Studies in experimental spinal cord trauma

Part 1: Alterations in catecholamine levels

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-The authors report a study of levels of norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT) in the traumatized spinal cords of dogs, and with alpha-methyltyrosine before and after injury. There was a significant elevation of DA 15 to 45 minutes after injury. NE was significantly reduced. Twenty-four hours of pretreatment with alpha-methyltyrosine depleted cord catecholamines and prevented trauma-induced DA elevation. Alpha-methyltyrosine given 15 minutes after the trauma did not prevent this trauma-related DA elevation. In a small pilot study in cats, DA was elevated and NE remained essentially normal.

KEY WORDS - spinal cord injury - alpha-methyltyrosine - dopamine - norepinephrine - catecholamines

REDUCTIONS in blood flow$^{0,12}$ and tissue $pO_2$$^{0,16}$ have been shown to follow experimental spinal cord trauma. Osterholm and Mathews$^{24}$ have demonstrated the production of hemorrhagic necrosis following injection of norepinephrine (NE) into spinal gray matter in cats. They also reported that a 500 gm-cm force injury to the cord of cats resulted in an increase in spinal cord NE$^{22}$ and that this increase was prevented by alpha-methyltyrosine ($\alpha$-MT) given 15 minutes after the trauma.$^{23}$ This treatment appeared to ameliorate trauma-induced paralysis in four animals. Levels of dopamine (DA) appeared to be lowered although no numerical data were given. All of these findings supported the hypothesis that the histological sequelae of spinal cord trauma are related to the release of NE in the cord.

The following study was performed to further evaluate this hypothesis using dogs as the experimental animal. Confirmation in another species would enhance the generalization of this hypothesis. To examine the mediator status more fully, NE, DA, and 5-hydroxytryptamine (5-HT) were measured in each sample.

Material and Method

A laminectomy was performed in 29 mongrel dogs weighing 15 to 20 kg and five cats, all under pentobarbital anesthesia, 30 mg/kg. The spinal cord was traumatized at T-10 through the unopened dura by a 420
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**FIG. 1.** Spinal cord dopamine (DA) levels at the lesion site and 1 cm above in μg/gm of tissue, at different time intervals after trauma in dogs. Values are shown as the mean with the standard error of the mean. Note that DA is significantly elevated throughout the time studied, with maximal elevation at 45 min, especially 1 cm above the lesion site.

**FIG. 2.** Spinal cord norepinephrine (NE) levels after trauma in the same dogs derived exactly as in Fig. 1. Note that NE remains normal or is minimally reduced except for slight elevation at 1.5 hrs after trauma.
Catecholamines in spinal cord injury

The catecholamine data resulting from these studies are shown in Figs. 1, 2, and 3, so that the course can be fully appreciated. The DA (Fig. 1) was dramatically elevated, particularly in the region above the trauma site, beginning within 15 min after trauma, peaking at 45 min, and persisting to some degree for 3 hrs. At these same times NE (Fig. 2) was undergoing a biphasic change, both at and above the trauma site, although the two were 90° out of phase. At 15 min, the NE level at the site of trauma was normal, while above the site the level was dropping. This relationship was reversed at 45 min. Both were normal at 1½ hrs, then falling together at 3 hrs. The “above site” return to normal NE corresponds with the “above site” peak DA level, while the “at site” return to normal NE corresponds to the more prolonged DA elevation for that region. Although it seems probable that some of the DA was being converted to NE at those times, the NE values never rose above control levels.

The data are shown again in Table 1 with the α-MT data and the results of statistical
TABLE 1

Concentration of catecholamines in traumatized spinal cord*

<table>
<thead>
<tr>
<th>Time</th>
<th>At Site</th>
<th>1 cm Above Site</th>
<th>At Site</th>
<th>1 cm Above Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (dogs)</td>
<td>.144 ± .012</td>
<td>(5)</td>
<td>.024 ± .010</td>
<td>(5)</td>
</tr>
<tr>
<td>After Injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>.153 ± .015</td>
<td>(5)</td>
<td>.109 ± .013†</td>
<td>(5)</td>
</tr>
<tr>
<td>45 min</td>
<td>.098 ± .013†</td>
<td>(5)</td>
<td>.149 ± .023</td>
<td>(5)</td>
</tr>
<tr>
<td>1½ hrs</td>
<td>.155 ± .040</td>
<td>(3)</td>
<td>.138 ± .018</td>
<td>(3)</td>
</tr>
<tr>
<td>3 hrs</td>
<td>.070 ± .013†</td>
<td>(3)</td>
<td>.081 ± .013†</td>
<td>(3)</td>
</tr>
<tr>
<td>45 min with 24-hr α-methyltyrosine pretreatment</td>
<td>.000 ± .000†</td>
<td>(5)</td>
<td>.020 ± .014†</td>
<td>(5)</td>
</tr>
<tr>
<td>45 min with α-methyltyrosine treatment 15 min after trauma</td>
<td>.109 ± .006‡</td>
<td>(3)</td>
<td>.101 ± .081†</td>
<td>(3)</td>
</tr>
<tr>
<td>Control (cats)**</td>
<td>.165 ± .031</td>
<td>(2)</td>
<td>.000 ± .000</td>
<td>(2)</td>
</tr>
<tr>
<td>After Injury**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1½ hrs</td>
<td>.288 (.88, .116, .480)</td>
<td>(3)</td>
<td>.109 (.068, .118, .141)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

* Number of animals is in parentheses.
† Statistically significant at p = .01 (students' t-test).
‡ Statistically significant at p = .02 (students' t-test).
§ Statistically significant at p = .05 (students' t-test).
** Values are given as means with the range in brackets except where values are given.

comparisons. It is apparent that the DA increase was statistically significant both at and above the site at 15 min, 45 min, and 1½ hrs, with the "at site" values becoming insignificant at 3 hrs. On the other hand, the NE reductions found in both regions were also significant statistically.

Twenty-four hours of pretreatment with α-MT resulted in almost complete depletion of cord catecholamines and prevented the trauma-induced DA elevation (Table 1). When α-MT was given 15 min after trauma, NE was reduced and DA was significantly elevated as in untreated dogs. This is clearly seen in Fig. 3, where all of the NE values fall at or below normal while all of the DA values are above normal.

In a pilot study on five cats, there was a slight but consistent elevation in DA 1½ hrs after trauma at 1 cm cephalad to the lesion site while NE values tended to be slightly lowered above it (Table 1). However, it is possible that other changes will be detected when a complete study of the time course is completed.

Discussion

The rapid elevation in DA accompanied by normal to reduced NE after spinal cord trauma in dogs is at variance with Östergren and Mathews' findings in cats, and becomes a matter of concern. Table 2, showing values reported for spinal cord catecholamines, reveals discrepancies in the literature. The DA values of McGeer and McGeer in the cat are exceptionally high compared to those from most reported studies in this and other species. We must suspect, along with others, that a technical fault exists in their method of measuring...
### TABLE 2

Concentration of catecholamines in normal spinal cord*

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Region</th>
<th>Cat Norepinephrine (μg/gm)</th>
<th>Cat Dopamine (μg/gm)</th>
<th>Dog Norepinephrine</th>
<th>Dog Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>McGeer &amp; McGeer (1962)</td>
<td>entire cord</td>
<td>.19 (.13 - .39)</td>
<td>.45 (.41 - .50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laverty &amp; Sharman (1965)</td>
<td>thoracic &amp; lumbar</td>
<td>.08</td>
<td>.003</td>
<td>.05</td>
<td>.003</td>
</tr>
<tr>
<td>Andén, et al. (1966)</td>
<td>lumbar</td>
<td>.23 (.13 - .34)</td>
<td>.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reis &amp; Gutnick (1970)</td>
<td>thoracic</td>
<td>.17 ± .03</td>
<td>.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lumbar</td>
<td>.37 ± .04†</td>
<td>.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voigt (1954)</td>
<td>anterior horns</td>
<td>--</td>
<td>.18 (.15 - .20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lateral horns</td>
<td>--</td>
<td>--</td>
<td>.19 (.11 - .24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>posterior horns</td>
<td>--</td>
<td>.12 ‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>King &amp; Jewett (1971)</td>
<td>cervical cord</td>
<td>.08 ± .05</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osterholm &amp; Mathews (1972)</td>
<td></td>
<td>.38 (.25 - .64)</td>
<td>.20**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hedeman, et al. (1973)</td>
<td></td>
<td>.16 (.03 - .31)§</td>
<td>0.0</td>
<td>.144 ± .012</td>
<td>.024 ± .010</td>
</tr>
</tbody>
</table>

* Values given as μg/gm of tissue with either the range in parentheses or ± the standard error of the mean.

† Extremes of diurnal range.
‡ Two identical values.
§ Spread probably resulting from small size compared to the dog.
** Read from graph on page 389 of ref. 23. No numerical data given.

DA and that this also may account for the high control DA value reported by Osterholm and Mathews.24

An additional discrepancy is found in the relatively high values of NE reported by Osterholm and Mathews. The most comparable values are those of Reis and Gutnick25 who found varying values throughout the day, with peak values reported at the T1–4 level in cat cord occurring between 0700 and 1300 hrs; values in other thoracic and lumbar regions were significantly lower at most times. It seems likely through these comparisons that the control values reported by Osterholm and Mathews are near or beyond the upper limits of normal. On the other hand, the values established in our study fall well within the range of most other published values for both spinal cord and other brain regions.26,27 The data in Table 2 also show that spinal cord catecholamine levels are comparable for cats and dogs, making it unlikely that the discrepancies between our data and those of Osterholm and Mathews are species-related.

Another area of disagreement between our data and those of Osterholm and Mathews24 is in the ability of α-MT to lower the amine levels when given after trauma. Our failure to find a blocking effect is not unexpected for two reasons. First, the DA rise occurs quite rapidly following trauma and would be essentially complete before effective inhibition could be achieved. Second, all evidence points to considerable interference with circulatory competence in the region of trauma, and raises the question of whether adequate concentrations of the inhibitor could reach this site.

From a chemical standpoint, the high degree of variability in catecholamine values in this study and in previous studies is of great concern.28 Variability was most notable in the traumatized animals in all studies, indicating the existence of uncontrolled variables. These studies utilized "light" barbiturate anesthesia and thus the level of anesthesia may have varied in individual animals. Since stress is known to cause alterations in brain catecholamine metabolism, it is possible that this may occur in the spinal cord as well. In addition, Reis and Gutnick25 reported daily segmental rhythms in NE concentrations in spinal cords of cats housed in a controlled light-dark cycle environment. No trauma study has attempted control of this factor. Therefore, it seems possible that the data spread
results in part from incomplete and variable suppression of the stress in these surgical and traumatizing procedures, and variability in the time of obtaining cord samples based on daily rhythms in spinal cord NE concentration. Other variables are undoubtedly present as well; these include variation in blood pressure, blood flow, temperature, and tissue oxygenation. All of these factors could affect catecholamine metabolism.

The source of trauma-related DA elevation is unknown. It may reflect the systemic adrenal response reported by Muelheims, et al., which occurs following spinal cord sectioning.21 Thus, the catecholamine elevation seen at the injury site could result from the uptake of NE and epinephrine from the blood. If this were true, however, one would expect NE to be the elevated catecholamine and the response to be more transient. Two other sites of possible catecholamine origin are the adrenergic neurons on blood vessels and catecholamine fiber tracts located in the dorsal, ventral, and lateral funiculi, described by histofluorescent techniques in rats.5 Anatomically, it is fairly well accepted that adrenergic fibers are abundant on subarachnoid and pial vessels in the brain, and that considerable diminution occurs as vessels become intraparenchymal.3 Anatomical studies may exist in the spinal cord since small numbers of adrenergic fibers have been described on intraparenchymal vessels there as well.5 From this it seems unlikely that such fibers could be an effective source of the DA increase seen in this study. We would, therefore, expect that the increase in DA arises from the descending catecholamine fiber tracts although most of these are noradrenergic. The observation that DA elevation was most marked 1 cm above the lesion site is probably related to the superior origin of catecholamines. However, present evidence does not permit us to speculate about the mechanism by which the DA increase occurs or why it should be maximal just proximal to the lesion site. However, it has been shown that peripheral noradrenergic endings will release DA subsequent to block of dopamine-β-oxidase.29 It is possible that the same may occur in the vicinity of the trauma site as a result of anoxic inhibition of dopamine-β-oxidase.

While we were unable to corroborate the findings of Osterholm and Mathews,23,24 we are in agreement that there is an adrenergic component in the response of the spinal cord to trauma. Our failure to find a decrease in amines with α-MT given after trauma suggests several experimental therapeutic implications. These include: 1) the necessity to increase trauma site-perfusion with α-MT; 2) a blockade of receptors with or without increasing perfusion; and 3) a combination of α-MT, receptor blockade, and increasing perfusion. Some of these studies have been performed and are described in a subsequent paper.15

Summary

A trauma-related DA elevation has been demonstrated in the spinal cords of dogs; the NE was normal or reduced depending on the timing. Treatment with α-MT 15 min after trauma was ineffective in preventing this elevation of DA. On the other hand, 24 hours of pretreatment with α-MT suppressed all cord catecholamine and prevented the trauma-related DA elevation.

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