Effect of aminophylline and isoproterenol on spinal cord blood flow after impact injury

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A study of the effects of spinal cord injury upon spinal cord blood flow was carried out in cats. A 400 gm-cm impact produced an overall reduction in spinal cord blood flow of 24% in the white matter and 30% in the gray matter, as determined by 14C-antipyrine autoradiography. At the level of the injury, white-matter flow was 8.1 ml/100 gm/min, a reduction of 49%, and in the gray matter, 12.5 ml/100 gm/min, a reduction of 76%. Treatment with aminophylline and isoproterenol improved the overall blood flow in the spinal cord. At the level of the injury, white-matter flow after this treatment was no longer significantly different from control values. The gray-matter flow remained decreased to 26.2 ml/100 gm/min, a reduction of only 47%. It is proposed that aminophylline and isoproterenol may increase cyclic adenosine monophosphate (AMP) and prevent platelet aggregation along the endothelial surfaces of the microcirculation, and may thereby help to maintain improved perfusion of the injured spinal cord.

KEY WORDS: spinal cord injury, spinal cord blood flow, cyclic AMP, aminophylline, isoproterenol

ALTHOUGH the actual cause of degeneration of the spinal cord after experimental impact injury is not known, ultrastructural and biochemical evidence indicates that ischemia is important in the development of the progressive lesion. Most investigators have noted a decrease in spinal cord blood flow in the white matter between 1 and 2 hours after injury.4,20,27 Several mechanisms have been proposed to explain the reduction in blood flow, including vasospasm,11 release of putative transmitters,8 platelet aggregation because of endothelial damage,3,9 and changes in effective perfusion pressure.8

Our experience with aminophylline and isoproterenol, two drugs capable of inhibiting platelet aggregation and relieving cerebral vasospasm by increasing plasma and vascular smooth muscle cyclic nucleotide levels, prompted us to investigate the effects of these agents on blood flow in the spinal cord after injury.5,10 Elevation of the cyclic nucleotide levels in animals with spinal cord injuries might decrease vascular spasm from extravasation of whole blood,9,10 as well as decrease platelet aggregation and release reaction, which are also influenced by cyclic adenosine monophosphate (AMP).24 The decreased blood flow secondary to any of these mechanisms might be ameliorated by administration of aminophylline and isoproterenol, and the progressive destruction of the spinal cord diminished.

Materials and Methods

Twenty adult mongrel cats of both sexes, weighing 2.5 to 3.5 kg, were sedated with Ketaject (ketamine HCl, 15 mg/kg), and maintained on N2O:O2 anesthesia (50:50 mixture) administered by tracheal intubation. All animals were continuously infused with Flaxedil (gallamine triethiodide, 30 mg/100 ml saline). Arterial blood gases were monitored from the femoral artery with a blood gas analyzer.* Body temperature was maintained at 37.0 ± 0.5° with a heat lamp.

A dorsal laminectomy was performed between the T-7 and T-11 vertebrae. Somatosensory evoked potentials (SEP's) were recorded before and after laminectomy. One-half hour after laminectomy, the spinal

*Blood gas analyzer (Model 113) manufactured by Instrumentation Laboratory, Inc., Lexington, Massachusetts.
cord was injured at T-9 in 10 animals by dropping a 20-gm weight from a height of 20 cm, to produce a 400 gm-cm impact injury. Ten sham-operated animals were operated on in an identical manner, but the spinal cord was not impacted. One-half hour before the impact injury and throughout the 2-hour experimental period, five injured and five sham-operated animals received an intravenous infusion of saline.

The remaining five cats in each group were given an infusion of aminophylline (55 mg/kg/hr), and isoproterenol HCl (Isupril HCl, 50 μg/kg/hr) in saline. If the mean blood pressure of these animals dropped below 70 mm Hg, the drug infusion was decreased until the pressure returned to baseline levels. The SEP's were recorded 1½ hours after injury. Two hours after injury, 100 μCi/kg of 14C-antipyrine in 5 ml saline was infused into the femoral vein over a 60-second interval. Arterial blood (0.4 ml) was sampled from the femoral artery in a heparinized syringe every 10 seconds during the isotope infusion. At the end of the 1-minute isotope infusion, 5 ml of saturated KCl solution was injected to sacrifice the animal. The spinal cord was quickly removed and cut in 1-cm segments, which were frozen in Freon. Three drops of blood from each syringe were placed into three preweighed vials containing filter paper discs, and the vials were reweighed. Beckman EP scintillation cocktail† (12 ml) was added to the vials, which were shaken overnight at room temperature. Radioactivity levels were determined the following day.

Sections of spinal cord were cut 20 μm thick, using a Slee cryostat,‡ and dried on a hot plate at 65°C for 5 minutes. The sections were placed with plastic standards (dosage equivalent 160 + 1350 nCi/gm, New England Nuclear) against Lo-Dose mammography film,§ and the film was exposed at room temperature for 2 weeks.

Following the development of the film, the densities of areas were determined by means of a Photovolt densitometer and a Heathkit voltohmeter. The voltages produced by the densitometer from the tissue autoradiographs were converted to the tissue concentrations of antipyrine by comparison with those produced from the autoradiographs of the plastic standards. The tissue concentration of the metabolically inert tracer (antipyrine) in a homogenous tissue at a given time, or Ci(T), is related to blood flow as follows:

\[
Ci(T) = \lambda k_i/\phi (Ca)e^{-ki(T-t)}dt,
\]

where Ci(T) is the concentration of the tracer substance in the tissue at time T, λ is the tissue blood partition coefficient for the tracer substance, \( k_i \) is the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that substance.

†Beckman Ready-Solv EP scintillation cocktail obtained from Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, California.
§Lo-Dose mammography film manufactured by Dupont Corp., Wilmington, Delaware.
| Photovolt densitometer manufactured by Photovolt Corp., New York, New York.
Spinal cord blood flow after injury

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cats</th>
<th>Blood Pressure (mm Hg)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>pCO₂ (mm Hg)</th>
<th>pO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no drug†</td>
<td>5</td>
<td>155 ± 5.0</td>
<td>37.0 ± 0.0</td>
<td>7.326 ± 0.01</td>
<td>31.8 ± 1.0</td>
<td>191 ± 15.9</td>
</tr>
<tr>
<td>control</td>
<td>5</td>
<td>121 ± 8.4</td>
<td>36.8 ± 0.5</td>
<td>7.334 ± 0.02</td>
<td>33.8 ± 1.2</td>
<td>194 ± 17.8</td>
</tr>
<tr>
<td>impact‡</td>
<td>5</td>
<td>93 ± 7.7</td>
<td>38.5 ± 0.4</td>
<td>7.328 ± 0.02</td>
<td>32.4 ± 1.0</td>
<td>250 ± 16.5</td>
</tr>
<tr>
<td>drugs§</td>
<td>5</td>
<td>74 ± 7.5</td>
<td>37.2 ± 0.3</td>
<td>7.334 ± 0.02</td>
<td>33.0 ± 1.0</td>
<td>245 ± 17.6</td>
</tr>
</tbody>
</table>

*Values are means ± standard error of the means.
†These animals received a saline infusion in place of the drug.
‡A 400 gm-cm impact was delivered to T-9.
§These animals received an infusion of aminophylline (55 mg/kg/hr) and isoproterenol (55 μg/kg/hr).

Thus, to calculate the blood flow in a homogeneous region of the spinal cord, three parameters were required: 1) the tissue-blood partition coefficient (λ, which is equated with 1); 2) the concentration of tracer substance in that region of the spinal cord at time T (which is determined autoradiographically); and 3) the time course of the change in arterial tracer concentration (which is determined by scintillation counting of the arterial blood).

Six determinations of blood flow rates were made for each of four areas of the white matter and two areas of the gray matter of the spinal cord at cervical, high thoracic, low thoracic, and lumbar levels, and at the impact site (T-9). All calculations were performed on a Texas Instrument Model 60 calculator. The data were analyzed for statistical significance using the Student's t-test.

**Results**

Although the mean arterial blood pressures were lower in the cats subjected to impact injury and those that received aminophylline and isoproterenol, the other physiological parameters, including temperature, pH, and arterial blood gases, were similar in all groups (Table 1).

Spinal cord blood flow was determined using the ¹⁴C-antipyrine autoradiographic technique. Sample autoradiographs of control, untreated, and treated cords at the impact site can be seen in Fig. 1. Examination of the average blood flow rates from each of the five segments of the entire spinal cord (Table 2) reveals little variation in white-matter blood flow (mean = 17.1 ml/100 gm/min) vertically along the length of the cord, and an average gray-matter blood flow (58.73 ml/100 gm/min). There was no difference in dorsal and lateral white-matter blood flow. Following impact injury, there were significant decreases in both white- and gray-matter blood flow at the level of injury and at sites proximal and distal to it (Table 2). The mean flow in the white matter after injury was 13.0 ml/100 gm/min, and in the gray matter it was 41.3 ml/100 gm/min for the cord as a whole. However, the blood flow in the cervical region of injured cats did not vary significantly from the control cervical blood flow rates.

Cats treated with aminophylline (55 mg/kg/hr) and isoproterenol (55 μg/kg/hr) and subjected to laminectomy only (drug-treated control animals) had spinal cord blood flows similar to those in the saline-treated cats.

### TABLE 2

**Spinal cord blood flow in five sham-operated and five injured cats 2 hours after injury***

<table>
<thead>
<tr>
<th>Cord Level</th>
<th>White Matter</th>
<th>Gray Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Impact</td>
</tr>
<tr>
<td>cervical</td>
<td>17.03 ± 0.71</td>
<td>16.30 ± 0.52</td>
</tr>
<tr>
<td>high thoracic</td>
<td>17.20 ± 0.74</td>
<td>14.40 ± 1.12</td>
</tr>
<tr>
<td>impact (T-9)</td>
<td>15.80 ± 0.71</td>
<td>8.10 ± 0.50</td>
</tr>
<tr>
<td>low thoracic</td>
<td>16.60 ± 0.79</td>
<td>13.30 ± 0.95</td>
</tr>
<tr>
<td>lumbar</td>
<td>18.40 ± 1.02</td>
<td>13.10 ± 1.08</td>
</tr>
<tr>
<td>mean</td>
<td>17.06 ± 0.43</td>
<td>13.04 ± 1.36</td>
</tr>
</tbody>
</table>

*Mean blood flows are given as ml/100 gm/min ± standard error of the mean. The injury was a 400 gm-cm impact at T-9. NS = not significant.
TABLE 3  
Spinal cord blood flow in five sham-operated and five injured cats 2 hours after injury and treatment with aminophylline and isoproterenol*

<table>
<thead>
<tr>
<th>Cord Level</th>
<th>White Matter</th>
<th>Gray Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Impact</td>
</tr>
<tr>
<td>cervical</td>
<td>16.7 ± 0.88</td>
<td>17.50 ± 0.88</td>
</tr>
<tr>
<td>high thoracic</td>
<td>16.9 ± 1.32</td>
<td>16.90 ± 1.30</td>
</tr>
<tr>
<td>impact (T-9)</td>
<td>15.0 ± 1.39</td>
<td>13.30 ± 1.60</td>
</tr>
<tr>
<td>low thoracic</td>
<td>14.6 ± 1.14</td>
<td>15.80 ± 1.20</td>
</tr>
<tr>
<td>lumbar</td>
<td>14.8 ± 1.24</td>
<td>16.80 ± 1.13</td>
</tr>
<tr>
<td>mean</td>
<td>15.6 ± 0.50</td>
<td>16.06 ± 0.74</td>
</tr>
</tbody>
</table>

*Mean blood flows are given as ml/100 gm/min ± standard error of the mean. The injury was a 400 gm-cm impact at T-9. NS = not significant.

control group (see Table 3). The mean blood flow in the white matter was 15.6 ml/100 gm/min, and in the gray matter was 53.0 ml/100 gm/min. Drug treatment abolished the decrease in white-matter blood flow following impact and minimized the decrease in gray-matter blood flow, particularly at the level of the injury. These changes can be expressed as the percent change in blood flow in the various areas of the injured animals by using the comparable values for the uninjured animals (Fig. 2). After injury, the blood flow in the gray matter of the untreated animals decreased along the length of the spinal cord (Fig. 2 left, open bars). Drug treatment (closed bars) minimized these decreases at all levels of the cord, especially at the impact site. In the white matter, injury caused reductions in blood flow from 4% to 47% (Fig. 2 right, open bars). Treatment with aminophylline and isoproterenol improved blood flow along the length of the spinal cord and prevented the decrease at the impact site.

In the injured segments, blood flows decreased 49% in white matter and 76% in the ventral gray matter in the untreated cats (Table 2). Both of these decreases are significant, p < 0.001. Drug treatment preserved the white-matter blood flow. After impact, there was no significant change in white-matter blood flow in animals that received aminophylline and isoproterenol, and ventral gray-matter blood flow was normalized. Although significant reductions in gray-matter blood flow were found in the treated animals, the decrease in gray-matter blood flow was not as profound as that seen in the untreated, injured animals (Table 3).

Discussion

Spinal cord blood flow in both white and gray matter at the impact site, as well as proximal and distal to it, was found to be decreased 2 hours after a 400 gm-cm impact injury. Treatment with aminophylline and isoproterenol restored blood flow to within the normal range in the white matter, and to almost one-half of normal in the gray matter.

A decrease in spinal cord blood flow after impact injury has previously been reported. The 49% decrease in white-matter blood flow and the 76% decrease in gray-matter blood flow are similar to other reported values following experimental spinal cord injury. Decreases both proximal and distal to the site of trauma have also been noted. These longitudinal decreases have been attributed to the effects of laminectomy; however, in the present study, the...
operative site was closed immediately after impact to minimize any decrease in local spinal cord temperature. Furthermore, such decreases in spinal cord blood flow were not seen in control animals that underwent only laminectomy.

The \(^{14}\text{C}\)-antipyrine technique proved to be a reliable method for distinguishing between gray-matter and white-matter blood flow. Major differences in blood flow values after trauma were seen in the gray and the white matter. The effects of aminophylline and isoproterenol were also quite different in these two areas. The use of other techniques for the determination of blood flow might have precluded the elucidation of the important differences in the responses of the gray and the white matter to trauma and drug therapy.

Senter, et al.,\(^{28}\) found no change in blood flow following the administration of aminophylline and isoproterenol, perhaps due to the limitations of the hydrogen clearance technique. Although several authors have used hydrogen clearance techniques for determining spinal cord blood flow, the volume sampled by the hydrogen electrode remains unknown. Readings from electrodes placed within the white matter may include some gray matter in the sampled zone. Kobrine, et al.,\(^{16}\) reported lower readings when the electrode was placed within the center of the cord than in various regions of the white matter.

One major limitation of the \(^{14}\text{C}\)-antipyrine technique is its lack of suitability for measurement of blood flow in fast-flow areas. Others have shown that blood flow through the frontal cortex is significantly lower using \(^{14}\text{C}\)-antipyrine as a tracer substance than it is when using \(^{14}\text{C}\)-ethanol.\(^{8}\) However, the levels of blood flow in the spinal cord are considerably lower than those in the brain, and the diffusion limitations of antipyrine are considered to be negligible,\(^{8}\) as are the differences in the partition coefficient between gray matter and white matter in the ranges of blood flow seen within the spinal cord.\(^{4,28}\)

The efficacy of aminophylline and isoproterenol in increasing cyclic AMP levels in cerebral blood vessels has been previously reported.\(^{10}\) Parenteral administration of these drugs is effective in preventing basilar artery spasm to whole blood and mechanical stimulation, and has been correlated with high levels of cyclic AMP in these vessels. The mechanism by which vasospasm is reversed involves a reduction in the levels of intracellular free Ca\(^{2+}\) through the action of cyclic AMP-dependent protein kinases. Phosphorylation of a serine residue in microsomal protein causes a decrease in the intracellular levels of free Ca\(^{2+}\) due to uptake into the intracellular stores.\(^{2,17}\) Decrease in the sarcoplasmic Ca\(^{2+}\) levels results in a relaxation of the Ca\(^{2+}\)-dependent contractions and a dilatation of the vessel. Triner, et al.,\(^{29}\) and Pöch and Kukovetz\(^{32}\) have shown that inhibition of phosphodiesterase (the enzyme responsible for converting cyclic AMP into 5'AMP) potentiates the increase in intracellular cyclic AMP. Aminophylline is an example of a phosphodiesterase-inhibiting drug.

In addition to affecting cyclic AMP in vascular smooth muscle, aminophylline and isoproterenol also modify platelet aggregation and the release reaction by decreasing platelet Ca\(^{2+}\) stores.\(^{13,14,24}\) Increased levels of Ca\(^{2+}\) cause platelets to release vesicles containing ADP and serotonin (5-HT), which further potentiate platelet aggregation and vasoconstriction.

In the injured spinal cord, the prevention of platelet aggregation at the sites of endothelial damage would help to maintain a patent microcirculation in the traumatized tissue. In addition, prevention of the release reaction would reduce the levels of vasoactive amines (5-HT and norepinephrine) released into the contused area following platelet aggregation. Zivin, et al.,\(^{31}\) and Němec, et al.,\(^{20}\) reported that, in the rabbit, 5-HT was significantly increased at the site of trauma in the white matter, and postulated that the release of the amine by aggregating platelets at the site of trauma contributed to the development of the lesion.

Our own studies and those of Nelson, et al.,\(^{19}\) using the scanning electron microscope, have shown that vessels in the long tracts show progressive changes in the endothelium from 1 or 2 hours after injury with a 400 gm-cm impact.\(^{5,19}\) Endothelial damage, as evidenced by the formation of craters and overriding of endothelial cell junctions, and platelet aggregation were noted at the impact site, as well as in adjacent areas of the spinal cord. This suggests that changes in the microvasculature of the spinal cord may be responsible for the reduction in flow observed both at the impact site and in more proximal and distal portions of the spinal cord. The changes in endothelial surfaces of the microvasculature within the spinal cord after trauma raises the possibility that the decrease in flow may also be produced by a breakdown in production of prostacyclin (PGI\(_2\)) by the vessel wall. This would leave the vasoconstricting and platelet-aggregating properties of thromboxane (TXA\(_2\)), which is formed in platelets, unopposed.\(^{12,19}\) Jonsson and Daniell\(^{15}\) have reported large increases in prostaglandin F (PGF) following injury to the spinal cord in cats. This prostaglandin is known to have potent vasoconstricting activity and stimulates platelet aggregation. Further study of the role of prostaglandins and their metabolites in spinal cord injury is necessary.

Since many investigators believe that ischemia is an important factor contributing to spinal cord degeneration after trauma, the prevention of ischemia in the white matter of the spinal cord may prevent the loss of motor and sensory function in the long tracts. The observation that aminophylline and isoproterenol can prevent the reduction of blood flow along the length of the spinal cord indicates that the decrease in perfusion after injury is not due to a disruption of the vascular bed. Rather, the obstruction to adequate blood flow...
may be due to micro-occlusions secondary to platelet aggregation, to decreased caliber of the vessels in response to trauma and extravasated blood, or to a combination of both. Modification of cyclic nucleotide levels in the spinal cord tissue and vessels could influence several of the possible causes of the decreased perfusion, and offer a vehicle for delivering therapeutic agents, such as steroids, as well as improving spinal cord blood flow itself.

References

This work was supported by National Institutes of Health Grant NINDS NS10164-05 to the Spinal Cord Clinical Research Center.

This paper was presented in part at the Annual Meeting of the American Association of Neurological Surgeons, Los Angeles, California, April 22-26, 1979.

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