The effect of biogenic monomines on rapid axonal transport in the rabbit optic nerve.

JOHN GUY, HARRY A. QUIGLEY, AND DOUGLAS R. ANDERSON.

Since l-dopa and serotonin have been reported to increase the rate of axonal transport in rat sciatic nerve, we decided to study the effect of these monoamines on rapid orthograde transport in the rabbit optic nerve. To do this, tritiated leucine was injected into the vitreous of both eyes of 56 albino rabbits, and arrival of radioactive labeled proteins at the superior colliculus was measured at various intervals by liquid scintillation counting. Rabbits were studied 24 hr after intraperitoneal injections of (1) Sinemet® + l-dopa, (2) Sinemet® + 5-hydroxytryptophan, or (3) pargyline. There were 14 rabbits in each group compared to 14 controls that received no monoamines. In the monoamine-treated groups, transported labeled proteins arrived at the superior colliculus earlier, and an increased amount of radioactivity accumulated during the next several hours. The maximum amount of radioactive proteins accumulating in drug-treated animals did not differ significantly from the maximum amount in control animals. As judged by autoradiographic densitometry, retinal ganglion cell synthesis was similar in control and drug-treated animals. We suspect that the rate of rapid axonal transport is increased by monoamines, although an increased rate of ganglion cell protein synthesis is another possibility.

Axonal transport of macromolecules and cellular organelles from the neuronal cell body to the axon terminal includes a rapid phase moving at 400 mm/day and a slow phase moving at 1 mm/day.1 With tritiated leucine as the amino acid substrate for incorporation into synthesis of transported proteins, the rapid component is mainly membrane-bound, and the slow component is predominantly soluble proteins.

The mechanism for axonal transport has been examined by studying the effects of alterations in the normal environment of nerve cells.1 Rapid transport ceases both with complete anoxia and total chemical depletion of energy supplied by ATP. Mechanical compression also interrupts movement of transported material in the axon. Rapid transport will also cease after treatment with various chemical agents whose actions seem to depend upon disruption of intracellular microtubules. Such factors are known to cause cessation of rapid transport, but there is little information to show whether there can be partial effects manifest by a change in the rate of rapid transport. Temperature changes can cause incremented alteration in the rate of rapid transport. Increases in rapid transport have also been reported in the sciatic nerve of rats systemically treated with L-dopa and 5-hydroxytryptophan.2

Such rate changes could have important effects on the functional state of the neuron. This study suggests the possibility of an increase in the rate of rapid transport in a central nervous system tract, the rabbit optic nerve.

Materials and methods. Albino rabbits of both sexes weighing 2.0 to 3.5 kg received intraperitoneal injections of (1) Sinemet® (6 tablets/kg) + l-dopa® (150 mg/kg), (2) Sinemet® (6 tablets/kg) + 5-hydroxytryptophan® (150 mg/kg), (3) Eutonyl® (150 mg/kg), or (4) no drug. Each drug was dissolved in saline for injection. At these high doses 50% of the animals died. Those who survived for 24 hr usually showed no residual effects and were used to study axonal transport. There were 14 survivors studied in each of the drug-treated groups and 14 control animals.

Axonal transport was measured by injection of 100 μCi of tritiated leucine (L-leucine-5-3H; New England Nuclear, Boston, Mass.; 50 Ci/mmol) in 100 μl into the vitreous of 112 eyes under indirect ophthalmoscopy, after anterior chamber paracentesis had been performed under topical proparacaine (E. R. Squibb & Co., Princeton, N.J.) anesthesia. The paracentesis was to prevent 3H-leucine loss though the scleral tract after the intravitreal injection. Rectal temperatures were measured immediately before the leucine injection and again before sacrifice and remained normal. Animals were sacrificed by intracardiac injection of 2 cc of pentobarbital at intervals of 1, 2, 3, 4, 6, 8, 12, and 24 hr after the intravitreal injection. Both eyes, superior colliculi, and a sample of cerebral cortex for determination of background radioactivity were speedily dissected out and fixed in 10% aqueous formalin. The superior colliculi and cortex were weighed with a Mettler H2OT balance.

1 Merck, Sharp & Dohme, West Point, Pa. Each tablet of Sinemet contains 25 mg of carbidopa (a peripheral decarboxylase inhibitor) and 250 mg of l-dopa.
2 HCN Pharmaceuticals, Inc., Cleveland, Ohio.
3 Abbott Laboratories (North Chicago, Ill.) brand of paraglyne, a monoamine oxidase inhibitor.
Fig. 1. Arrival of radioactively labeled materials at the rabbit superior colliculus in drug-treated animals compared to controls. A, Sinemet + 5-hydroxytryptophan group. B, Sinemet + L-dopa group. C, Pargyline group. At each time is shown the mean and standard error of the mean.
Table I. Arrival of labeled protein at the superior colliculus (dpm/mg tissue)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Control Mean</th>
<th>S.E.M.</th>
<th>Control S.E.M.</th>
<th>Sinemet + 5-HTP Mean</th>
<th>S.E.M.</th>
<th>Sinemet + 5-HTP S.E.M.</th>
<th>Sinemet + L-dopa Mean</th>
<th>S.E.M.</th>
<th>Pargyline S.E.M.</th>
<th>Sinemet + L-dopa p*</th>
<th>Sinemet + 5-HTP p*</th>
<th>Control p*</th>
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<tr>
<td>2</td>
<td>107</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>&lt;0.02</td>
<td>1</td>
<td>0.4</td>
<td>&lt;0.05</td>
<td>-1</td>
<td>0.3</td>
<td>&lt;0.05</td>
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<tr>
<td>3</td>
<td>148</td>
<td>17</td>
<td>144</td>
<td>13</td>
<td>&lt;0.02</td>
<td>172</td>
<td>23</td>
<td>&lt;0.01</td>
<td>188</td>
<td>35</td>
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<tr>
<td>4</td>
<td>199</td>
<td>40</td>
<td>1,007</td>
<td>145</td>
<td>&lt;0.005</td>
<td>743</td>
<td>450</td>
<td>ns</td>
<td>428</td>
<td>135</td>
<td>ns</td>
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<td>6</td>
<td>698</td>
<td>177</td>
<td>1,265</td>
<td>264</td>
<td>ns</td>
<td>1,711</td>
<td>359</td>
<td>&lt;0.02</td>
<td>994</td>
<td>132</td>
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<td>1,441</td>
<td>444</td>
<td>1,635</td>
<td>443</td>
<td>ns</td>
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<td>739</td>
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<td>2,859</td>
<td>717</td>
<td>ns</td>
<td>1,952</td>
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<tr>
<td>24</td>
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<td>357</td>
<td>994</td>
<td>463</td>
<td>ns</td>
<td>2,341</td>
<td>675</td>
<td>ns</td>
<td>2,238</td>
<td>471</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

*p*By 2-tailed t test, compared to control group at same time.

Table II. Ganglion cell autoradiographic densitometry in 17 rabbits (expressed as % light transmittance*)

<table>
<thead>
<tr>
<th></th>
<th>At 1 hr</th>
<th>At 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of eyes</td>
<td>M</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>57.9</td>
</tr>
<tr>
<td>Sinemet + 5-HTP</td>
<td>5</td>
<td>56.6</td>
</tr>
<tr>
<td>Sinemet + L-dopa</td>
<td>4</td>
<td>33.0</td>
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<td>Pargyline</td>
<td>3</td>
<td>73.8</td>
</tr>
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</table>

*Inversely related to density of grains in the autoradiograph.

(Mettler Instrument Corp., Princeton, N.J.) and subsequently dissolved in 0.5 cc of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) for 3 days at 45° C. One drop of 4M ammonium hydroxide and 15 cc of Liquiflor (New England Nuclear) in toluene were added to the samples, which were then counted twice in a Packard Tricarb 3375 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downer’s Grove, Ill.) at a mean counting efficiency of 43%. The method of internal standardization was used to detect any quenching.

To measure radioactively labeled protein in ganglion cell bodies, autoradiographic densitometry was used on 29 eyes of 17 rabbits. Retinal tissue obtained 1 or 2 hr after tritiated leucine precursor injection was postfixed in 2% phosphate-buffered osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin. Sections (2μm) were cut on an ultramicrotome, placed on glass slides, and coated with Kodak NTB-2 emulsion (Eastman Organic Chemicals, Rochester, N. Y.). After a 2-week exposure they were developed in Kodak D19 for 2 min and fixed in Rapid Fix. At a magnification of 1,250×, individual ganglion cells were centered under a slit (diameter 2 mm) of the densitometer. The light passing through the cell and the autoradiographic grains overlying it was directed into the densitometry slit and measured on a scale from 0 to 100% light transmittance. The more grains and, therefore, the greater the radioactive protein in each cell, the lower the transmittance. Between 40 and 60 ganglion cells were measured in each eye, with the observer unaware of whether the eye being examined was drug-treated or control.

**Results.** The effects of biogenic amines on rapid axonal transport are shown in Fig. 1 and Table I. In all three drug-treated groups, the labeled material was detected in the superior colliculus 1 hr earlier than in the controls. Over the next several hours the amount of accumulated radioactivity in the superior colliculus was higher in the drug-treated groups. However, the maximum level representing the equilibrium between arriving material and catabolic turnover was the same in all groups.

The effect on rapid transport was more striking when exogenous monoamines were given (Fig. 1, A and B), compared to pargyline treatment (Fig. 1, C) which increases endogenous serotonin and dopamine levels. Since pargyline was given only 24 hr prior to transport measurement, it is possible that the monoamine levels were not as high as with exogenous administration. To explore whether an increased rate of synthe-
sis might explain the increased arrival of labeled protein (an alternative to the hypothesis that the results are explained by more rapid transport), microdensitometry of silver grains overlying ganglion cells in autoradiographs was performed on specimens obtained 1 and 2 hr after the \(^3\)H-leucine injection. The results shown in Table II indicate that there might have been an increased rate of ganglion cell synthesis at 1 hr in the Sinemet + L-dopa animals (\(p = 0.05\), one-tailed rank test) but not in the other two experimental groups.

Discussion. The arrival of labeled macromolecules at the rabbit superior colliculus is dependent on at least two factors. The first is the rate of synthesis in the ganglion cells. At a higher rate of synthesis, more labeled material might enter the axon earlier, therefore arriving at the superior colliculus sooner. The second is the rate of axonal transport of labeled macromolecules from the ganglion cell body to its axon terminal, which would increase the rate of arrival of labeled material even if the rate of synthesis is unchanged.

An increase in the gross rate of synthesis was not detected at 1 or 2 hr in two out of three experimental groups, although in the Sinemet + L-dopa group it seems that an increased rate of synthesis by retinal ganglion cells might have occurred. Interpretation of the results is difficult because cell body synthesis includes a wide spectrum of macromolecules, each of which may have its own rate of transport.\(^3\) Only a small amount of the synthesized protein will leave the cell by rapid transport. Our method, which is limited to observing total synthesis, might not be adequate to detect small but important changes in the amount of rapidly transported protein, which is the component studied when the first-arriving radioactivity is measured at the superior colliculus. Furthermore, serotonin and dopamine might specifically increase synthesis of the faster-moving proteins that have already departed from the ganglion cell at the time of the autoradiograph, or there may be increased synthesis of only certain of the rapidly transported macromolecular species. In this regard, it is notable that in the Sinemet + L-dopa group, there is more radioactivity (i.e., less light transmittance) in the ganglion cell at 1 hr than there was at 2 hr (Table II), as if indeed there was an increase in synthesis of rapidly transported material or one of its major components. Our method of measuring total labeled protein arrival at the superior colliculus is not able to distinguish shifts in specific protein species transported. Thus, although we have demonstrated an increased rate of arrival of labeled material at the superior colliculus, further work would be needed to determine whether this effect was due to an increased rate of rapid transport, an increased rate of ganglion cell synthesis, or an increase in synthesis of a specific protein species.

If monoamines cause an increased transport rate, one might ask how they do so. Although biogenic amines can act as neurotransmitters, neither serotonin nor dopamine is reported to be the transmitter involved in this system.\(^4\) There are two possible explanations. First, there may be an indirect effect on transport caused by altered synaptic transmission in the rabbit superior colliculus. Dopamine and serotonin have been reported to decrease synaptic transmission in the cat lateral geniculate nucleus.\(^5\) In the mollusc Aplysia californica, these monoamines likewise impair cholinergic neurotransmission by decreasing the functional availability of neurotransmitter at the presynaptic nerve endings.\(^6\) Since materials required for synaptic transmission at the axon terminal are supplied from the cell body via rapid axonal transport, the rate of transport might be responsive to the functional level of neurotransmitter by a feedback system. Thus, if dopamine and serotonin cause a decrease in available transmitter in the rabbit superior colliculus, it is reasonable that the rate of transport or the rate of ganglion cell synthesis could speed up in an effort to restore normal synaptic transmission. Second, there could be a change in transport rate by a direct effect of monoamines on the microtubules. Serotonin has been reported to cause movement of cilia, whose motion is generated by their microtubular substructure. Conceivably, serotonin could have a similar stimulating effect on neuronal microtubules, which seem to be involved in the mechanism of axonal transport.

Alterations in normal axonal transport have been demonstrated in a number of experimental models of optic nerve disease. After transection of the goldfish optic nerve, axonal transport may increase in rate coincident with the regeneration of axons, but this does not seem to occur in primate optic nerve, which does not regenerate.\(^8\) In experimental disk edema and acute experimental glaucoma, axial transport is slowed or obstructed at the optic nerve head. Possibly the induced abnormality in transport in these latter two conditions might be reversed to some degree by pharmacologic manipulation with monoamines. We are presently evaluating the effect of monoamines on the rate of transport in primate optic nerve and on...
the transport blockade produced by acute intraocular pressure elevation.

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Key words: axonal transport rate, rabbit, optic nerve, retinal ganglion cell, serotonin (5-hydroxytryptophan), L-dopa, pargyline, superior colliculus

REFERENCES

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