1044-7393/94/2301—0013 $06 80

Lipid Alterations Following Impact Spinal Cord Injury in the Rat

ERIC J. MURPHY,[1,5] DONALD BEHRMANN,[2] CARLTON M. BATES,[3] AND LLOYD A. HORROCKS*,[1]

Departments of [1]Medical Biochemistry, [2]Surgery, and [3]Pediatrics, and the [4]Neuroscience Program, The Ohio State University, 1645 Neil Ave., Columbus, OH 43210; and [5]Current Address: Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX

Received September 27, 1993; Accepted July 7, 1994

ABSTRACT

A computer-controlled impactor was used to produce a severe spinal cord injury in the rat thoracic spinal cord. Cords were rapidly frozen *in situ* at 5, 15, 30, and 60 min and 6, 12, and 24 h postinjury. Control cords were noninjured cords from animals having undergone a laminectomy and allowed to recover for 90 min postlaminectomy. The cords were assayed for alterations in lipid metabolism. Specifically, there were rapid increases in prostaglandin $F_{2\alpha}$ and thromboxane, with a peak increase in thromboxane levels at 30 min. Prostaglandin $F_{2\alpha}$ levels peaked at 15 min with levels remaining nearly constant for 12 h. There were no detectable changes in phospholipid levels, although diacylglycerol levels and free fatty acid levels were increased. Total free fatty acids were increased at 12 and 24 h postinjury by 2.3- and 3.2-fold over control levels, respectively. Arachidonic acid levels were not significantly elevated at early time points, however, these early time points correspond to elevated eicosanoid synthesis and this may account for the lack of early detectable increases in arachidonic acid. After 6 h postinjury, arachidonic acid levels were 20-fold greater than control levels and remained elevated at 24 h. There were minimal

*Author to whom all correspondence and reprint requests should be addressed.

decreases in cholesterol and no decrease in either choline or ethanolamine plasmalogen levels. These results suggest a rapid turnover of arachidonic acid following spinal cord injury with a concomitant increase in vasoconstrictive eicosanoid synthesis. The lack of changes in major membrane constituents suggests the mechanisms may not involve general membrane degradation, but an over-stimulation of phospholipase A_2-linked membrane receptors.

Index Entries: Spinal cord injury; prostaglandins; thromboxane; diacylglycerols; free fatty acids; arachidonic acid; cholesterol; phospholipids; plasmalogens; choline plasmalogen; ethanolamine plasmalogen.

INTRODUCTION

The initial sites of injury following spinal cord injury appear to be the cellular and subcellular membranes (Saunders and Horrocks, 1987). A severe compression injury of the spinal cord in cats results in a rapid increase in free fatty acid, diacylglycerol, and eicosanoid levels (Demediuk et al., 1985a,b). However, these increases in polyunsaturated fatty acid levels are rapidly reduced beginning within 30 min postinjury (Demediuk et al., 1985a,b). Surprisingly, there are no decreases in phospholipid levels except for ethanolamine plasmalogen (Demediuk et al., 1985a,b), although using a rat weight-drop model, a reduction in phospholipid content occurs 24 h postinjury accompanied by elevated fatty acid levels (Faden et al., 1987).

Following spinal cord injury, there is a rapid increase in eicosanoid production. Levels of thromboxane B_2, a metabolite of thromboxane A_2 that is a potent vasoconstrictor and platelet aggregant, increase during spinal cord injury (Demediuk et al., 1985a,b, 1988; Hsu et al., 1985, 1986). Levels of prostacyclin, a vasodilator and platelet antiaggregant, do not increase during spinal cord injury (Demediuk et al., 1985a,b, 1988; Hsu et al., 1985, 1986). However, levels of other vasoconstrictive prostaglandins increase, including PGE_2 and $PGF_{2\alpha}$ (Demediuk et al., 1985a,b). Peptidoleukotrienes are increased in the cat (Saunders et al., 1987) but not in the rat (Demediuk et al., 1988) following spinal cord injury.

Previous studies on altered lipid metabolism following spinal cord injury have focused on severe injuries produced using weight-drop methods and compression methods. In this study a reproducible computer controlled impact model was used to determine whether lipid metabolism, including increases in vasoconstrictive eicosanoids, is altered in rat spinal cord following a severe impact injury. Times postinjury varied from 5 min to 24 h in order to determine the involvement of early alterations in lipid metabolism during spinal cord injury compared to late changes that may reflect tissue necrosis.

MATERIALS AND METHODS

Materials

Sprague-Dawley rats (albino females, 3–6 mo of age) were purchased from Harlan Laboratories (Indianapolis, IN). All solvents were reagent grade or better. For high-performance liquid chromatography (HPLC), all solvents were purchased from E. M. Science (Cherry Hill, NJ) and were HPLC grade. Unisil activated silicic acid was purchased from Clarkson Chemical Co. (Williamsport, PA). All enzyme immunoassy kits were purchased from Cayman Chemical (Ann Arbor, MI).

Injury

All rats (n = 40) underwent a T-8 laminectomy after administration of ketamine (80 mg/kg) and xylazine (10 mg/kg). Additional doses of anesthetic (one-half of the original dose) were given immediately prior to injuring the animals and harvesting their spinal cords. The rats were allowed to recover 90 min after laminectomy to permit adequate recovery of any membrane perturbations caused by the surgery (Demekiuk et al., 1985a,b). The spinal cords of the control animals (n = 5) were then immediately removed. The remaining 35 rats underwent spinal cord contusion using The Ohio State University injury device. The injury chosen for this study (mean ± SD; spinal cord displacement = 1.18 ± 0.01 mm; force = 360.8 ± 21.4 kdyn) has previously been shown to result in a central hemorrhagic lesion of the cord and animals with an incomplete recovery of neurologic function (Stokes et al., 1992; Behrmann et al., 1992). The spinal cords were frozen *in situ* with liquid nitrogen and harvested at the following times after the injury (n = 5 per group): 5 min; 15 min; 30 min; 60 min; 6 h; 12 h; and 24 h. Three spinal segments, inclusive of the injured segment, were wrapped in foil and stored at −80°C to minimize autolysis of the tissue. Prior to extraction of the lipids, the wet weight of the cords was measured and recorded.

Lipid Extraction

Total spinal cord lipids were extracted using n-hexane:2-propanol 3:2 (v/v) containing butylated hydroxytoluene 0.01% (w/v) (Hara and Radin, 1978; Demediuk et al., 1985a,b). The amount of solvent used was 18 mL/g of tissue. Samples were kept in solvent and homogenized at 4°C. The homogenate was transferred to screwtop test tubes and the protein pelleted by centrifugation in an International Equipment Company (Needham Heights, MA) HN-S table top centrifuge. The supernatants containing the total lipid extracts were saved and stored under nitrogen at −80°C.

Column Chromatography

The total lipid fractions were separated on a 1 g Unisil silicic acid column. The neutral lipid fraction was eluted with 100 mL chloroform, eicosanoids eluted with 100 mL of methyl formate, glycolipids eluted with 100 mL of acetone and phospholipids eluted with 100 mL of methanol (Saunders and Horrocks, 1984). The neutral lipid, eicosanoid, and phospholipid fractions were retained for analysis. Volumes were reduced by rotoevaporation and the samples dissolved in *n*-hexane:2-propanol (3:2 v/v) containing 0.01% butylated hydroxytoluene. The neutral lipid and phospholipid fractions were filtered prior to HPLC through a 0.2 μm nylon filter (Rainin, Woburn, MA) to remove any silicic acid particles.

Phospholipid Analysis

The phospholipids were separated on a Dupont Zorbax silica column (Wilmington, DE) using a gradient of *n*-hexane:2-propanol (3:2 v/v) and *n*-hexane:2-propanol (3:2 v/v) containing 5.5% water by volume (Dugan et al., 1986). Column temperature was maintained at 34°C using a Jones Chromatography column heater (Littleton, CO). The flowrate was 1.5 mL/min with initial solvent proportions of 55 and 45%. This separation resolves all major phospholipid classes, including lysophosphatidylcholine and lysophosphatidylethanolamine. The eluant was monitored using ultraviolet light absorbance of 205 nm. Phospholipid classes were collected and quantitated by assay of lipid phosphorus (Rouser et al., 1969). The HPLC system consisted of a Beckman 420/421 controller (Fullerton, CA), two Altex (Berkeley, CA) 100A pumps, a Beckman 210 injector, and an ISCO V_4 uv/vis detector (Lincolon, NE).

Neutral Lipid Analysis

The neutral lipid fraction was separated on a Dupont Zorbax silica column (4.6 mm × 250 mm, 5 μm) using an isocratic system using two solvents: *n*-hexane with 1.2% 2-propanol containing 0.1% acetic acid and hexane at proportions of 90 and 10%, respectively. The column temperature was maintained at 55°C using a Jones Chromatography column heater. The flow rate was 0.6 mL/min. Analog data were collected with a Nelson Analytical (Cupertino, CA) 760 series intelligent interface and converted to digital data. Nelson 2600 software was used to calculate peak areas. Cholesterol levels were monitored using an ISCO V_4 detector using UV absorbance at 205 nm and α-tocopherol levels were monitored using a Shimadzu RS-535 fluorescence detector with excitation at 295 nm and emission at 340 nm. The free fatty acid and diacylglycerol fractions were collected and analyzed by gas liquid chromatography. The HPLC system consisted of a Beckman 420/421 controller, a Beckman 210 injector, two Beckman 114 M pumps, an ISCO V_4 uv/vis detector, and a Shimadzu RS-535 fluorescence detector.

Fatty Acid and Diacylglycerol Analysis

The free fatty acid fractions were converted to methyl esters by heating at 65°C for 4 h in methanol:toluene 1:1 containing 2% sulfuric acid (Åkesson et al., 1970). The diacylglycerols were quantitated by converting the esterified fatty acids to methyl esters and injected onto a Shimadzu GLC 14-A gas chromatograph. The diacylglycerol fraction was incubated in 1*M* potassium hydroxide in methanol at 37°C for 10 min (Brockerhoff, 1975). Both reactions are quantitative.

The samples were quantitated using a Shimadzu GLC-14A equipped with two Supelco SP-2330 capillary columns (0.32 mm id × 30 m long, Bellefonte PA). Column linearity ($r^2 = 0.985$ or better) and retention times were determined using fatty acid methyl ester standards from NuChek Prep (Elysian, MN). Peak area data were collected using a Nelson 760 series intelligent interface and area calculated using Nelson Analytical 2600 software.

Eicosanoid Analysis

Samples were evaporated and 1 mL of enzyme immunoassay buffer added. Samples were run in duplicate using 50 μL of sample per well per assay.

Statistics

Statistical significance was determined using Crunch® and Instat II® computer programs. One-way analysis of variance was used with the Newman-Kuels post-test, $p \leq 0.05$.

RESULTS

Phospholipids

There were no significant decreases in phospholipid levels following spinal cord injury (Table 1). There was a trend toward decreased ethanolamine glycerophospholipids, choline glycerophospholipids, and phosphatidylserine. These trends did not reach statistical significance.

Ethanolamine and choline plasmalogen levels were also unaffected by spinal cord injury although there was a trend toward decreased levels of ethanolamine plasmalogen (Table 2).

Neutral Lipids

Diacylglycerol levels were statistically elevated at 6 h compared to control, 5, 15, 30, and 60 min, $p \leq 0.05$ (Table 3). Levels at 12 and 24 h were elevated but were not statistically significant.

Table 1
Effect of Spinal Cord Injury on Phospholipid Levels

	Time after injury							
Phospholipid	Control	5 min	15 min	30 min	1 h	6 h	12 h	24 h
EtnGpl	40.4 ± 2.5	39.5 ± 2.9	42.7 ± 0.6	38.2 ± 1.0	37.5 ± 3.5	35.1 ± 2.2	33.8 ± 3.0	33.4 ± 1.5
Ptd_2Gro	0.1 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
PtdIns	2.0 ± 0.1	2.3 ± 0.3	1.9 ± 0.3	2.3 ± 0.2	1.9 ± 0.4	1.8 ± 0.2	1.9 ± 0.3	2.1 ± 0.3
lysoEtnGpl	2.3 ± 0.3	1.9 ± 0.3	4.1 ± 2.1	2.6 ± 0.2	2.5 ± 0.5	2.7 ± 0.5	1.9 ± 0.4	2.5 ± 0.4
PtdSer	8.1 ± 0.9	8.7 ± 0.7	8.1 ± 1.1	8.0 ± 0.7	8.4 ± 0.8	6.3 ± 0.5	6.2 ± 0.6	6.2 ± 0.5
ChoGpl	21.5 ± 1.0	21.7 ± 1.2	24.4 ± 0.5	21.4 ± 1.0	21.8 ± 1.8	19.3 ± 2.0	19.3 ± 1.4	18.4 ± 0.9
CerPCho	9.3 ± 0.5	9.5 ± 0.6	9.1 ± 0.4	8.4 ± 0.4	9.1 ± 1.1	7.8 ± 0.7	7.2 ± 0.6	7.8 ± 0.5
PtdIns-4*P*	0.7 ± 0.5	0.7 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.2
lysoChoGpl	0.4 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	0.4 ± 0.2	2.1 ± 1.1

[a] Values are expressed as μmol/g ww and represent means ± SEM, $n = 5$ except for 15 min, $n = 4$. There were no statistically significant differences between the values for any time points at $p < 0.05$.

Table 2
Plasmalogen Levels
Following Spinal Cord Injury[a]

	Ethanolamine	Choline
con	29.8 ± 2.1	5.5 ± 0.5
5 min	31.3 ± 2.6	5.9 ± 0.5
15 min	30.9 ± 0.6	7.4 ± 1.1
30 min	28.3 ± 0.9	5.0 ± 0.6
1 h	28.7 ± 2.9	4.8 ± 0.5
6 h	26.3 ± 1.4	4.3 ± 0.8
12 h	25.2 ± 2.5	5.7 ± 0.7
24 h	23.4 ± 1.8	5.2 ± 0.4

[a] Values are expressed as μmol/g ww and represent means ± SEM, $n = 5$ except for 15 min where $n = 4$. There were no statistical differences in choline or ethanolamine plasmalogen levels with increasing time postinjury.

Table 3
Diacylglycerol Levels
Following Spinal Cord Injury[a]

	Diacylglycerol
con	12.4 ± 1.4
5 min	16.0 ± 4.6
15 min	14.6 ± 1.0
30 min	11.3 ± 0.7
1 h	19.4 ± 2.8
6 h	42.0 ± 13.6[b]
12 h	30.8 ± 9.9
24 h	30.9 ± 6.4

[a] Values are expressed as nmol/g ww and represent means ± SEM, $n \geq 3$.

[b] Indicates that the value for 6 h > the value for control, 5, 15, 30, and 60 min, $p < 0.05$. There were no other significant differences.

Cholesterol levels tended to decrease with increasing time following injury (Table 4). Only levels at 24 h were statistically lower than levels of earlier time points, however, levels at 12 h were lower but not statistically significant.

Total free fatty acid levels, the combined saturated, monounsaturated and polyunsaturated fatty acid levels, were increased only at 12 and 24 h postinjury (Fig. 1). Total polyunsaturated fatty acid levels were increased

Table 4
Cholesterol and α-Tocopherol
Levels Following Spinal Cord Injury[a]

	Cholesterol, mg/g wet wt	α-tocopherol, μg/g wet wt
con	42.9 ± 1.6	9.7 ± 2.3
5 min	45.5 ± 0.4	6.9 ± 0.8
15 min	40.9 ± 1.3	5.3 ± 0.5
30 min	39.3 ± 1.3	6.1 ± 0.7
1 h	46.1 ± 3.2	8.3 ± 2.7
6 h	41.1 ± 2.5	5.2 ± 1.3
12 h	37.4 ± 3.2	4.7 ± 0.7
24 h	37.0 ± 1.3[b]	6.0 ± 0.6

[a]Values are expressed as mg/g ww for cholesterol and μg/g ww for α-tocopherol and are means ± SEM, $n \geq 3$.

[b]Indicates value at 24 h < 5 min and 1 h, $p < 0.05$.

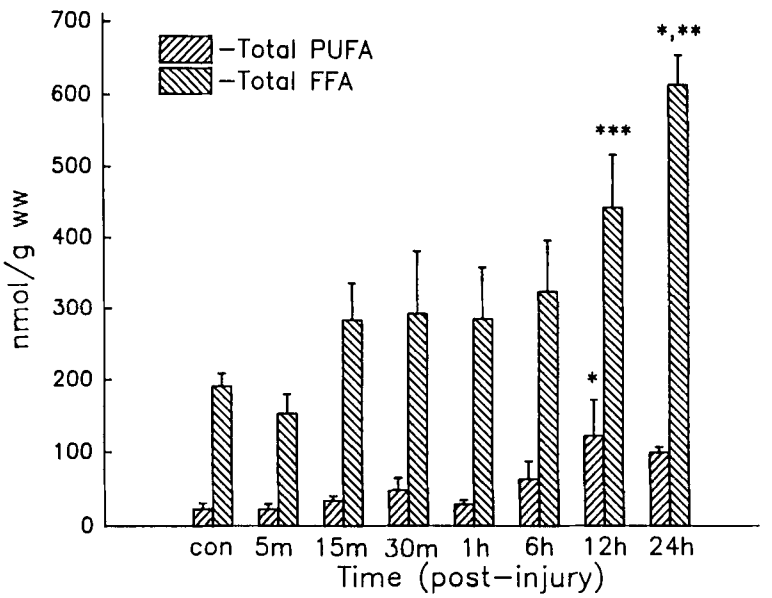

Fig. 1. Total free fatty acid and polyunsaturated fatty acid content in rat spinal cord following spinal cord injury. Values are expressed as nmol/g ww and are means ± SEM, $n \geq 4$. For total free fatty acids, the * indicates that 24 h > control and 5 min, $p < 0.01$. The ** indicates that 24 h > 15, 30, 60 min, and 6 h, $p \leq 0.05$. The *** indicates that 12 h > 5 min, $p \leq 0.05$ and is marginally significant compared to control values, $p \leq 0.10$. For total polyunsaturated fatty acids, the * indicates 12 h > control, 5, 15, and 60 min, $p \leq 0.05$.

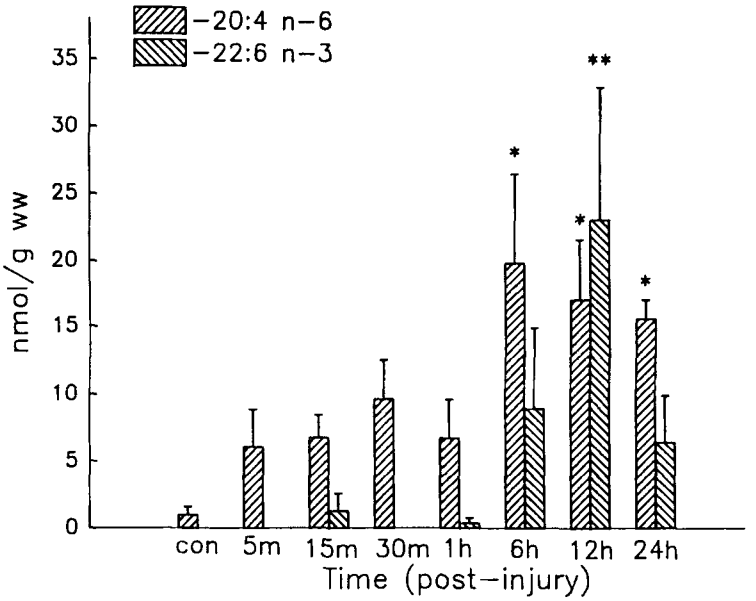

Fig. 2. Arachidonic acid (20:4 n-6) and docosahexaenoic fatty acid (22:6 n-3) values are expressed as nmol/g ww and are means ± SEM, $n \geq 4$. For 20:4 n-6, * indicates values are significant from control values, $p < 0.05$. Levels at 6 h are marginally significant from 5, 15 min, and 1 h, $p \leq 0.10$. For 22:6 n-3, the ** indicates values are significant from control values, $p \leq 0.05$.

at 12 h, with elevated levels at 24 h that did not reach statistical significance (Fig. 1). Arachidonic acid levels (20:4 n-6) were increased at 6, 12, and 24 h postinjury (Fig. 2). Docosahexaenoic acid levels were increased at 12 h, with increased levels at 6 and 24 h that were not statistically significant as a result of a large standard deviation (Fig. 2).

Eicosanoids

Thromboxane B_2 levels were dramatically increased during the first few minutes following spinal cord injury (Fig. 3). Levels at 5, 15, 30, and 60 min postinjury were all significantly greater than control levels. Thromboxane levels were 5.6- and 11.9-fold greater than control values at 5 and 30 min postinjury, respectively. By 1 h postinjury, levels were significantly decreased, and after 6 h concentrations returned to control values.

Levels of $PGF_{2\alpha}$, another potent vasoconstrictor, were also increased soon after onset of injury (Fig. 3). Unlike thromboxane, levels of $PGF_{2\alpha}$ remained elevated up to 12 h postinjury. Levels were 2.1- and 2.3-fold greater than control levels after 15 min and 12 h postinjury, respectively. After 24 h, $PGF_{2\alpha}$ concentrations returned to control levels.

The stable metabolite of prostacyclin, 6-keto $PGF_{1\alpha}$, was also assayed (Fig. 3). There were no large increases in prostacyclin levels following injury, although levels at 6 h were 1.8-fold greater than control levels. This increase in prostacyclin was transitory, and by 12 h, levels were back to control values.

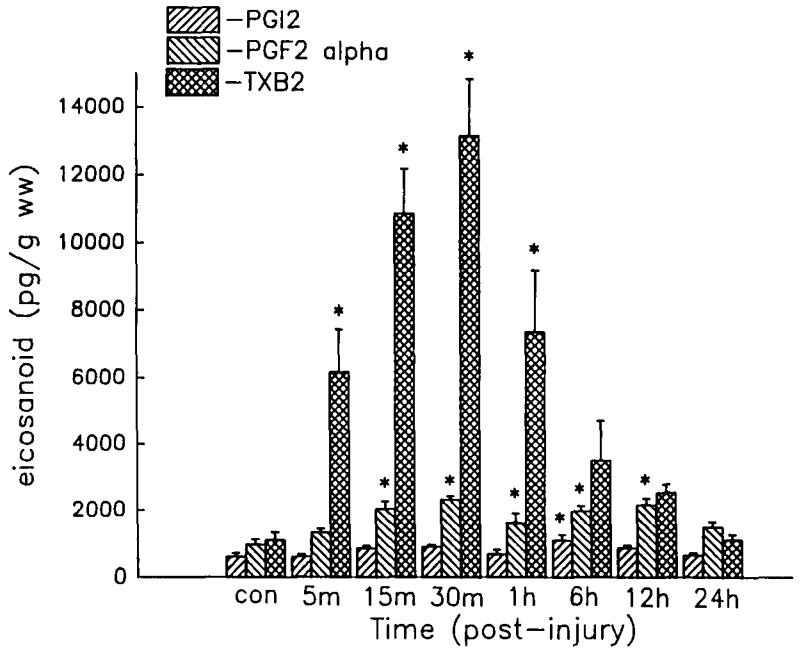

Fig. 3. Eicoanoids are expressed as pg/g ww and are means ± SEM, $n \geq 4$. For prostacyclin, levels at 6 h were statistically significant from control, 5 min, 1 h, and 24 h, $p \leq 0.05$. For $PGF_{2\alpha}$ levels at 15 min, 30 min, 6 h, and 12 h > control, $p \leq 0.05$ and 1 h > control, $p \leq 0.10$. Also, levels at 30 min > 5 min and 24 h, $p \leq 0.05$ and significantly greater than 1 h, $p \leq 0.10$. Levels at 12 h > 5 min, $p \leq 0.05$ and 15 min > 5 min, $p \leq 0.10$. Thromboxane levels at 5, 15, 30, and 60 min > control, $p \leq 0.05$. Levels at 5 min and 1 h > 12 and 24 h, $p \leq 0.05$; at 15 and 30 min > 1, 6, 12, and 24 h, $p \leq 0.05$; and at 30 min > 1, h, $p < 0.05$.

Levels of leukotriene B_4 and leukotriene C_4 were not significantly increased following spinal cord injury in the rat (data not shown).

DISCUSSION

We, like others, found that spinal cord injury resulted in a rapid increase in eicosanoid formation (Saunders et al., 1987). Unlike previous studies with cats (Demediuk et al., 1985a,b), the rats did not have an early increase in free fatty acid levels, with significant increases occurring only 6 h post-injury. Furthermore, there were no significant increases in diacylglycerol levels following injury, except at 6 h. These results are radically different from those with the cat, where both diacylglycerol and free fatty acid levels increased rapidly following compression injury and then decreased with increasing recovery time (Demediuk et al., 1985a,b).

The rapid increase in eicosanoid levels, specifically vasoconstrictive eicosanoids, suggests the rapid liberation of arachidonic acid. In our severe injury model, TXB_2 was maximally elevated at 30 min and did not return to control levels until 6 h after injury. These results agree with the reported peak of thromboxane formation in rats at 15–20 min postinjury (Hsu et al., 1986). Others have shown levels to be statistically elevated up to 7 d postinjury using a rat impact model (Demediuk et al., 1988). The magnitude of these increases in eicosanoid synthesis appears to be closely related to the severity of injury (Demediuk et al., 1988; Hsu et al., 1986). Our results also showed that $PGF_{2\alpha}$ levels remain elevated up to 12 h postinjury. These increases, combined with elevated thromboxane levels, undoubtedly contribute to the events causing the decrease in spinal cord blood flow associated with spinal cord injury (Banik et al., 1987). No increases in peptidoleukotrienes were shown to occur in the rat following spinal cord injury (Demediuk et al., 1988). Our results also showed no increases in leukotriene following injury in the rats (data not shown).

Cholesterol content was not decreased in the rat, but in the cat there was a rapid loss of this membrane constituent (Demediuk et al., 1985a,b). Because these decreases in cholesterol were preventable by pretreating the animals with α-tocopherol or methylprednisolone sodium succinate, the involvement of a free radical mechanism has been proposed (Anderson et al., 1985). Marginal, but not statistically significant, decreases in α-tocopherol are found following impact injury in the rat (Faden et al., 1987). We found no decreases in α-tocopherol using fluorescent detection of the HPLC eluent, even though this method has a wide detectable range (Table 4). Further, ethanolamine plasmalogen levels were not decreased in the rat although levels were decreased in the cat (Demediuk et al., 1985a,b). Ethanolamine plasmalogen has been postulated to act as an endogenous antioxidant, absorbing free radicals at the vinyl bond (Zoeller et al., 1988; Morand et al., 1988). These results, combined with the lack of decrease in cholesterol at early time points, suggest that a lipid peroxidative mechanism does not occur in the rat following impact injury.

The very rapid increase in arachidonic acid metabolites indicates a rapid release of arachidonic acid that is specifically in a pool for eicosanoid synthesis. This pool is rapidly metabolized into eicosanoids because after 5 min postinjury, no statistically significant increases in 20:4 n-6 levels occur, whereas TXB_2 levels increase above control levels. These results are consistent with two possible hypotheses: mechanical damage that elicits a response at the level of the membrane, or a receptor-mediated increase in phospholipase activity.

The first hypothesis has been supported by previous work with the cat (Demediuk et al., 1985a,b). Early indications of arachidonic acid liberation are seen in the rat (Demediuk et al., 1988; Hsu et al., 1986), but no evidence for the dramatic changes in membrane lipid metabolism is seen using rat injury models (Faden et al., 1987). The results with the rat are consistent with a receptor-mediated increase in arachidonic acid,

independent of general membrane breakdown. Our results are consistent with this hypothesis.

Glutamate levels increase rapidly during spinal cord injury. Following moderate injury in rabbits, glutamate and aspartate levels both increase over 2.5-fold and return to control levels 40 min postinjury (Panter et al., 1990). A more severe trauma causes a threefold increase in excitatory amino acid levels that remain elevated for over 50 min (Panter et al., 1990). Using MK-801, a noncompetitive glutamate antagonist, the decreased motor function caused by a 40 g-cm injury was reversed, whereas *N*-methyl-D-aspartate (NMDA) injections exacerbated the injury (Panter et al., 1990). This evidence strongly supports an excitotoxic component in the injury cascade following spinal cord injury.

In mixed neuronal cultures from spinal cord, glutamate causes a receptor-mediated increase in diacylglycerol lipase and monoacylglycerol lipase activities (Farooqui et al., 1993). These lipases will remove any diacylglycerol produced by the phospholipase C or phospholipase D signal transduction mechanisms. These diacylglycerols are often rich in 20:4 n-6, thus such breakdown by the combined action of these two lipases would liberate arachidonic acid for potential eicosanoid biosynthesis. In striatal neurons, glutamate stimulates the release of arachidonic acid through the joint activation of both ionotropic and metabotropic receptors (Dumuis et al., 1990). The Ca^{2+}-component may involve a Ca^{2+}-sensitive phospholipase A_2, whereas the metabotropic phospholipase C component may involve the combined activities of diacylglycerol and monoacylglycerol lipases. Rat hippocampal neurons prelabeled with [^{3}H]20:4 n-6 release radioactivity, presumed to be 20:4 n-6, when stimulated with NMDA (Sanfeliu et al., 1990). This release was blocked by NMDA receptor antagonists as well as by Mg^{2+}, indicating a receptor-mediated process. Recent evidence in our laboratory has shown that murine spinal cord neurons prelabeled with [^{3}H]20:4 n-6 release radioactivity following stimulation with glutamate (Horrocks et al., 1993). Thus, in numerous neuronal cell types, glutamate stimulation results in the release of arachidonic acid and/or stimulates potential mechanisms for the release of arachidonic acid.

The combined evidence suggests an excitatory amino acid component in the injury cascade following spinal cord injury in the rat. Our results are also consistent with a receptor-mediated cascade. The very rapid release of arachidonic acid combined with no early increase in diacylglycerols suggests a very rapid turnover of lipid intermediates into eicosanoids. The rapid rise in vasoconstrictive eicosanoids will contribute to a disruption in normal blood flow, causing areas of the injured cord to become ischemic. The later increases in free fatty acids can be attributed to increased acylhydrolase activity possibly associated with cellular necrosis. We did not find any significant early decreases in cholesterol, plasmalogens or α-tocopherol, suggesting a limited role for lipid peroxidation in the rat model.

ACKNOWLEDGMENTS

The authors thank Cindy Murphy for the typed preparation of the manuscript. This work was supported by NIH research grant NS-10165.

REFERENCES

Akesson B., Elovsson J., and Arvidsson, G. (1970) Initial incorporation into rat liver glycerolipids of intraportally injected [^{3}H]glycerol. *Biochim. Biophys. Acta* **210,** 15–27.

Anderson D. K., Saunders R. D., Demediuk P., Dugan L. L., Braughler J. M., Hall E. D., Means E. D., and Horrocks L. A. (1985) Lipid hydrolysis and peroxidation in injured spinal cord: Partial protection with methylprednisolone or vitamin E and selenium. *Cent. Nerv. Sys. Trauma* **2,** 257–267.

Banik N. L., Hogan E. L., and Hsu C. Y. (1987) The multimolecular cascade of spinal cord injury: Studies on prostanoids, calcium, and proteinases. *Neurochem. Pathol.* **7,** 57–77.

Behrmann D. L., Bresnahan J. C., Beattie M. S., and Shah B. R. (1992) Spinal cord injury produced by consistent mechanical displacement of the cord in rats: behavioral and histological analysis. *J. Neurotrauma* **9,** 197–217.

Brockerhoff H. (1975) Determination of the positional distribution of fatty acids in glycerophospholipids. *Methods Enzymol.* **35B,** 315–323.

Demediuk P. and Faden A. I. (1988) Traumatic spinal cord injury in rats causes increases in tissue thromboxane but not peptidoleukotrienes. *J. Neurosci. Res.* **20,** 115–121.

Demediuk P., Saunders R. D., Anderson D. K., Means E. D., and Horrocks L. A. (1985a) Membrane lipid changes in laminectomized and traumatized cat spinal cord. *Proc. Natl. Acad. Sci. USA* **82,** 7071–7075.

Demediuk P., Saunders R. D., Clendenon N. R., Means E. D., Anderson D. K., and Horrocks L. A. (1985b) Changes in lipid metabolism in traumatized spinal cord. *Prog. Brain Res.* **63,** 211–226.

Dugan L. L., Demediuk P., Pendley II C. E., and Horrocks L. A. (1986) Separation of phospholipids by high pressure liquid chromatography: All major classes including ethanolamine and choline plasmalogens, and most minor classes, including lysophosphatidylethanolamine. *J. Chromatogr.* **378,** 317–327.

Dumuis A., Pin J. P., Oomagari K., Sebben M., and Bockaert J. (1990) Arachidonic acid released from striatal neurons by joint stimulation of ionotropic and metabotropic quisqualate receptors. *Nature* **347,** 182–184.

Faden A. I., Chan P. H., and Longar S. (1987) Alterations in lipid metabolism, Na^+,K^+-ATPase activity, and tissue water content of spinal cord following experimental traumatic injury. *J. Neurochem.* **48,** 1809–1816.

Farooqui A. A., Anderson D. K., and Horrocks L. A. (1993) Effect of glutamate and its analogs on diacylglycerol and monoacylglycerol lipase activities of neuron-enriched cultures. *Brain Res.* **604,** 180–184.

Hara A. and Radin N. S. (1978) Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **90,** 420–426.

Horrocks L. A., Wells K., and Anderson D. K. (1993) Glutamate neurotoxicity and arachidonate release in primary cultures of fetal mouse spinal cord. *J. Neurochem.* **61,** S272D.

Hsu C. Y., Halushka P. V., Hogan E. L., Banik N. L., Lee W. A., and Perot P. L. (1985) Alteration of thromboxane and prostacyclin levels in experimental spinal cord injury. *Neurology* **35,** 1003–1009.

Hsu C. Y., Halushka P. V., Hogan E. L., and Cox R. D. (1986) Increased thromboxane level in experimental spinal cord injury. *J. Neurol. Sci.* **74,** 289–296.

Morand O. H., Zoeller R. A., and Raetz C. R. H. (1988) Disappearance of plasmalogens from membranes of animal cells subjected to photosensitized oxidation. *J. Biol. Chem.* **263,** 11597–11606.

Noyes D. H. (1987) Electromechanical impactor for producing experimental spinal cord injury in animals. *Med. Biol. Eng. Comput.* **25,** 335–340.

Panter S. S., Yum S. W., and Faden A. I. (1990) Alteration in extracellular amino acids after traumatic spinal cord injury. *Ann. Neurol.* **27,** 96–99.

Rouser G., Siakotos A., and Fleischer S. (1969) Quantitative analysis of phospholipids by thin layer chromatography and phosphorus analysis of spots. *Lipids* **1,** 85–86.

Sanfeliu C., Hunt A., and Patel A. J. (1990) Exposure to *N*-methyl-D-aspartate increases release of arachidonic acid in primary cultures of rat hippocampal neurons and not in astrocytes. *Brain Res.* **526,** 241–248.

Saunders R. D. and Horrocks L. A. (1984) Simultaneous extraction and preparation for HPLC of prostaglandins and phospholipids. *Anal. Biochem.* **143,** 71–75.

Saunders R. and Horrocks L. A. (1987) Eicosanoids, plasma membranes, and molecular mechanisms of spinal cord injury. *Neurochem. Pathol* **7,** 1–22.

Saunders R. D., Dugan L. L., Demediuk P., Means E. D., and Horrocks L. A. (1987) Effects of methylprednisolone and the combination of alpha tocopherol and selenium on arachidonic acid metabolism and lipid peroxidation in traumatized spinal cord tissue. *J. Neurochem.* **49,** 24–31.

Somerson S. K. and Stokes B. T. (1987) Functional analysis of an electromechanical spinal cord injury device. *Exp. Neurol.* **96,** 82–96.

Stokes B. T., Noyes D. H., and Behrmann D. L. (1992) An electromechanical spinal injury technique with dynamic sensitivity. *J. Neurotrauma* **9,** 187–195.

Zoeller R. A., Morand O. H., and Raetz C. R. H. (1988) A possible role for plasmalogens in protecting animal cells against photosensitized killing. *J. Biol. Chem.* **263,** 11590–11596.