A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia

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A pharmacological analysis was carried out to determine the possible role of aberrant calcium fluxes, vasoactive arachidonic acid metabolites, and microvascular lipid peroxidation in the development of posttraumatic spinal cord white matter ischemia. Pentobarbital-anesthetized cats were treated intravenously 30 minutes before a 500-gm-cm contusion injury to the lumbar spinal cord with one of the following test drugs: the Ca++ channel antagonists verapamil, diltiazem, or nifedipine; the cyclo-oxygenase inhibitors ibuprofen or meclofenamate; the thromboxane A2 (TXA2) synthetase inhibitor furegrelate sodium; or the stable epoprostenol (prostacyclin, or PGI2) analogue ciprosten calcium alone or in combination with furegrelate sodium. Another group of animals was pretreated for 5 days before spinal injury with a combination of the antioxidants vitamin E and selenium in high doses. The hydrogen clearance technique was used to make repeated measurements of spinal cord blood flow (SCBF) in the dorsolateral funiculus of the injured segment before and for 4 hours after injury. In 11 untreated uninjured cats, the mean preinjury SCBF was 12.7 ± 1.5 ml/100 gm/min. Following contusion, there was a progressive decline in SCBF to 6.8 ± 0.4 ml/100 gm/min, or 53.5% of the preinjury level at 4 hours. In comparison, the Ca++ antagonists diltiazem and nifedipine (but not verapamil) prevented a significant posttraumatic decrease in SCBF. Similarly, both cyclo-oxygenase inhibitors (ibuprofen and meclofenamate) maintained SCBF within normal limits (10 ml/100 gm/min or greater). However, neither TXA2 synthetase inhibition nor the stable PGI2 analogue alone had a significant effect in preventing ischemia, whereas a combination of the two agents did serve to support SCBF. The most impressive preservation of posttraumatic SCBF, however, was observed in the antioxidant-treated animals. Based upon these results, a hypothesis is presented concerning the pathogenesis of posttraumatic central nervous system ischemia which integrates an injury-induced rise in intracellular Ca++, the increased synthesis of vasoactive prostanoids (such as prostaglandin F2, and TXA2), and progressive microvascular lipid peroxidation.

KEY WORDS - spinal cord injury - ischemia - thromboxane - prostacyclin - peroxidation - cat
promote recovery following spinal cord injury, an attempt must be made to retard posttraumatic ischemia. This is particularly true for the white matter since sensorimotor recovery primarily depends upon the preservation of the long ascending and descending spinal tracts.

A number of pathophysiological factors have been postulated to play a role in the fall in central nervous system (CNS) blood flow after injury. A certain amount of mechanical disruption of the microvasculature by the injury force takes place, especially in gray matter areas which have the highest microvascular density, as is evidenced by gray matter petechial hemorrhage.

However, within the white matter, blood flow is initially within normal limits and ischemia develops progressively over the ensuing hours, suggesting the role of secondary mediators. Clearly, a successful definition of the most important of these would aid in the development of a rational acute therapy for CNS trauma. Therefore, we have recently undertaken a series of investigations to pharmacologically dissect out the contribution of certain putative ischemic mediators. Based upon evidence from past biochemical and physiological studies, we have focused upon the roles of three possible mediators: aberrant posttraumatic calcium fluxes, formation of vasoconstrictor and/or platelet-aggregating prostanooids, and microvascular lipid peroxidation. Accordingly, we have treated cats with individual agents that block with high selectivity the endogenous production or action of these, and have examined the subsequent effects on the decline in spinal cord white matter blood flow (SCBF) after severe contusion injury.

To examine the role of aberrant calcium fluxes, we tested the anti-ischemic effects of three structurally different calcium channel antagonists: verapamil, diltiazem, and nifedipine. To assess the contribution of vasoactive prostanooids, we compared the actions of the cyclo-oxgenase inhibitors ibuprofen or meclofenamate, and the selective thromboxane A$_2$ (TXA$_2$) synthetase inhibitor furylafate sodium (U-63557A). In addition, we tested the effects of a stable analogue of the vasodilator platelet aggregation-inhibiting prostanooid epoprostanol (PGI$_2$, or prostacyclin), namely, ciprostene calcium. Finally, we studied the potential role of free-radical-induced lipid peroxidative microvascular damage in the ischemic phenomenon by examining the possible benefits of intensive pretreatment with a combination of the antioxidants vitamin E and selenium (Se$^{++}$).

**Materials and Methods**

*Surgical Procedure*

Adult mongrel cats of either sex (each weighing between 2.2 and 3.7 kg) were anesthetized with sodium pentobarbital (30 mg/kg injected into the saphenous vein) and supplemental doses were given as needed throughout the subsequent experiment to just maintain a near total suppression of the corneal reflex. A tracheotomy was performed, a catheter was inserted into the right carotid artery for blood pressure monitoring, and a dorsal laminectomy was carried out to expose the spinal cord at the L-3 segment. The animal was then placed in a prone position in a Kopf 1780 spinal unit and rigidly fixed in place by means of hip spikes, a clamp on the L-2 spinous process, and a stereotactic head holder. Blood pressure and heart rate were monitored continuously. Rectal temperature was maintained at approximately 37°C.

**Neurophysiological Recordings**

Somatosensory evoked potentials (SEP's) were recorded from the right somatosensory cortex via a pair of bipolar platinum-iridium (90%/10%) electrodes (interelectrode distance 2 mm) resting gently upon the burr hole-exposed dura mater. The SEP's were evoked by bipolar supramaximal (approximately 1.0 V, 1 Hz, 0.4-msec duration) stimulation of the contralateral exposed sciatic nerve. At certain times during the experiment, 200 successive SEP's were amplified 1000 times, summed on a 1550-signal averager, and recorded on Polaroid film for later measurement of the latency (in msec) and amplitude (in mV) of the initial positive wave. Three SEP's were obtained at 30-minute intervals before spinal cord injury, and a mean latency and amplitude were calculated for each cat.

**Spinal Cord White Matter Blood Flow Measurement**

Local SCBF was determined using the H$_2$ clearance technique which allows for repeated measurements in the same animal. The SCBF electrode was constructed of 178-μ triple Teflon-coated platinum-iridium (90%/10%) wire coated with fingernail polish. The electrode tip was inserted into the dorsolateral funiculus of the spinal cord through a small hole in the meninges. The electrode was polarized to +350 mV with respect to a subcutaneous reference electrode. The reference electrode consisted of a silver/silver chloride pellet resting in a glass syringe plugged at the tip with agar and filled with a saturated KCl solution. Hydrogen was administered to the cats for 3 minutes by feeding H$_2$ gas directly into the intake line of a Harvard 607B respirator at a flow rate that did not result in significant hypoxia. As the H$_2$ was taken up by the spinal tissue...
and oxidized at the electrode tip, a current was generated which was monitored on a polarograph. Following H₂ inspiration, the current declined as the tissue concentration of H₂ fell. The initial 10-minute segment of the H₂ decay curve was analyzed by taking measurements at 1-minute intervals starting approximately 1 minute after the peak. The H₂ decay curve values were approximated to a monoexponential function using a Hewlett-Packard 41C computer. A program calculated the correlation coefficient and a slope value; the slope value was multiplied by 100 to give the SCBF (in ml/100 gm/min). Decay curves with correlation coefficients of less than 0.990 were discarded. Less noisy H₂ decay curves were obtained by thinly coating the spinal tissue electrodes with fingernail polish and daily snipping off the tip to expose fresh wire. Mean preinjury SCBF was determined for each cat before and after drug administration. The electrode was removed just prior to contusion injury. Arterial blood gases (pO₂ and pCO₂) and pH were measured using a pH/blood gas analyzer.* However, this instrument was not available until near the end of the study when it was used to assess the stability of blood gases and pH over the course of four of the untreated injury experiments.

Spinal Cord Injury Technique

A 500-gm-cm contusion injury (a 50-gm weight dropped 10 cm) or a 300-gm-cm contusion injury (a 50-gm weight dropped 6 cm) was produced at the exposed L-3 spinal cord segment as previously described.10,25,26 Immediately following the contusion, the SCBF electrode was reinserted into the dorsolateral funiculus at the injury site and SCBF determinations were made at 10 and 30 minutes and 1, 2, 3, and 4 hours postinjury. The uniformity and completeness of the injury were verified by an immediate and total abolition of the SEP and by an acute increase in the mean arterial blood pressure (MAPB) of at least 50 mm Hg.

Drug Treatments

All test drugs except vitamin E and Se⁺⁺ were given into the cephalic vein as a bolus and/or a constant infusion as described below.

Calcium Channel Antagonists. Three structurally distinct calcium channel antagonists were investigated in separate groups of animals in doses that produced a significant, but not drastic, cardiovascular effect (that is, a decrease in MABP). Verapamil HCl and diltiazem HCl were both dissolved in 0.9% saline. The former was given as a 150-μg/kg bolus 30 minutes before injury, then by a constant 5-μg/kg/min infusion. The latter was given as a 100-μg/kg bolus, then by a 5-μg/kg/min infusion. Nifedipine was initially dissolved in absolute ethanol and then diluted to 7% ethanol with distilled water. A 10-μg/kg bolus of nifedipine was administered at 30 minutes before injury, followed by a constant 1-μg/kg/min infusion.

Cyclo-Oxygenase Inhibitors. Two different cyclooxygenase inhibitors were studied. Ibuprofen with Na salt was given 30 minutes before injury as a 10-mg/kg bolus (10 mg/ml in 0.9% saline), followed by a second 5-mg/kg bolus 1.5 hours after injury. Meclofenamate with Na salt was given as a single 2-mg/kg bolus (2 mg/ml in 0.9% saline) 30 minutes before injury, based on its greater potency and longer duration of action than ibuprofen.20

Thromboxane Synthetase Inhibitor. The selective thromboxane synthetase inhibitor furegrelate sodium (sodium 5-(3'-pyridinylmethyl)-2-benzofuran carboxylate, monohydrate; U-63557A)22 was injected as a 10-mg/kg bolus (10 mg/ml in 0.9% saline) 30 minutes before injury.

Stable Prostacyclin. The stable analogue of epoprostenol (PGI₂), ciprostene calcium (9-methyl-(5Z)-6a-carboxaprostaglandin I₂, calcium salt, hydrate)6 was dissolved in saline and given as a constant infusion of either 1 or 3 μg/kg/min begun 30 minutes before injury and continued throughout the experiment. The total infusion volume in both series was 17 ml. In a third series, a 10-mg/kg bolus of the thromboxane synthetase inhibitor furegrelate sodium was given 30 minutes before injury, followed immediately by 1-μg/kg/min constant infusion of ciprostene calcium.

Antioxidant Agents. One group of four cats was orally pretreated once daily 5 days before experimentation with vitamin E (d-alpha-tocopherol, 1000-IU capsules) and Se⁺⁺ (50-μg tablets). This particular regimen has been shown to protect cat spinal cord from peroxidative damage by ferrous chloride microinjection.1

In the case of vitamin E, its antioxidant properties are believed to reside in an ability of the lipid-soluble vitamin E to intercalate between the susceptible polyunsaturated fatty acid moieties of cell membrane phospholipids, thus protecting them from free-radical attack by acting as a reducing agent.41,45 In addition, vitamin E may function as a free-radical scavenger by combining with lipid peroxide radicals to form tocopherol quinone. Selenium, on the other hand, is a cofactor for glutathione peroxidase, an enzyme which acts to remove intracellular hydrogen peroxide and lipid hydroperoxides.15

Statistical Analysis

Significant preinjury drug effects were identified by comparing the pre- and post-drug values of SCBF or MABP, using a paired t-test. Because of the slow progressive nature of the posttraumatic fall in SCBF, significant effects of drug treatment on ischemia development were identified by comparing the 4-hour SCBF values after drug administration to the 4-hour untreated
TABLE 1
Comparison of the effects of a 300- and 500-gm-cm spinal cord contusion

<table>
<thead>
<tr>
<th>Factor</th>
<th>Preinjury</th>
<th>4 Hrs Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td>300-gm-cm injury (5 cats)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>SEP's present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCBF (ml/100 gm/min)</td>
<td>12.8 + 1.9</td>
<td>10.8 + 1.4</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>119.3 + 4.2</td>
<td>94.0 + 13.7†</td>
</tr>
<tr>
<td>500-gm-cm injury (11 cats)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>SEP's present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCBF (ml/100 gm/min)</td>
<td>12.7 + 1.5</td>
<td>6.8 + 0.4†</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>130.1 + 4.6</td>
<td>95.3 + 5.2</td>
</tr>
<tr>
<td>blood analysis (4 cats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.43 + 0.01</td>
<td>7.41 + 0.01</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>21.7 + 1.4</td>
<td>19.5 + 0.7</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>103.0 + 4.2</td>
<td>100.8 + 4.3</td>
</tr>
</tbody>
</table>

* Effects on somatosensory evoked potential (SEP) conduction, spinal cord white matter blood flow (SCBF), and mean arterial blood pressure (MABP) are summarized. Values are means ± standard error.
† Significance: p < 0.05 by paired t-test (two-tailed) versus preinjury mean.

Values using a one-way analysis of variance (ANOVA, Fisher's method of least significant difference). In addition, the 10-minute postinjury value was compared with later values using a paired t-test to identify decreases in SCBF within a treatment group. Significant changes in the number of cats showing a 4-hour SCBF of less than 10 ml/100 gm/min, as compared to the untreated group at 4 hours, were tested using chi-square analysis. Regarding changes in posttraumatic hypotension, the values for the entire posttraumatic period (that is, MABP values from 10 minutes to 4 hours after injury) following drug treatment were compared to those in untreated cats by means of repeated-measures ANOVA.

Results

Effects of Contusion Injury

Acute Neurophysiological and Cardiovascular Response. Acute contusion of the lumbar spinal cord with either a 300- or a 500-gm-cm injury force resulted in immediate neurophysiological and cardiovascular changes. The conduction of the SEP's was completely eliminated immediately after both injuries. However, Table 1 shows that, in the 300-gm-cm injury group, SEP's returned in two of five cats by 4 hours after injury, while in the 11 animals with 500-gm-cm injury, SEP conduction did not return in any. The immediate effect of lumbar spinal contusion on the cardiovascular system was to cause a dramatic increase in the MABP. However, the hypertensive episode was transient, and after 3 to 5 minutes the MABP fell to 20 to 25 mm Hg below the preinjury value. This posttraumatic hypotension persisted for the remainder of the experiment, as also shown in Table 1, and was equivalent in both the 300- and 500-gm-cm injury groups. Although statistically significant, the lowered MABP was not considered to be of sufficient magnitude to contribute to spinal cord ischemia development. Furthermore, this effect reached a maximum within 1 hour after injury (not shown), while the time course of the decrease in SCBF was slow, as discussed below. Arterial blood gases and pH as measured in four of the 500-gm-cm-injured cats were not affected by the injury.

Spinal Cord Blood Flow Response. Table 1 also shows that the degree of posttraumatic spinal cord white matter ischemia, like the persistence of SEP loss, was related to the injury force. The mean 4-hour SCBF in the 300-gm-cm cats was not significantly less than the paired preinjury value, while in the more severely injured animals SCBF did fall significantly (p < 0.05 by paired t-test versus preinjury SCBF). Similar results have been published by Lohse, et al., who concluded that, in general, blunt spinal injuries which do not cause significant white matter ischemia do not result in permanent paraplegia.

Figure 1 compares the time courses of posttraumatic ischemia development in the dorsolateral funiculus of the injured spinal cord segment of untreated cats following either a 300- or a 500-gm-cm contusion. As seen, there was evidence of an initial hyperemia in the 300-gm-cm animals as the mean SCBF was increased from a preinjury control level of 12.8 ± 1.9 (mean ± standard error) to 15.9 ± 1.5 ml/100 gm/min at 10 minutes after injury, representing a 24.2% increase. By
Effects of Drug Treatment

Normal SCBF and MABP. Table 2 presents the acute effects of the various test drugs on the normal SCBF and MABP. Although some drugs did appear to affect the preinjury SCBF, none of the changes were significant by paired t-test. The most consistent effect was observed with ibuprofen, which caused an increase in SCBF in all four cats tested; however, since the increase was variable in magnitude, ranging from 0.3 to 2.3 ml/100 gm/min, it did not reach statistical significance. In contrast, meclofenamate, the other cyclooxygenase inhibitor tested, caused a mean decrease of 1.6 ml/100 gm/min. A similar dichotomy was observed among the three Ca++ antagonists tested, with verapamil causing a 21.6% decrease and nifedipine producing an 18.0% increase in normal SCBF.

Concerning the MABP, the larger dose of the stable PGI2 analogue ciprostene calcium (3 μg/kg/min) caused a significant, but mild, hypotensive effect. Predictable as well was the hypotensive action of each of the three Ca++ antagonists, with diltiazem causing a significant decrease in the MABP. None of the changes in SCBF seemed to parallel a cardiovascular action of the test drug in question.

Posttraumatic Spinal Cord Ischemia and Hypotension. The influence of pretreatment with various test drugs on the fall in SCBF recorded after 500-gm-cm contusion injury is shown in Table 3. Of the three Ca++ antagonists examined, diltiazem and nifedipine significantly preserved normal SCBF over the course of the experiment. The mean 4-hour SCBF values remained stable over the entire course of the experiment; the ischemic threshold of 10 ml/100 gm/min, as opposed to all 11 untreated animals. In the case of verapamil, although the difference between values at 10 minutes and 4 hours postinjury was only 14.4%, none of the verapamil-treated cats had a 4-hour SCBF of 10.0 ml/
Effects of various test drugs given or started at 30 minutes before spinal cord contusion on posttraumatic white matter ischemia development

<table>
<thead>
<tr>
<th>Intravenous Drug Treatment</th>
<th>No. of Cats</th>
<th>SCBF After Injury (ml/100 gm/min)</th>
<th>4-Hr SCBF (% of 10-min SCBF)</th>
<th>No. of Cats With 10-min Ischemia at 4 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>verapamil, 150 μg/kg + 5 μg/kg/min (Ca++ channel blocker)</td>
<td>11</td>
<td>11.4 ± 1.1</td>
<td>9.5 ± 1.1</td>
<td>8.0 ± 0.6†</td>
</tr>
<tr>
<td>diltiazem, 100 μg/kg + 5 μg/kg/min (Ca++ channel blocker)</td>
<td>5</td>
<td>9.9 ± 1.4</td>
<td>7.9 ± 1.1†</td>
<td>10.2 ± 1.3</td>
</tr>
<tr>
<td>nifedipine, 10 μg/kg + 1 μg/kg/min (Ca++ channel blocker)</td>
<td>4</td>
<td>11.7 ± 1.3</td>
<td>12.2 ± 0.9</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>ibuprofen, 10 mg/kg + 5 mg/kg 2 hrs later (cyclo-oxygenase inhibitor)</td>
<td>4</td>
<td>14.3 ± 0.7</td>
<td>14.0 ± 1.6</td>
<td>12.0 ± 1.5</td>
</tr>
<tr>
<td>meclofenamate, 2 mg/kg (cyclo-oxygenase inhibitor)</td>
<td>4</td>
<td>12.5 ± 0.8</td>
<td>11.5 ± 0.9</td>
<td>12.7 ± 1.5</td>
</tr>
<tr>
<td>furegrelate sodium, 10 mg/kg (TXA2 synthetase inhibitor)</td>
<td>4</td>
<td>14.1 ± 2.0</td>
<td>12.4 ± 1.2</td>
<td>13.5 ± 1.9</td>
</tr>
<tr>
<td>ciprofibrate calcium, 1 μg/kg/min (stable PGI2)</td>
<td>4</td>
<td>13.1 ± 0.3</td>
<td>10.2 ± 0.4</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>ciprofibrate calcium, 3 μg/kg/min</td>
<td>4</td>
<td>11.6 ± 1.6</td>
<td>10.0 ± 1.1</td>
<td>8.2 ± 1.9†</td>
</tr>
<tr>
<td>furegrelate sodium, 10 mg/kg + ciprofibrate calcium, 1 μg/kg/min</td>
<td>5</td>
<td>12.7 ± 1.1</td>
<td>10.9 ± 1.1</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>vitamin E, 1000 IU + Se**, 50 μg orally for 5 days</td>
<td>4</td>
<td>16.1 ± 2.0</td>
<td>13.4 ± 2.4</td>
<td>13.5 ± 2.3†</td>
</tr>
</tbody>
</table>

* SCBF = spinal cord blood flow (values are mean ± standard error). TX = thromboxane; PGI2 = prostacyclin.
† Significance: p < 0.05 by paired t-test (two-tailed) versus 10-minute value in same treatment group.
‡ Significance: p < 0.05 by one-way ANOVA (Fisher’s method of least significant difference) versus untreated group at 4 hours.
§ Significance: p < 0.05 by chi-square versus vehicle group.

100 gm/min or above. However, the results with verapamil are complicated by an apparent reduction of the initial (10-minute) postinjury SCBF.

Both of the cyclo-oxygenase inhibitors ibuprofen and meclofenamate attenuated posttraumatic ischemia development. After administration of both drugs, the mean 4-hour SCBF value was still greater than 80% of the paired 10-minute postinjury level. Moreover, only one of the four animals in either cyclo-oxygenase inhibitor-treated group showed a 4-hour SCBF value below the ischemic threshold.

On the other hand, the selective TXA2 synthetase inhibitor furegrelate sodium alone did not prevent ischemia development, as the mean SCBF values in that group at 2 and 4 hours after injury were not significantly different from those in the untreated cats. In fact, the rate of ischemia development was increased, as shown by the occurrence of a significant fall in SCBF between 10 and 30 minutes after injury in this treatment group, but in none of the others.

A constant 1-μg/kg/min intravenous infusion of the stable PGI2 analogue ciprofibrate calcium by itself only modestly improved postinjury SCBF. Raising the infusion rate to 3 μg/kg/min did not augment the effect. However, pretreatment with the TXA2 synthetase inhibitor furegrelate sodium followed by a 1-μg/kg/min infusion of the PGI2 analogue resulted in a maintenance of SCBF in the normal range in all of five animals tested.

The most dramatic maintenance of SCBF was seen in the cats pretreated with vitamin E and Se++. In the antioxidant-treated group, the SCBF consistently remained well above the 10-ml/100 gm/min ischemic threshold. Furthermore, the mean values were significantly higher than in the untreated group at both the 2- and 4-hour measurement times. There was only a 7.4% decrease in SCBF between 10 minutes and 4 hours after trauma. However, correlating the 4-hour postinjury mean to its paired preinjury control level of 13.5 ± 1.0 ml/100 gm/min revealed a slight increase in SCBF. Figure 2 graphically displays a statistical comparison of the SCBF values at 4 hours after injury in the untreated versus each of the drug-treated groups.

In Table 4, the effects of the different test drugs on posttraumatic hypotension are shown. All three Ca++ antagonists appeared to intensify posttraumatic hypotension, diltiazem and nifedipine significantly. This is in line with the known hypotensive effects of these agents; a similar action was also apparent in the preinjury MABP (Table 2) as discussed above. The combination of the TXA2 synthetase inhibitor furegrelate sodium and the stable PGI2 analogue (1 μg/kg/min) also significantly lowered the posttraumatic MABP over the course of the experiment, while neither drug alone had any apparent effect. Since diltiazem, nifedipine, and the furegrelate sodium-PGI2 combination were three of the most effective inhibitors of posttraumatic ischemia development, it is clear that the increased posttraumatic hypotension was not of a sufficient magnitude to compromise spinal cord perfusion.

Furthermore, although arterial blood gases and pH were not measured in the drug-treated animals, the fact...
TABLE 4

Effects of various test drugs given or started 30 minutes before spinal cord contusion on posttraumatic hypotension

<table>
<thead>
<tr>
<th>Intravenous Drug Treatment</th>
<th>No. of Cats</th>
<th>Mean Arterial Blood Pressure (mm Hg) Pre- &amp; Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preinjury</td>
</tr>
<tr>
<td>untreated group</td>
<td>11</td>
<td>130.1+4.6</td>
</tr>
<tr>
<td>verapamil, 150 µg/kg + 5 µg/kg/min (Ca++ channel blocker)</td>
<td>5</td>
<td>105.3+7.8</td>
</tr>
<tr>
<td>diltiazem, 100 µg/kg + 5 µg/kg/min (Ca++ channel blocker)</td>
<td>4</td>
<td>100.0+12.2</td>
</tr>
<tr>
<td>nifedipine, 10 µg/kg + 1 µg/kg/min (Ca++ channel blocker)</td>
<td>4</td>
<td>100.8+4.4</td>
</tr>
<tr>
<td>ibuprofen, 10 mg/kg + 5 mg/kg 2 hrs later (cyclo-oxygenase inhibitor)</td>
<td>4</td>
<td>113.8+6.8</td>
</tr>
<tr>
<td>meclofenamate, 2 mg/kg (cyclo-oxygenase inhibitor)</td>
<td>4</td>
<td>137.1+7.7</td>
</tr>
<tr>
<td>furegrelate sodium, 10 mg/kg (TXA2-synthetase inhibitor)</td>
<td>4</td>
<td>123.8+14.1</td>
</tr>
<tr>
<td>ciprostene calcium, 1 µg/kg/min (stable PGI2)</td>
<td>4</td>
<td>135.5+9.0</td>
</tr>
<tr>
<td>ciprostene calcium, 3 µg/kg/min</td>
<td>4</td>
<td>120.4+6.2</td>
</tr>
<tr>
<td>furegrelate sodium, 10 mg/kg + ciprostene calcium, 1 µg/kg/min</td>
<td>5</td>
<td>117.3+5.4</td>
</tr>
<tr>
<td>vitamin E, 1000 IU + Se++, 50 µg, orally for 5 days</td>
<td>4</td>
<td>118.3+12.0</td>
</tr>
</tbody>
</table>

* Values are mean ± standard error. TX = thromboxane; PGI2 = prostacyclin.
† Significance: p < 0.05 by repeated-measures ANOVA versus vehicle group for 10 minutes to 4 hours postinjury.

that SCBF was affected independently of posttraumatic changes in the MABP argues against a significant role of blood gas or pH effects. For instance, if a drug-induced elevation in pCO2 were to have been a factor in the support of SCBF, one would also expect a pCO2-induced increase in the MABP. Instead, with nifedi-
pine and the furegrelate sodium-ciprostene calcium or the vitamin E-Se++ combinations, posttraumatic ischemia was prevented while hypotension was actually increased. Such a response pattern is inconsistent with a possible elevation in plasma pCO2. Moreover, other unpublished studies in this laboratory have shown that doses of the cyclo-oxygenase inhibitor ibuprofen identical to those used in the present study have no effect on pCO2, pO2, or pH in anesthetized cats. This is noteworthy since, of all the experimental treatments employed, a large dose of cyclo-oxygenase inhibitor, which could theoretically reduce pulmonary PGI2 formation (with a consequent increase in arteriole constriction), would be the most likely to trigger an increase in pCO2.

The SEP's never returned over the 4-hour experimental period in any of the animals, regardless of treatment. While it may appear incongruous that postinjury SCBF was maintained within normal limits but SEP conduction did not return, the explanation may simply reside in the fact that in the present study a contusion force was employed that was greater than that used by other investigators reporting acute SEP recovery within 3 to 4 hours, together with a maintenance of white matter SCBF.29,60

Discussion

The present series of investigations has demonstrated three pharmacological mechanisms by which progressive posttraumatic spinal cord white matter ischemia can be prevented: 1) calcium channel blockade; 2) prevention or antagonism of the formation of vasoactive prostanoïd metabolites of arachidonic acid (such as prostaglandin F2α, (PGF2α) or TXA2); and 3) atten-
ulation of microvascular lipid peroxidation induced by oxygen-free radicals. While other factors may also contribute to ischemia development, the fact that preinjury pharmacological antagonism of any of these three events can essentially block the fall in SCBF suggests their probable preeminence in the progressive ischemic response to blunt injury.

One of the very earliest events that appear to follow blunt spinal cord injury, which is quite conceivably related to the subsequent fall in SCBF, is a fall in extracellular Ca\(^{++}\).\(^{26,61}\) and its subsequent accumulation in the intracellular space.\(^{28}\) An increase in intracellular Ca\(^{++}\), assuming that it could occur in various cell types, could trigger a variety of pathophysiological phenomena relevant to ischemia development. For instance, elevated Ca\(^{++}\) levels in vascular smooth-muscle cells may increase contractility and thus reduce arteriolar patency directly (by microvascular spasm). Providing that excess Ca\(^{++}\) may enter after injury, in large part via voltage-dependent channels, the administration of Ca\(^{++}\) channel antagonist may be expected to ameliorate the fall in SCBF. Indeed, both diltiazem and nifedipine were found to prevent significant posttraumatic ischemia development. Interestingly, the ability of these agents to maintain postinjury tissue perfusion did not appear to be limited by a concomitant decrease in systemic arterial pressure, an action presumably due to the negative inotropic and vascular smooth-muscle relaxant effects of these drugs.\(^{29}\) For example, diltiazem prevented ischemia concomitant with an MABP (over a 4-hour period) in the range of 67.1 to 82.5 mm Hg. It may be noted that the lack of a significant beneficial effect of verapamil on posttraumatic ischemia development did not appear to be due to an inadequate dose of the drug since posttraumatic hypotension was enhanced by the drug, indicating a significant microvascular action. Rather, a greater peripheral versus cerebral vascular selectivity may perhaps explain the difference.

A second mechanism by which a reduction in the postinjury intracellular accumulation of Ca\(^{++}\) could lead to a maintenance of SCBF may involve a decreased activation of Ca\(^{++}\)-dependent membrane phospholipases, thereby preventing the production of vasoconstrictor and/or platelet-aggregating arachidonic acid metabolites such as PGF\(_{2\alpha}\) and TXA\(_2\). There is, in fact, a growing amount of information showing that the CNS tissue levels of certain vasoactive prostanoids are dramatically elevated after blunt brain or spinal injury. For example, experimental spinal cord contusion\(^{32}\) or compression\(^{49}\) injury in cats has been demonstrated to cause a large increase in the spinal tissue levels of the potent cerebral vasoconstrictor PGF\(_{2\alpha}\).\(^{56}\) Similarly, PGF\(_{2\alpha}\) has been shown to increase by 30-fold in rat cortical tissue after freezing injury, an effect that parallels a decrease in brain functional activity as measured by glucose utilization.\(^{43}\) In addition to PGF\(_{2\alpha}\), TXB\(_2\) (the stable metabolite of TXA\(_2\)) has been found to be increased in the injured spinal cord of both cats\(^{49}\) and rabbits.\(^{31}\) The increase in PGF\(_{2\alpha}\) and TXB\(_2\) in the injured cat spinal cord is apparent as early as 5 minutes postinjury.\(^{49}\) On the other hand, very little increase, if any, seems to take place in the spinal level of the PGI\(_2\) metabolite 6-keto PGF\(_{1\alpha}\).\(^{49}\)

The effect of pretreatment with either of the cyclooxygenase inhibitors ibuprofen or meclofenamate in reducing posttraumatic ischemia is consistent with the possibility that PGF\(_{2\alpha}\) and TXA\(_2\) are important mediators of the decline in SCBF. In contrast, selective inhibition of TXA\(_2\) synthetase by furegrelate sodium prior to injury did not affect the decline in SCBF. Indeed, the rate of the decline may actually have been speeded. The most straightforward explanation may be that a blockade of injury-induced TXA\(_2\) formation alone without an effect on PGF\(_{2\alpha}\) is insufficient to maintain SCBF. Furthermore, the very limited support of SCBF by a constant intravenous infusion of the stable vasodilator antiplatelet-aggregating PGI\(_2\) analogue ciprostene calcium can be most simply explained in terms of effective antagonism by the accumulated PGF\(_{2\alpha}\) and TXA\(_2\). In contrast, when PGI\(_2\) is utilized against a background of TXA\(_2\) synthesis inhibition, a significant preservation of SCBF becomes apparent. Others have shown that furegrelate sodium can improve the action of the PGI\(_2\) precursor eicosapentaenoic acid to antagonize posts ischemic hypoperfusion in the gerbil, while neither agent alone is effective.\(^{7}\) An augmentation of the relaxant effects of PGI\(_2\) on canine cerebral vessels by meclofenamate\(^{11}\) has also been reported. Intuitively, the selection of a cyclooxygenase inhibitor as an adjunct to PGI\(_2\) infusion would be preferable to a TXA\(_2\) synthetase inhibitor since, in the former instance, the production of both PGF\(_{2\alpha}\) and TXA\(_2\) would be reduced. The combined use of indomethacin and PGI\(_2\) has actually been shown to enhance the neurological recovery of cats after spinal cord contusion injury.\(^{27}\) However, that study and its interpretation were complicated by the simultaneous use of a third agent, the anticoagulant heparin. In any case, the known anti-hemostatic actions of any of the three agents strongly suggest a maintenance of SCBF as the basis for the facilitated sensorimotor recovery.

Demopoulos and coworkers\(^{13}\) have hypothesized that posttraumatic spinal cord ischemia development is ultimately due to lipid peroxidative damage to the spinal microvasculature induced by hypoxic free radicals. Using a variety of biochemical approaches, they and others have provided evidence of oxygen-free radical generation and spinal tissue lipid peroxidation occurring within an hour (in some studies as early as 5 minutes) after blunt injury.\(^{12,14,21,25,35,44}\) The present results showing the anti-ischemic efficacy of intensive pretreatment with the antioxidant combination of vitamin E and Se\(^{++}\) strongly support a critical role of lipid peroxidation in the posttraumatic development of ischemia in the spinal cord.

**Hypothesis of Posttraumatic Ischemia Development**

Figure 3 displays a hypothesized scheme defining the
probable evolution of posttraumatic spinal cord ischemia, with microvascular lipid peroxidation viewed as the ultimate mediator of the decline in SCBF. The sequence of events is very likely of equal relevance in moderate to severe brain injury. In brief, blunt spinal injury causes two immediate events conceivably related to the subsequent fall in SCBF. These include the occurrence of petechial hemorrhage in the gray matter and the accumulation of calcium in the intracellular space. The latter event, in addition to increasing microvascular smooth-muscle tone, would be expected to lead to an activation of cell membrane phospholipases which would, in turn, liberate various phospholipids. The most notable of these phospholipids may be arachidonic acid, which was recently shown by Saunders, et al., to increase in the cat spinal cord by 5 minutes after compression injury. As a consequence, certain prostanoids such as PGF\textsubscript{2\alpha} from the vascular wall and TXA\textsubscript{2} from platelets would be liberated and cause local ischemia via their vasoconstrictor and/or platelet-aggregating actions. Then, due to the resulting local ischemia, generation of hypoxic free radicals and lipid peroxidative damage would ensue.

The iron (such as hemoglobin) and copper complexes derived from the petechial hemorrhages associated with the mechanical injury force would contribute greatly to the oxidative tissue damage by catalyzing the formation of free-radical species and lipid peroxides. In addition, the early and dramatic increase in prostanoid production would be promoted by the ability of either thrombin or PGF\textsubscript{2\alpha} in a positive feedback manner, to stimulate phospholipase A\textsubscript{2} activity.

Intuitively, it would be expected that the injury-induced phospholipase activation would also cause an increase in PGI\textsubscript{2} production, thereby counteracting the ischemic influence of the other prostanoids. However, PGI\textsubscript{2} synthesis would be compromised by early lipid peroxidative damage to the synthetic vascular endothelium, as hypothesized by Demopoulos, et al. Indeed, the trauma-induced rise in cat spinal cord PGI\textsubscript{2} has been shown to be slight in comparison to the concomitant large increase in PGF\textsubscript{2\alpha} and TXA\textsubscript{2}.

The above sequence of events appears to occur initially in the central gray matter of the injured spinal cord. For instance, the greater microvascular density and the immediate petechial hemorrhages produced in that area after injury would represent more vessels and platelets as sources of PGF\textsubscript{2\alpha} and TXA\textsubscript{2}, and also more iron-containing hemoglobin to promote free-radical generation and lipid peroxidation. Furthermore, the higher cellular density of the gray matter would provide more mitochondria, which under conditions of ischemia are perhaps one of the earliest generation sources of hypoxic free radicals. Lipid peroxidation, however, is a self-perpetuating process that would gradually spread from the central gray matter to the circumferential white matter tracts. There it would irreversibly damage myelin and axons directly and indirectly due to the ischemia caused by peroxidative white matter microvascular destruction. The slow progressive nature of the fall in SCBF is consistent with this latter conclusion.

Finally, from a therapeutic standpoint, it would appear that the principal goal of pharmacological treatment of acute spinal cord injury, and probably of CNS trauma in general, should be to retard generation of oxygen-free radicals (which is due in large part to vasoactive prostanoid generation) which would lead to localized gray matter ischemic hypoxia and the associated spread of neuronal and microvascular lipid peroxidation. Interestingly, two agents which have been shown to reduce posttraumatic spinal cord white matter ischemia and to promote neurological recovery when given in large doses early after blunt cord injury are methylprednisolone and naloxone. Both of these have been demonstrated to inhibit lipid peroxidation.

References


\[\text{References}]

\[\text{Fig. 3. Diagram showing a hypothetical pathogenesis of posttraumatic spinal cord ischemia. PG = prostaglandin; TX = thromboxane.}\]
of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and prevention of Fe\textsuperscript{2+} initiated free radical-induced lipid peroxidation in spinal cord by methylprednisolone. *Soc Neurosci Abst* 9:351, 1983 (Abstract)


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