa)	13.69 ± 0.34	1.27 ± 0.26‡	14.35 ± 0.61
MBP (mm Hg)	79 ± 3	60 ± 7‡	82 ± 4
HR (beats/min)	207 ± 15	79 ± 11‡	223 ± 11
Allopurinol (1 mmol/kg)			
pH	7.420 ± 0.03	6.913 ± 0.031‡	7.407 ± 0.036
P_{CO_2} (kPa)	5.50 ± 0.46	11.88 ± 0.61‡	5.72 ± 0.49
P_{O_2} (kPa)	12.64 ± 0.64	1.85 ± 0.49‡	12.21 ± 0.73
MBP (mm Hg)	76 ± 6	51 ± 11‡	78 ± 6
HR (beats/min)	228 ± 16	88 ± 8‡	230 ± 11

* Saline (n = 7), ibuprofen (n = 7), and allopurinol (n = 6) were injected 45 min before the first measurements. Values are mean ± SEM. MBP, mean blood pressure; HR, heart rate.

† Measurements were taken 10 s before the end of the 5-min asphyxia period.

‡ $p < 0.05$ compared with preasphyxia value.

factoring for group and time. In addition, for values that did not fit a normal distribution (photopic a-wave amplitude and implicit time for allopurinol-treated animals), nonparametric analysis was performed using the Mann-Whitney U-Test and the Wilcoxon two-sample test. Statistical significance was set at $p < 0.05$.

RESULTS

The blood pressure, heart rate, and arterial blood gases and pH before asphyxia, 10 s before the end of the asphyxial period, and 1 h after asphyxia are shown in Table 1. Asphyxia produced hypoxia, as well as a metabolic and respiratory acidosis associated with a decrease in blood pressure and heart rate, which resolved 1 h after reventilation.

ERG patterns. Neither ibuprofen nor allopurinol produced a significant change in the preasphyxial ERG (Fig. 1). In saline-treated animals, the amplitude of the a-wave measured under scotopic conditions decreased 1 h after asphyxia (Fig. 1, Table 2). Ibuprofen completely prevented this change in the a-wave amplitude and allopurinol attenuated it. However, the a-wave implicit time after dark adaptation and the amplitude and implicit time after light adaptation did not change after asphyxia, regardless of the treatment.

In saline-treated animals, the amplitude of the b-wave measured for the combined rod/cone response as well as for the the selective cone response significantly decreased after asphyxia ($p < 0.01$) (Fig. 1, Table 2). In addition, the b-wave implicit times under scotopic and photopic conditions increased after asphyxia. In contrast, ibuprofen and allopurinol prevented the postasphyxial changes in b-wave amplitudes and diminished the delays in implicit times; the amplitude of the combined rod/cone and the selective cone responses did not change (not different than 0) after asphyxia in ibuprofen- and allopurinol-treated animals (Fig. 1, Table 2). Ibuprofen and allopurinol also did not differ as to their effects on the a- and b-waves.

MDA, hydroperoxide, $PGF_{2\alpha}$, and PGE_2 measurements. In saline-treated animals, MDA, hydroperoxide, $PGF_{2\alpha}$, and PGE_2 concentrations in the retina increased markedly after asphyxia ($p < 0.01$) (Table 3). Ibuprofen and indomethacin significantly reduced the levels of $PGF_{2\alpha}$ and PGE_2 ($p < 0.01$) and did not

Table 2. *Difference between preasphyxial and postasphyxial ERG a- and b-wave amplitudes and implicit times in piglets treated with saline, ibuprofen, and allopurinol**

	Saline ($n = 7$)	Ibuprofen (194 μmol/kg, $n = 7$)	Allopurinol (1 mmol/kg, $n = 6$)
a-Wave			
Scotopic			
Amplitude (μV)	−25.3 ± 6.5†	−2.4 ± 2.6‡	−8.2 ± 3.3†‡
Implicit time (ms)	1.7 ± 0.8	1.4 ± 0.4	−0.1 ± 1.0
Photopic			
Amplitude (μV)	−6.4 ± 4.3	−3.3 ± 1.6	−3.6 ± 1.8
Implicit time (ms)	1.7 ± 0.8	1.4 ± 0.4	−0.1 ± 1.0
b-Wave			
Scotopic			
Amplitude (μV)	−51.4 ± 9.2†	−1.6 ± 6.9‡	−5.8 ± 12.5‡
Implicit time (ms)	6.8 ± 0.9†	4.3 ± 0.8†‡	3.4 ± 1.0†‡
Photopic			
Amplitude (μV)	−47.5 ± 10.5†	−10.2 ± 5.5‡	−24.8 ± 9.9‡
Implicit time (ms)	3.5 ± 0.8†	2.5 ± 0.7†	1.7 ± 0.7‡

* Values are mean ± SEM, calculated as the value of amplitide and implicit time 1 h after a 5-min asphyxial period minus that before asphyxia in the same animal.

† $p < 0.02$ compared with a value of 0.

‡ $p < 0.05$ compared with saline-treated animals.

Table 3. *Concentrations of MDA, hydroperoxides, $PGF_{2\alpha}$, and PGE_2 in retina before and 1 h after 5-min period of asphyxia in piglets treated with saline, ibuprofen, indomethacin, and allopurinol**

	Preasphyxia	1 h Postasphyxia
Saline		
MDA (nmol/mg protein)	12.4 ± 0.5	27.4 ± 0.2†
Hydroperoxides (nmol/mg protein)	2.9 ± 0.5	103.6 ± 10.5†
$PGF_{2\alpha}$ (pmol/g protein)	118.9 ± 10.5	215.3 ± 8.7†
PGE_2 (pmol/g protein)	27.2 ± 10.5	44.9 ± 11.6†
Ibuprofen (194 μmol/kg)		
MDA (nmol/mg protein)	13.8 ± 0.9	11.9 ± 0.8
Hydroperoxides (nmol/mg protein)	2.8 ± 0.4	4.9 ± 1.5
$PGF_{2\alpha}$ (pmol/g protein)	24.2 ± 3.1‡	22.8 ± 5.9
PGE_2 (pmol/g protein)	5.1 ± 1.7‡	6.2 ± 1.4
Indomethacin (28 μmol/kg)		
MDA (nmol/mg protein)	13.3 ± 1.6	11.7 ± 1.3
Hydroperoxides (nmol/mg protein)	3.1 ± 0.9	2.5 ± 0.8
$PGF_{2\alpha}$ (pmol/g protein)	11.0 ± 2.2‡	5.4 ± 1.7
PGE_2 (pmol/g protein)	6.8 ± 2.6‡	9.7 ± 2.7
Allopurinol (1 mmol/kg)		
MDA (nmol/mg protein)	9.1 ± 0.6‡	7.9 ± 0.8
Hydroperoxides (nmol/mg protein)	<2	4.6 ± 1.8
$PGF_{2\alpha}$ (pmol/g protein)	ND	ND
PGE_2 (pmol/g protein)	ND	ND

* Each value is a mean ± SEM of three to four experiments. ND, not determined.

† $p < 0.01$ compared with preasphyxia value.

‡ $p < 0.01$ compared with corresponding value after saline treatment.

change the preasphyxial concentrations of MDA and hydroperoxides (compared with saline, $p > 0.2$) but maintained the latter two at constant levels after asphyxia. Allopurinol decreased the levels of MDA and hydroperoxides and kept them stable after asphyxia.

DISCUSSION

Our findings suggest that, in the retina, lipid peroxides originate to a large extent from the cyclooxygenase pathway and contribute significantly to the deterioration in retinal electrophysiologic function of the newborn animal. Cyclooxygenase inhibitors, like free radical scavengers, blocked the increase in MDA and hydroperoxides and significantly attenuated the deterioration in retinal function occurring after asphyxia.

Lipid peroxidation involves the formation and propagation of lipid radicals after the generation of superoxide and/or hydroxyl radicals that abstract a hydrogen atom from an unsaturated fatty acid. Hydroperoxides are by-products and MDA is a further breakdown product of lipid peroxidation (25, 29). After asphyxia, there was an increase in the levels of MDA and a larger increase in the levels of hydroperoxides in the retina, and these changes were abolished by free radical scavenging doses of allopurinol (Table 3) (17); the sensitivity of hydroperoxide measurements is greater than that of MDA (27). Similar increases of other indices of lipid peroxidation, namely fluorescent compounds and conjugated dienes, have been observed in the brain after asphyxia (2).

Free radicals arise from several sources; these include the metabolism of xanthine to uric acid via xanthine oxidase, as well as the metabolism of arachidonic acid via the cyclooxygenase and lipoxygenase pathways (9, 10). In certain tissues, such as the heart, xanthine oxidase contributes significantly to free radical formation after an oxidative stress such as ischemia (30). However, in the CNS, of which the retina is an integral part, xanthine dehydrogenase does not appear to convert readily to its oxidase form (31). These latter observations concur with ours. We found that PG increased concomitantly with the propagation of lipid radicals after asphyxia (high levels of hydroperoxides, Table 3), and that two structurally unrelated cyclooxygenase inhibitors, ibuprofen and indomethacin, completely inhibited the postasphyxial increase in retinal MDA and hydroperoxides. These findings suggested that the cyclooxygenase pathway, but not the xanthine oxidase or the lipoxygenase pathways (32), was the principal source of free radicals in the retina after asphyxia in the newborn animal (Table 3). Finally, basal MDA and hydroperoxide levels were not altered by ibuprofen and indomethacin; it is therefore unlikely these agents exerted a direct effect on lipid peroxide formation by chelating iron or scavenging free radicals to block lipid peroxide production (33).

The increases in retinal MDA and hydroperoxides observed after asphyxia were associated with a decrease in the b-wave amplitude and an increase in the implicit time of the ERG of saline-treated animals (Fig. 1, Table 2); the a-wave was generally minimally affected (7, 8), as previously reported. Ibuprofen, like allopurinol, which blocked the postasphyxial increases in lipid peroxides (Table 3), prevented the changes in the ERG that were observed after asphyxia in untreated animals (Fig. 1, Table 2). These findings further support the notion that in the retina the cyclooxygenase pathway contributes to free radical formation and functional deterioration after asphyxia.

Free radical scavengers and cyclooxygenase inhibitors have been shown to reduce neuronal damage and electrophysiologic function and to improve outcome after various types of oxidative stresses, such as asphyxia, ischemia, and hypoxia (13, 14, 34–38). These findings in the brain are consistent with ours in the retina, which reveal that a cyclooxygenase inhibitor, ibuprofen, and a free radical scavenger, allopurinol (17), similarly attenuated the retinal electrophysiologic damage produced by asphyxia (Fig. 1, Table 2).

In the present study, we could not clearly identify whether the effects of asphyxia were caused by PG or by free radicals because both increased after asphyxia (Table 3). Nonetheless, certain inferences can be made. Free radicals have been shown to exert adverse effects on the retina, including the ERG pattern (7, 8). Similarly, doses of PG nearly 10 000-fold higher than those

normally present, applied directly to the vitreous in proximity to the retina have also been shown to decrease the b-wave amplitude (39); however, the physiologic significance of such excessive doses is unclear. Indeed, primary PG administered at more physiologic concentrations have not been shown to cause direct tissue damage; on the contrary, PGE_2 and PGI_2 may be cytoprotective (3, 4). It would thus appear that the free radicals, rather than the PG, are major factors in the deterioration of the ERG pattern after asphyxia.

Asphyxia, which commonly occurs in sick preterm newborns because of respiratory difficulties, can potentially cause an increase in free radicals (Table 3), which have been implicated in the development of injury to the immature retina (15, 16). In other neural tissues, cyclooxygenase inhibitors improve clinical and electrophysiologic recovery after oxidative insults (37, 38). Some of these drugs such as ibuprofen, which markedly enhances the regulation of oxygen delivery to the retina (40), may ultimately become clinically useful to help protect retinal function from deteriorating after oxidative stresses. However, this speculation cannot apply to all cyclooxygenase inhibitors; for instance, indomethacin impairs retinal hemodynamics (21) and has even been associated, although not conclusively, with the development of retinopathy of prematurity (41).

In conclusion, the cyclooxygenase inhibitor ibuprofen, like the neuroprotective free radical scavenger allopurinol (14, 17), significantly attenuated the deterioration in the response of rods and cones to light after asphyxia and blocked the increase in retinal lipid peroxides. Our findings suggest that in the retina after asphyxia free radicals seem to originate primarily from the cyclooxygenase pathway and contribute to the deterioration in retinal function of the newborn animal. Long-term effects of specific cyclooxygenase inhibitors, such as ibuprofen, in reducing oxidative damage to the immature retina are currently under investigation.

Acknowledgment. The authors thank F. Ménard Inc. (Ange-Gardien, De Rouville, Quebec) for its generosity in supplying the newborn pigs.

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