Biogenic amine concentrations in traumatized spinal cords of cats

Effect of drug therapy

N. Eric Naftchi, Ph.D., Margaret Demeny, Ph.D., Vincent DeCrescito, John J. Tomasula, Eugene S. Flamm, M.D., and James B. Campbell, M.D.

The Laboratory of Biochemical Pharmacology, Department of Rehabilitation Medicine, and the Department of Neurosurgery, the Milbank Research Laboratories, New York University Medical Center, 550 First Avenue, New York, New York

Concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and histamine (HIST) were determined in spinal cords of five groups of cats. One group underwent laminectomy only; a second untreated group received a 400 gm-cm impact at the T-9 level. These were compared with three groups treated with epsilon aminocaproic acid (EACA), methyl prednisolone sodium succinate (MP), and a combination of EACA and MP after similar trauma. The biogenic amines were measured in three 1-cm segments of the cord, rostral, middle, and caudal, 1 hour after trauma. There was no change in NE concentration in any of the three segments after impact compared with laminectomized controls, nor was the NE concentration in the impacted (middle) area higher than that in the rostral or caudal sites. Although the NE content of the cord in treated animals decreased compared to that of laminectomized controls, the decrease was not significant. The concentration of DA, however, significantly increased after impact and significantly decreased after treatment with EACA and EACA plus MP. There was no significant change in 5-HT levels, but the level of HIST increased significantly after impact and was lowered by treatment with EACA and EACA with MP. The implications of these changes in biogenic amines in spinal cord trauma are discussed.

KEY WORDS: spinal cord injury • epsilon aminocaproic acid • methyl prednisolone sodium succinate • norepinephrine • dopamine • histamine

Recent publications have suggested that biogenic amines released within the spinal cord after trauma contribute to the formation of the lesion. The mechanism proposed for this enhancement of deterioration following the traumatic injury is based, in part, on the general knowledge of the necrotizing effect of norepinephrine when infiltrated in tissues.\(^\text{13,14}\) We have previously shown that catecholamine metabolism is altered in paraplegia.\(^\text{11,12,17}\) The similarity between the protection afforded by alpha methyl-p-tyrosine in the work of Osterholm and Mathews\(^\text{14}\) and by epsilon aminocaproic
Biogenic amines in traumatized spinal cord

Acid (EACA) and methyl prednisolone sodium succinate (MP) in our work prompted us to undertake a quantitative measurement of biogenic amines in traumatized spinal cords.

Materials and Methods

Fifty immunized mongrel cats of either sex, ranging in weight from 2.5 to 2.8 kg, were used in equal numbers in five different experimental groups.

Preparation of Animals

Intravenous pentobarbital (30 mg/kg) was used for anesthesia. Animals underwent laminectomy from T-7 to T-11. A 400 gm-cm force was delivered to the exposed dura at the ninth thoracic segment in the experimental groups.

Arterial blood pressure was continuously monitored in all animals. The blood pressure was permitted to stabilize for 1 hour before laminectomy and trauma. Averaged evoked cortical sensory action potentials (EP) were recorded from the region of the sigmoid gyrus during stimulation of the contralateral peroneal nerve before and after impact to ascertain the adequacy of the trauma.

One hour after impact, a 3-cm portion of the cord, centered at the level of the impact, was removed in a temperature-controlled room (4°C). The specimens were divided into three equal portions, labeled rostral, middle, and caudal.

Concentrations of norepinephrine, dopamine, serotonin, and histamine in the spinal cords were determined for the five groups of cats. One group underwent laminectomy only. A second untreated group received a 400 gm-cm impact at the T-9 level. These animals were compared with three groups treated with EACA, MP, and a combination of EACA and MP after similar trauma. One hour after trauma, the biogenic amines were measured in each of the three 1-cm cord segments.

In animals treated with drugs, each received an intravenous priming dose of EACA (1 gm) and/or MP (3 mg) 1 hour before contusion. Animals were then maintained on a continuous infusion of EACA (350 mg/hour) and MP (1.5 mg/hour), separately, or in combination until the time of sacrifice.

Determination of Biogenic Amines

Analysis of Norepinephrine and Dopamine. This method is based on several fluorometric techniques described previously.1,2,5,7,9,16,18,20 The tissues were homogenized in acidified butanol. After centrifuging the homogenate at 2000 rpm for 15 minutes, a portion of the supernatant butanol fraction was shaken in heptane and water and centrifuged at 2000 rpm. A solution of 2 M sodium acetate and 0.1 gm alumina was added to the water phase, and the pH adjusted to 7.5. After shaking the mixture for 5 minutes and centrifuging, the effluent was discarded. Catecholamines were then eluted for alumina with 0.25 M acetic acid. For development of the fluorophores, 150 μl of 0.1 M EDTA were added to 50 μl of the eluate followed by 50 μl of 0.1 N iodine reagent. Two minutes later, alkaline sulfite was added, and after 2 more minutes, was acidified with 6 N acetic acid. The tube containing the mixture was heated at 100°C for 4 minutes, then cooled in ice water, and the fluorescence of norepinephrine was read immediately at 485 μm (activated at 385 μm). The mixture was set aside for 1 hour, after which the dopamine content was measured at 370 μm (activated at 320 μm).

Analysis of Serotonin and Histamine. The butanol extract was washed with 0.1 M borate buffer (pH 10) saturated with NaCl. To an aliquot of the washed butanol phase, heptane and 0.1 N HCl were added, shaken, and centrifuged. The fluorescence of serotonin and histamine were determined in the water phase by O-phthalaldehyde condensation. Fluorescence of serotonin was measured at 470 μm (activated at 360 μm) and that of histamine was measured at 440 μm (activated at 350 μm). In all determinations, internal and external standards and faded blanks were similarly determined.

Results

There were 10 animals in each group listed below. The results are summarized in Tables 1-4. The concentrations of biogenic amines measured in the treated groups were
Naftchi, Demeny, DeCrescito, Tomasula, Flamm and Campbell

TABLE 1
Norepinephrine levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Rostral (µg/gm)</th>
<th>Middle (µg/gm)</th>
<th>Caudal (µg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: laminectomy</td>
<td>0.164 ± 0.015</td>
<td>0.150 ± 0.015</td>
<td>0.137 ± 0.014</td>
</tr>
<tr>
<td>2: impact</td>
<td>0.156 ± 0.012</td>
<td>0.138 ± 0.015</td>
<td>0.138 ± 0.019</td>
</tr>
<tr>
<td>3: EACA</td>
<td>0.138 ± 0.012</td>
<td>0.070 ± 0.016</td>
<td>0.145 ± 0.009</td>
</tr>
<tr>
<td>4: MP</td>
<td>0.125 ± 0.014</td>
<td>0.121 ± 0.017</td>
<td>0.153 ± 0.009</td>
</tr>
<tr>
<td>5: EACA &amp; MP</td>
<td>0.158 ± 0.017</td>
<td>0.121 ± 0.018</td>
<td>0.167 ± 0.022</td>
</tr>
</tbody>
</table>

TABLE 2
Dopamine levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Rostral (µg/gm)</th>
<th>Middle (µg/gm)</th>
<th>Caudal (µg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: laminectomy</td>
<td>0.149 ± 0.029</td>
<td>0.115 ± 0.017</td>
<td>0.107 ± 0.017</td>
</tr>
<tr>
<td>2: impact</td>
<td>0.232 ± 0.045</td>
<td>0.223 ± 0.046</td>
<td>0.224 ± 0.043</td>
</tr>
<tr>
<td>3: EACA</td>
<td>0.089 ± 0.010</td>
<td>0.094 ± 0.013</td>
<td>0.086 ± 0.011</td>
</tr>
<tr>
<td>4: MP</td>
<td>0.074 ± 0.011</td>
<td>0.119 ± 0.020</td>
<td>0.103 ± 0.027</td>
</tr>
<tr>
<td>5: EACA &amp; MP</td>
<td>0.166 ± 0.042</td>
<td>0.068 ± 0.011</td>
<td>0.083 ± 0.020</td>
</tr>
</tbody>
</table>

TABLE 3
Histamine levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Rostral (µg/gm)</th>
<th>Middle (µg/gm)</th>
<th>Caudal (µg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: laminectomy</td>
<td>0.166 ± 0.047</td>
<td>0.092 ± 0.024</td>
<td>0.106 ± 0.019</td>
</tr>
<tr>
<td>2: impact</td>
<td>0.287 ± 0.076</td>
<td>0.248 ± 0.075</td>
<td>0.267 ± 0.095</td>
</tr>
<tr>
<td>3: EACA</td>
<td>0.146 ± 0.018</td>
<td>0.109 ± 0.021</td>
<td>0.106 ± 0.020</td>
</tr>
<tr>
<td>4: MP</td>
<td>0.206 ± 0.045</td>
<td>0.109 ± 0.021</td>
<td>0.200 ± 0.058</td>
</tr>
<tr>
<td>5: EACA &amp; MP</td>
<td>0.136 ± 0.027</td>
<td>0.109 ± 0.017</td>
<td>0.101 ± 0.013</td>
</tr>
</tbody>
</table>

TABLE 4
Serotonin levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Rostral (µg/gm)</th>
<th>Middle (µg/gm)</th>
<th>Caudal (µg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: laminectomy</td>
<td>0.693 ± 0.044</td>
<td>0.684 ± 0.045</td>
<td>0.672 ± 0.074</td>
</tr>
<tr>
<td>2: impact</td>
<td>0.625 ± 0.106</td>
<td>0.692 ± 0.159</td>
<td>0.614 ± 0.074</td>
</tr>
<tr>
<td>3: EACA</td>
<td>0.765 ± 0.104</td>
<td>0.668 ± 0.107</td>
<td>0.530 ± 0.067</td>
</tr>
<tr>
<td>4: MP</td>
<td>0.746 ± 0.043</td>
<td>0.732 ± 0.062</td>
<td>0.639 ± 0.055</td>
</tr>
<tr>
<td>5: EACA &amp; MP</td>
<td>0.715 ± 0.127</td>
<td>0.623 ± 0.062</td>
<td>0.629 ± 0.051</td>
</tr>
</tbody>
</table>

compared with those of the impacted, but untreated, animals. Results were only considered significant if, by comparison with the untreated animals, the probability was below 5% (p < 0.05). The results are expressed as mean ± standard deviation for each group of 10 animals.

Group 1: Laminectomy

A concentration gradient, proceeding along a rostral caudal axis, was observed for norepinephrine, dopamine, and histamine (Fig. 1). Serotonin levels were the same in all three segments.
Biogenic amines in traumatized spinal cord

Fig. 1. Concentration gradients for norepinephrine (upper), dopamine (center), and histamine (lower) content of the cat spinal cord. Each bar represents mean ± SD in μg of norepinephrine per gram tissue in 10 cats. After laminectomy from T-7 to T-11 the animal's spinal cord was contused by dropping a 20 gm weight from a height of 20 cm at T-9, the middle segment. Each segment represents 1 cm of the spinal cord. Upper: A concentration gradient for norepinephrine exists along the rostral caudal axis. This gradient is disrupted after contusion of the spinal cord but is re-established during therapy with EACA and MP, only in the middle contused 1 cm segment. Center: The gradient for dopamine present before contusion of the spinal cord is abolished. Dopamine content of all these segments rises significantly to approximately the same level. Administration of EACA and MP re-establishes the gradient; the dopamine concentration in all segments, especially in the middle (contused) and caudal segments, is significantly reduced. Lower: The histamine content of the spinal cord shows similar patterns of changes as those described for dopamine. Note, however, that after treatment with EACA and MP the concentration of the rostral segment is decreased below the levels found during laminectomy controls; this decrease is not statistically significant.
Group 2: Impact

The gradient for biogenic amines was not present after impact. The norepinephrine concentration was the same in all three segments (Fig. 1 upper). The concentration of dopamine (Fig. 1 center) was significantly increased after impact in all three segments of the cords compared with specimens obtained after laminectomy alone (Group 1). Similarly, a significant rise in histamine concentrations was observed (Fig. 1 lower). The levels of serotonin, however, remained unchanged in all segments of the cord (Tables 1-4).

Group 3: Epsilon Aminocaproic Acid (EACA)

Treatment with EACA produced a significant decrease in the concentration of norepinephrine in the impacted (middle) segment (Table 1) and significantly decreased both dopamine and histamine concentrations below that of untreated animals (Tables 2 and 3). Again, the concentrations of serotonin remained unchanged in all segments (Table 4).

Group 4: Methyl Prednisolone Sodium Succinate (MP)

The results with MP treatment were similar to those obtained with EACA alone (Group 3) with the exception of histamine, the concentration of which was significantly reduced only at the impacted site in the cord (Table 3).

Group 5: Combined EACA and MP

There was no significant change in concentrations of norepinephrine and serotonin (Fig. 1, and Tables 1 and 4). This form of therapy produced a significant reduction of the concentration of dopamine in all segments compared with Group 2. The concentration of histamine was significantly lower in all three segments of the cord (Table 3).

Discussion

The results of this study are not in agreement with those reported by others. A rise in the concentration of norepinephrine 1 hour after impact was not observed, nor was there a concomitant decrease in levels of dopamine. By contrast, the concentration of dopamine increased significantly and norepinephrine remained unchanged in this period.

The significant rise in concentrations of dopamine within the cord substance may be explained by the fact that dopamine-B-hydroxylase (DBH), the enzyme responsible for the conversion of dopamine to norepinephrine, exerts its biosynthetic effect in the central nervous system only when membrane-bound. Trauma to the cord may alter this state and thereby reduce DBH activity. Dopamine and norepinephrine, released from the storage vesicles after trauma, are subject to degradation by monoamine oxidase and catechol-o-methyl transferase. Dopamine levels remain higher than those of norepinephrine because its turnover to norepinephrine is curtailed due to reduction in DBH activity within the traumatized spinal cord. The biosynthesis of dopamine, however, continues since dopa decarboxylase is not membrane-bound. In addition, tyrosine hydroxylase activity is increased due to decrease in the free intraneuronal norepinephrine available to reduce its activity through end-product feedback inhibition.

These results may partially explain the elevated urinary levels of homovanillic acid, the major metabolite of dopamine, found in man as early as 2 days after spinal cord injury. The urinary levels may reflect changes in peripheral metabolism of biogenic amines after trauma.

It has been shown that treatment with MP preserves membrane integrity. This may explain the reduction in dopamine concentration after treatment with MP. Under these conditions, DBH may remain in a relatively bound form and the turnover of dopamine to norepinephrine is not appreciably altered.

EACA, by virtue of its antiproteolytic activity, may also preserve membrane integrity by neutralizing lysosomal enzymes and thus act synergistically with MP. This is supported by histological studies previously reported.

Elevated levels of dopamine and histamine after trauma and the role these...
Biogenic amines in traumatized spinal cord

substances may play in the development of a spinal cord lesion are not yet known. Possibly research already underway may determine their significance.

Acknowledgment

Gratitude is expressed to Mr. Fred A. Holmes for his surgical assistance in these experiments.

References


The work from the Laboratory of Biochemical Pharmacology, Department of Rehabilitation Medicine, was supported by the Edmund A. Guggenheim Research Endowment. The work from the Department of Neurosurgery was supported by U. S. Army Medical Service, Research and Development Command, Office of The Surgeon General, Contract DADA 17-73-C-3021, and National Institutes of Health Grant 5-P01-NS04257-09.

Address reprint requests to: N. Eric Naftchi, M.D., Institute of Rehabilitation Medicine, 400 East 34th Street, New York, New York 10016.