Comparison of Nerves to Cerebral and Extracerebral Blood Vessels: A Differential Effect of Alpha Methyl Tyrosine on Norepinephrine Content

WILLIAM I. ROSENBLUM, M.D., AND MELISSA CHEN, M.S.

SUMMARY Alpha methyl tyrosine (AMT), an inhibitor of norepinephrine (NOR) synthesis, was injected intraperitoneally (200 mg/kg) in Sprague Dawley rats, kept in a cold room, or at room temperature for 16 hours. Using formaldehyde induced NOR fluorescence, nerve counts were made on whole mounts of cerebral and femoral arterioles 14-300 μm in diameter, utilizing a grid superimposed on the vessels. Cold had no effect on the number of visible (i.e., fluorescing) nerves. AMT had an appreciable effect but only on nerves to femoral arterioles, where a significant reduction in nerve count was observed in both cold stressed and non stressed rats, when compared with animals not given AMT. Since the counting technique is sensitive only to large depletions of NOR, we cannot conclude that AMT failed to affect NOR content in cerebrovascular nerves. However, if such an effect was present, it was much less than the effect of AMT on nerves to femoral vessels.

We suggest that the differential effect of AMT on these 2 vascular beds may indicate a lower basal level of NOR release from cerebrovascular nerves, which would correlate with the difficulty of demonstrating basal sympathetic tone in this vascular bed.

Methods

Male rats, Sprague Dawley strain, were used. Four were injected with 200 mg/kg alpha methyl tyrosine (AMT) and immediately placed one to a cage, as recommended by Gordon et al., in a cold room at 4-6 degrees C. Four others were similarly treated, except that they were injected with only the diluent for AMT. Two rats were given AMT but kept, one to a cage, at room temperature and two were similarly housed but were injected only with diluent and kept at room temperature (24-26°C). All animals were sacrificed 16 hours after injection. Rats were killed by a blow at the base of the skull, and were immediately decapitated. The basal artery, posterior communicating, proximal middle cerebral artery and branches of the latter were spread as whole mounts and the nerves in the adventitia of these vessels were examined by fluorescence histochemistry using the standard technique of Falck and others. Nerves in the adventitia of the branches from the femoral vessels were similarly prepared and studied. A quantitative technique was used which entails counting each fluorescent nerve as it crosses a grid superimposed on the whole mounted vessel. Values are expressed in terms of nerve counts per unit length of grid line. Counts are diminished if NOR is reduced to levels below those required to make the nerves fluoresce. This method is insensitive to diminutions in NOR fluorescence unless they are extreme enough to diminish the nerve count. The vessel branches were broken down into 20 size categories, based on diameter (14-300 μm). Analysis according to size category failed to show a relationship between size and counts. Therefore, the counts were averaged over all size classes in each animal. Thousands of nerve crossings were observed in obtaining the data from which these averages were calculated. The mean scores for animals in each group were analyzed according to an unbalanced analysis of variance, using a 2 X 2 factorial design for each of three dependent variables (cerebral scores, femoral scores, difference between cerebral and femoral scores) for each animal.

Results

Although rats were exposed to cold for 16 hours we were unable to find a depletion of NOR in the cold treated animals as compared with controls, even after AMT (200 mg/kg intraperitoneal) was used, as recommended by Gordon et al. The AMT blocks NOR synthesis and thereby makes it easier to demonstrate NOR depletion by preventing accelerated synthesis from compensating for the NOR release. Perhaps the small sample size mitigated against showing an affect of cold. However, we were able to demonstrate another, unexpected, phenomenon. Both control and cold-stressed rats showed an affect of AMT, greater on the nerves to femoral branches than on nerves to cerebral vessels. The NOR was significantly depleted only from nerves to the former. This is reflected in the nerve counts shown in the accompanying table.

Discussion

The data suggest either (1) a difference in synthesis or release rates for NOR in nerves to CBV as compared to ECV, or else (2) a difference in concentration of AMT in nerves to the former as compared to the latter. If there were
a low level of NOR release from nerves to the CBV this would account for the low or absent neurogenic tone reported by many workers, and would provide an explanation for our data, since release might be so slow that inhibition of synthesis would fail to deplete NOR sufficiently to be reflected as a diminution of nerve counts. Alternatively, AMT levels might remain lower in nerves to CBV than in nerves to ECV, and, therefore, AMT would have a lesser affect on the former. The latter hypothesis is amenable to an experimental test. But even if correct, it would not rule out a reduction of the effects of drugs on NOR concentration in cerebrovascular nerves since concentration of AMT in the nerve might be related to NOR release from nerves to the CBV this would account for the low or absent neurogenic tone a low level of NOR release from nerves to the CBV this would account for the low or absent neurogenic tone reported by many workers, and would provide an explanation for our data, since release might be so slow that inhibition of synthesis would fail to deplete NOR sufficiently to be reflected as a diminution of nerve counts. Alternatively, AMT levels might remain lower in nerves to CBV than in nerves to ECV, and, therefore, AMT would have a lesser affect on the former. The latter hypothesis is amenable to an experimental test. But even if correct, it would not rule out a reduction of the effects of drugs on NOR concentration in cerebrovascular nerves since concentration of AMT in the nerve might be related to NOR turnover. In any case, it appears of special interest that the reduced effects of drugs on NOR concentration in cerebrovascular nerves do not appear limited to the effects of reserpin as reported earlier, but are also seen with AMT, as reported here.

References
2. Rosenblum WI: Further notes on the binding of norepinephrine by nerves to cerebral blood vessels. Stroke 4: 813, 1973

Sudden Death from Stroke

LAWRENCE H. PHILLIPS II, M.D., JACK P. WHISNANT, M.D., AND THOMAS J. REAGAN, M.D.

SUMMARY Sudden death is defined as any death that occurs less than 24 hours after the onset of first symptoms. Strokes account for 10 to 20% of all sudden deaths. The records of all residents of Rochester, Minn., who had their first stroke during the period 1955 through 1969 were analyzed. Among 255 deaths caused by the first stroke, 52 were sudden. Twenty-six of the deaths were due to primary intracerebral hemorrhage, and 20 to primary subarachnoid hemorrhage. Only two of the sudden deaths were caused by infarction: one by pontine and cerebellar infarct and the second by a cortical infarct, which resulted in death from status epilepticus. Among the nine patients who died within 2 hours of the onset of symptoms, six had primary subarachnoid hemorrhage. Hypertension was noted in 23 of the 26 patients (88%) who died of primary intracerebral hemorrhage; 8 patients with primary intracerebral hemorrhage were on long-term oral anticoagulant therapy, and all 8 were hypertensive.

SUDDE death is commonly defined as any death that occurs less than 24 hours after the onset of clinical symptoms and is not attributable to trauma or known preexisting illness. The most frequent cause is cardiovascular disease, specifically coronary artery disease. It has been reported to have been responsible for between 50 and 90% of all sudden deaths. Neurologic disease, although not a common cause, has been responsible for many sudden deaths. The most common disease of the central nervous system responsible for sudden death is stroke. In a review that pooled the results of 10 studies of sudden death, 14% of all deaths were found to have a neurologic basis, and the major, if not the only, diagnosis was some type of cerebrovascular disease. In general, most studies have found that stroke accounts for 10 to 20% of sudden deaths, but are also seen with AMT, as reported here.

References
2. Rosenblum WI: Further notes on the binding of norepinephrine by nerves to cerebral blood vessels. Stroke 4: 813, 1973

TABLE 1 Fluorescent Nerve Counts* on Rat Cerebral and Femoral Arterioles

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cold</th>
<th>AMT + Room temp</th>
<th>Cold + AMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral</td>
<td>0.46 ± 0.18</td>
<td>0.44 ± 0.17</td>
<td>0.45 ± 0.20</td>
<td>0.37 ± 0.10</td>
</tr>
<tr>
<td>Femoral</td>
<td>1.13 ± 0.25</td>
<td>1.43 ± 0.12</td>
<td>0.34 ± 0.14**</td>
<td>0.58 ± 0.36**</td>
</tr>
</tbody>
</table>

*Alpha methyl tyrosine significantly diminished number of fluorescing nerves only in femoral artery bed (p < .01).
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