Nerve Terminal Damage in Cerebral Ischemia: Greater Susceptibility of Catecholamine Nerve Terminals Relative to Serotonin Nerve Terminals

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SUMMARY The energy-dependent uptake of \(^3^H\)-dopamine (DA), \(^3^H\)-norepinephrine (NE) and \(^3^H\)-serotonin (5-HT) was measured in synaptosomes isolated from either the whole cerebral hemispheres or the striata of gerbils after cerebral ischemia. Ischemic stroke was induced in the Mongolian gerbil by left common carotid ligation. Uptake values in the affected hemisphere (expressed as a percent of the corresponding control hemisphere) were 32.6% for DA, 35.1% for NE, and 52.0% for 5-HT, 16 hours after stroke. The differential reduction in uptake of the catecholamines relative to 5-HT was significant (p < 0.005). This differential persisted when measures were made on isolated striata from the ischemic and control hemispheres. In the latter measurements, uptake of DA was 20.7% of control and uptake of 5-HT was 44.7% of control. Uptake of both DA and NE were significantly reduced in animals exhibiting milder circling behavior, while uptake of 5-HT was not. There was no significant reduction of uptake in animals subjected to left common carotid ligation not exhibiting signs of stroke. These studies indicate a selective sensitivity of catecholamine nerve terminals to damage in ischemic stroke.

PRIOR STUDIES IN THIS LABORATORY examined the active uptake of dopamine, GABA and glutamate into synaptosomes that were prepared from gerbils at 16 hours after unilateral carotid ligation. The high-affinity uptake of neurotransmitters by synaptosomes is an energy-dependent process that requires the integrity of neuronal membrane function. No changes in uptake were noted for up to 8 hours after carotid ligation, even though animals exhibited signs of stroke. However, by 16 hours, the uptake of dopamine (DA) into synaptosomes prepared from the ischemic hemisphere was reduced to 15.2% of the unaffected hemisphere. GABA uptake was reduced to 28.0% and glutamate uptake to 47.5%. The greater effect of ischemia on the uptake of dopamine relative to glutamate was significant (p < 0.001). Potassium-stimulated release of radiolabelled transmitter from the synaptosomal preparation, following labeling of the tissue via uptake, was not affected. This indicated that the residual uptake of transmitter was into normally functioning synaptosomes and that the reduction in uptake was most probably due to loss of nerve terminals.

Marked reductions in the levels of the catecholamines DA and norepinephrine (NE) have been observed in the ischemic hemispheres of gerbils exhibiting stroke after unilateral carotid ligation. Layne et al and Meyer et al have proposed that increased release of NE and 5-HT occurs during ischemia, and accounts for the decreased levels in the brain. However, this would not explain why 5-HT levels are not reduced to the same extent as dopamine and NE in cerebral ischemia. Therefore, we studied the high-affinity active uptake of DA, NE and 5-HT into synaptosomes prepared from gerbils 16 hours after unilateral carotid ligation, in order to determine if there was a differential susceptibility of these neuronal populations to the effects of ischemia.

Materials and Methods

Male gerbils weighing 50 to 70 gm were anesthetized with approximately 35 mg pentobarbital/kg (i.p.), with the dosage titrated to the stage of surgical anesthesia for each animal. Animals could breath spontaneously without a respirator. The left common
carotid artery was exposed in the paratracheal region by blunt dissection so that there was no blood loss. After the animals exhibited partial recovery from anesthesia to the extent that they were responsive to leg pinch, the carotid artery was ligated with two ties. This method has been employed by Lust et al.3 to provide animals in whom behavioral changes associated with clinical stroke could be readily observed.

At 16 hours after carotid ligation, the gerbils were decapitated with a guillotine. The cerebrum was removed and separated into left and right hemispheres, which were immediately immersed in ice cold isotonic (0.9%) saline. This procedure took approximately 30 seconds. Each hemisphere was weighed and then homogenized in 10 ml of 0.32M sucrose. Centrifugation was performed in a Sorvall RC 2B refrigerated centrifuge with a SS 34 head. The homogenates were centrifuged at 3000 rpm (1100 x g), for 10 minutes, to remove blood and cellular debris. The supernatant was centrifuged at 15,000 rpm (27,000 x g) for 30 minutes and the pellet retained. The pellet was then resuspended at a concentration of 150 mg wet weight of tissue per ml in Krebs-Ringer phosphate buffer, pH 7.4, containing 0.05 mM pargyline and 1.7 mM ascorbic acid. This preparation has been used previously to provide a synaptosomal suspension which contains mitochondria, but is relatively free of glial elements and other subcellular particles.1,6,7 For measurements with synaptosomes isolated from striata, the striata were obtained by dissection over ice, weighed and homogenized in 3.0 ml of 0.32M sucrose. The synaptosomal pellet obtained by differential centrifugation was resuspended in the Krebs-Ringer phosphate buffer at a concentration of 10 mg wet weight of tissue/ml.

Uptake measurements1 were carried out in triplicate. Aliquots of 200 µl of the synaptosomal preparation in either 5 ml (whole cerebrum) or 2.5 ml (striata) of the Krebs-Ringer phosphate buffer were preincubated for 10 minutes at 37°C in a Precision Scientific GCA shaker bath. Radiolabeled neurotransmitters were added to a final concentration of 5 x 10⁻⁶M (cerebrum) or 2 x 10⁻⁶M (striatum). After 10 minutes, one ml aliquots were removed from replicate flasks and passed through a 0.65 µm Millipore filter by means of suction filtration to isolate the synaptosomes. The filters were washed quickly with 1.0 ml saline. They were then resuspended in 10 ml Scintiverse solution for scintillation counting of the radioactivity; radioactivity measurements were made 8–24 hours later in a Packard Model 2450 Tricarb Scintillation Spectrometer. The uptake of radiolabeled transmitters, corrected for radioactivity retained on the filter papers,1 was expressed as the CPM per mg wet weight of original tissue divided by the CPM in an equivalent volume of incubation medium, that is, the tissue-to-medium ratio. The uptake of transmitter in the ischemic left side versus the control right side was compared by expressing results as a ratio of left/right (L/R ratio). Statistical comparisons were made by paired Student “t” test for uptake of transmitter in each animal.

Radiolabeled neurotransmitters were obtained from New England Nuclear (Boston, Mass): ³H-dopamine (22 Ci/mmol), ³H-NE (13.7 Ci/mmol) and ³H-5HT (26.5 Ci/mmol). Scintiverse was from Fisher Scientific (Springfield, N.J.). Gerbils were obtained from Tumblebrook Farms (West Brookfield, Mass.)

Results

Neurotransmitter uptake was measured in synaptosomes prepared from the ischemic left cerebral hemisphere and control right cerebral hemisphere of gerbils subjected to left carotid ligation (table 1). Animals were separated into the following groups: those with no behavioral change (unaffected), those with intermediate behavioral change (circling) and those with hemiparesis (stroke). Uptake was measured at 16 hours after carotid ligation.

Uptake of DA by synaptosomes from the ischemic left hemisphere of 6 stroke animals was reduced to 30.3 ± 7.3% of that of the control right hemisphere (table 1, L/R ratio). NE uptake was similarly reduced to 35.1 ± 8.8%. 5-HT uptake was also reduced, but to only 52.0 ± 10.4% of control. The greater decrease in uptake of both DA and NE relative to 5-HT was significant (p < 0.005).

In seven gerbils with milder circling behavior, uptake of DA by synaptosomes from the ischemic hemisphere of 6 stroke animals was reduced to 30.3 ± 7.3% of that of the control right hemisphere (table 1, L/R ratio). Uptake of NE was similarly reduced to 69.5 ± 6.7%. Again, uptake of 5-HT was reduced to a lesser extent; 86.2 ± 5.3% of control. This was significantly different from the catecholamines (p < 0.05). In 10 gerbils with no observable signs of stroke, there was no decrement in uptake of the neurotransmitters in the hemisphere ipsilateral to the carotid ligation relative to the control hemisphere.

Uptake of DA and 5-HT was also studied in synaptosomes prepared from isolated striata from both the ischemic and control hemispheres (table 2). These studies were conducted in order to assess whether or not the previously observed differences (Table 1) could be ascribed to regional variations in circulation or to differential sensitivity of the nerve terminals in the same brain region. In striata from 5 gerbils with severe stroke, uptake of DA was reduced to 20.7 ± 9.3% of control (L/R ratio), while uptake of 5-HT was affected to a lesser degree, 44.7 ± 8.0% of control (p < 0.02). There was no decrement in uptake of synaptosomes from striata on the side ipsilateral to carotid ligation in 4 unaffected gerbils. The lesser effect on 5-HT uptake in stroke animals was similar to that seen in the whole hemisphere (table 1). Therefore, the observed differences between 5-HT and the catecholamines appear to be intrinsic to the neuronal types and independent of variations in cerebral circulation.

Discussion

The current study shows that the catecholamine nerve terminals utilizing NE and DA as transmitters are more susceptible to damage by cerebral ischemia than nerve terminals utilizing 5-HT. The selectivity in damage could not be attributed to regional variations in
The uptake of DA, NE and 5-HT into synaptosomes from the ischemic (left) hemisphere and control (right) hemisphere is shown at 16 hours after left carotid ligation. Uptake is expressed as the tissue to medium ratio; the counts per minute (cpm)/mg wet weight of tissue divided by the cpm is an equivalent volume of incubation medium. Figures in parentheses are the S.E.M.

The raw uptake values shown for L and R underestimate the tissue-to-medium ratio because the wet weight of original tissue was used in the denominator; actually, the synaptosomal pellet contains only approximately 25% of the original protein. However, the ratio (L/R) provides a direct comparison of the ischemic versus control hemisphere in individual gerbils and is unaffected by the method of calculation for raw values.

Uptake of all transmitters in the left (ischemic) hemisphere was significantly reduced relative to the right in stroke animals (paired "t"-test, \( p < 0.001 \) for DA and NE, \( p < 0.01 \) for 5-HT). In circling animals, uptake of DA and NE were significantly reduced in the ischemic left hemisphere relative to the right (\( p < 0.05 \)), but uptake of 5-HT was not significantly reduced. Uptake in the control right hemisphere of stroke animals was not significantly reduced relative to the right hemisphere of unaffected animals, indicating that there was no bilateral effect of ischemia on uptake.

The left:right ratios should be compared to examine the differences between transmitters. The ratios for DA and NE were similar and were significantly more affected than those for 5-HT in both stroke (\( p < 0.005 \)) and circling (\( p < 0.05 \)) groups.

### Table 1: Uptake of Dopamine, Norepinephrine and Serotonin into Synaptosomes Prepared from Cerebral Hemispheres

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>DA</th>
<th>NE</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left/right ratio</td>
</tr>
<tr>
<td>Stroke</td>
<td>6</td>
<td>1.02</td>
<td>3.01</td>
</tr>
<tr>
<td></td>
<td>(0.42)</td>
<td>(0.53)</td>
<td>(0.073)</td>
</tr>
<tr>
<td>Circle</td>
<td>7</td>
<td>1.32</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>(0.18)</td>
<td>(0.10)</td>
<td>(0.065)</td>
</tr>
<tr>
<td>Unaffected</td>
<td>10</td>
<td>2.39</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>(0.28)</td>
<td>(0.25)</td>
<td>(0.025)</td>
</tr>
</tbody>
</table>

The concentration of dopamine in the rodent striatum is in the range of 10 \( \mu \)g/g, or about 65 \( \mu \)M. This value is reduced by 70% in the ischemic gerbil (2), to about 20 \( \mu \)M. Even if all of this dopamine appeared in the medium of the diluted synaptosomal preparation, (approximately 0.8 mg wet weight per ml), the final concentration would be \( 1.6 \times 10^{-4} \)M.
lation through a pial window, both cause vasoconstriction and regional ischemia. However, NE produces histologic damage to neurons at concentrations that are not sufficient to produce ischemia. 5-HT, on the other hand, does not damage the neurons in this model even when ischemia is induced. It is possible, therefore, that the extraneuronal catecholamines observed by fluorescence microscopy may be toxic to adjacent neurons. Furthermore, the uptake in vivo of released catecholamines by nerve terminals would expose the catecholamines to monoamine oxidase (MAO) in mitochondria. MAO has been shown to remain active in the gerbil stroke model despite ischemia: that is, the levels of oxygen present are sufficient to support MAO activity. This enzyme produces the potentially toxic product, H$_2$O$_2$, during the oxidative deamination of catecholamines. H$_2$O$_2$ may, therefore, represent an additive toxic factor that contributes to destruction of catecholamine nerve terminals in ischemia. It is not clear why 5-HT terminals would not also be subject to the same stress. However, a recent study has demonstrated a cytoplasmic protein that binds 5-HT and limits the amount of 5-HT metabolized by MAO.

Our studies have demonstrated a selective sensitivity of catecholamine nerve terminals, relative to glutamate and 5-HT nerve terminals, to ischemic damage. These studies suggest that catecholamines may play a role in the mechanism of irreversible neuronal destruction in ischemic stroke.

**References**

Nerve terminal damage in cerebral ischemia: greater susceptibility of catecholamine nerve terminals relative to serotonin nerve terminals.

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