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CONCENTRATION DEPENDENCE OF RAPID AXONAL TRANSPORT: A STUDY OF THE TRANSPORT KINETICS OF [^{35}S]METHIONINE-LABELED PROTEIN IN POSTGANGLIONIC SYMPATHETIC FIBERS OF THE BULLFROG[1]

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Abstract

The kinetics of transport of radiolabeled proteins in sympathetic axons of the bullfrog sciatic nerve were examined after injection of [^{35}S]methionine into the S9 sympathetic ganglion. Under resting conditions at 20°C, the fastest moving material was carried distally at 5.7 ± 0.3 mm/hr. Various manipulations of temperature in the proximal part of the nerve were used to alter the amount of protein transported into the distal region, which was always kept at 20°C. The velocity in this test region was found to increase to over 9 mm/hr when material that had accumulated at a cold block for 4 hr was released by rewarming. This acceleration was transient, and base line velocity was regained after 2 hr. In order to increase the local concentration of transported protein by a second method, the proximal part of several nerves was warmed to 28°C. Maximal transport velocity in the 20°C test region rose to 6.2 ± 0.12 mm/hr. To decrease the local concentration of transported protein, the proximal part of other nerves was cooled to 15°C. Maximal transport velocity in the 20°C test region fell to 4.7 ± 0.7 mm/hr. We conclude that there is a small but real tendency for the velocity of rapid axonal transport in this neural system to be positively related to the availability of material suitable for transport.

Although great effort has been made to understand how proteins and organelles are translocated in axons, little can be said with assurance about the mechanism of rapid axonal transport. The various theories attempting to incorporate presently available data are quite diverse in the anatomical substrates and physiological mechanisms invoked. They do not even agree on the fundamental question of whether transported materials must associate with a specific part of the transport machinery or whether they merely move passively in a stream of thixotropic fluid. This state of affairs is well reviewed in recent articles and monographs (Schwartz, 1979; Grafstein and Forman, 1980; Ochs, 1982).

To formulate reasonable theories of a physiologic process, one must have a firm grasp of its basic properties. In the case of rapid axonal transport, the morphological framework, metabolic requirements, effects of temperature, and the role of ions must all be defined. It is particularly important to determine how the rate of transport depends upon the availability of material to be transported, since this relationship constitutes the "substrate kinetics" of the transport system.

Although the concentration of transported material is difficult to manipulate experimentally, there have been reports that this variable influences the velocity of transport. For example, Goldberg et al. (1976, 1978) found that the velocity of rapid transport of [^{3}H]serotonin in single giant axons of *Aplysia californica* varied directly with the axonal concentration of serotonin-containing vesicles. On the other hand, experiments in this laboratory (Brimijoin, 1979) were unable to detect a change in transport velocity of dopamine β-hydroxylase when the concentration of enzyme moving down the rabbit sciatic nerve was altered.

Such divergent findings could reflect the existence of different mechanisms for axonal transport in different species, but this explanation seems unlikely. Alternatively, the discrepancies could reflect differences in the techniques of measurement, in the behavior of single axons as compared with large populations, or in the neuronal handling of exogenous transmitter and endogenous protein.

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The purpose of the present investigation was to clarify this issue by re-examining the relationship between the concentration of transported material and the velocity of transport. We chose the frog for study since much is known about the kinetics of rapid axonal transport in this species. A technique was developed to inject radiolabeled amino acid into the sympathetic ganglia and then, by manipulating the local temperature, to alter the flux of protein into adjacent regions of the sciatic nerve. Our results demonstrated a dependence of transport velocity on the amount of material available for transport.

Materials and Methods

Animals. Bullfrogs (*Rana catesbeiana*) with hindlegs longer than 6 inches were obtained from Nasco Biological Supply (Fort Atkinson, WI) or Central Valley Biologicals (Clovis, CA); they were kept in Plexiglas containers with fresh water at room temperature (20 to 22°C) and were used within 3 weeks of arrival. The animals were killed by decapitation, skinned immediately, and immersed in a bath containing an oxygenated frog Ringer's solution consisting of: NaCl, 11 mM; KCl, 1.9 mM; $CaCl_2$, 1.1 mM; $MgSO_4$, 1.6 mM; NaH_2PO_4, 0.45 mM; Na_2HPO_4, 0.26 mM; and glucose, 11 mM, at pH 7.4. The back and hindlimbs with the spinal cord, sciatic plexus, accompanying sympathetic nervous system, and sciatic nerve were isolated and kept intact (Fig. 1).

Injection. After the initial dissection, the S9 sympathetic ganglion was located on each side and freed from the surrounding tissue, avoiding damage to its capsule. As shown in Figure 1, it is this ganglion which contributes most of its postganglionic fibers to the sciatic plexus and nerve. A glass micropipette was drawn to a tip diameter of ~40 μm and beveled to a 60° angle. The micropipette was backfilled with [^{35}S]methionine (~1200 Ci/mmol; New England Nuclear Corp., Boston, MA) which had previously been lyophilized and reconstituted in 0.9% NaCl at a concentration of 10 μCi/μl. The capsule of the ganglion was impaled with the tip of the micropipette and 1.0 μl of fluid was injected into the capsule in 10 to 15 sec. The injection was monitored visually and was considered adequate when ballooning of the capsule could be seen.

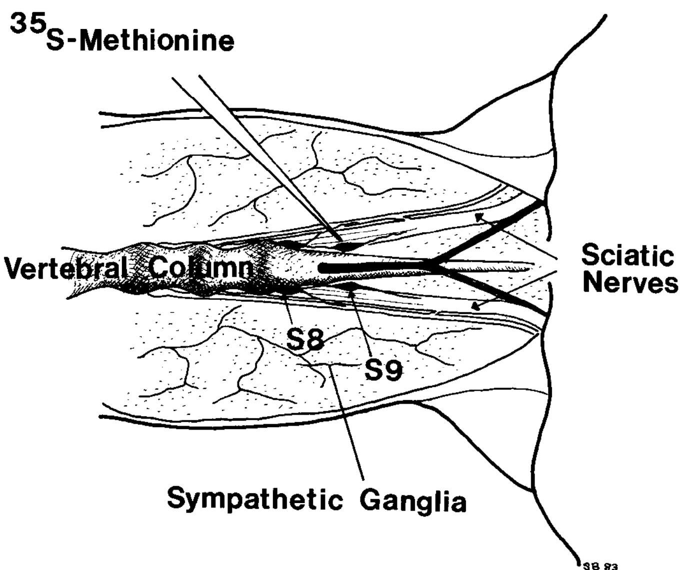

Figure 1. Anatomical relation of the sympathetic ganglia and sciatic plexus in the bullfrog. Ventral view. Overlying viscera have been removed, except for the distal portion of the abdominal aorta lying over the tail bone. The terminal ganglia of the sympathetic chain are drawn in *black* for emphasis. Postganglionic branches of S9 are shown contributing to the sciatic plexus and nerve. By means of a glass micropipette (indicated diagrammatically), [^{35}S]methionine was injected into both S9 ganglia. Later, a ligature was tied between S9 and the spinal cord. The ganglion, plexus, and sciatic nerve were then transferred to incubation chambers (see the text).

Apparatus. After injection of the ganglion, the preparation remained in the dissection bath for either 15 or 60 min. Then the S9 sympathetic ganglion with its efferents intact was carefully dissected free, along with the sciatic plexus and nerve. The preparation was ligated proximally (above the ganglion) and distally (below the bifurcation of the tibial and peroneal nerves) with 5-0 nylon thread. The isolated length (usually 9 to 11 cm) was determined after applying the minimal amount of tension needed to straighten the nerve. Depending on the experiment, the nerve was placed in one of three different chambers. For studies of the base line velocity, nerves were incubated for 3 to 12 additional hr in a 250-ml container filled with oxygenated frog Ringer's solution at a temperature of 20°C, monitored by a thermistor probe. For experiments involving temperature gradients, nerves were placed in two-compartment chambers. As previously described (Brimijoin and Helland, 1976), these chambers consisted of Plexiglas tubes with inlets at either end and a central slit for outflow of bathing solution. By controlling the temperature at the inlets, any desired temperature differential could be obtained, with the change confined to the outflow region. For stop-flow experiments, in which transport was temporarily halted by a cold block, three-compartment chambers were used (Brimijoin, 1975). In these chambers, the proximal part of each preparation was maintained at 28°C, the distal part was kept at a uniform 20°C, and a 3-mm intermediate segment was cooled to 5°C for 4 hr. Subsequently, the midsegment was rewarmed to 20°C and the preparations were incubated for an additional 1, 2, or 4 hr.

Extraction and measurement of labeled protein. In temperature gradient and stop-flow experiments, the position of the temperature boundary was marked lightly with graphite powder while the preparation was still in the apparatus. After removal, all nerves were stretched to their reference length and immediately cut into consecutive 3-mm segments. Individual segments were placed in small glass vials with 1 ml of 10% (w/v) trichloroacetic acid and kept overnight at 5°C. The acid, containing "soluble" radioactivity, was then discarded and the nerve segments were dissolved overnight in 125 μl of Soluene-100 (Packard) at room temperature. The solubilized samples were diluted with 5 ml of Dimilume-30 (Packard) and the amount of ^{35}S-labeled material was determined by liquid scintillation spectroscopy.

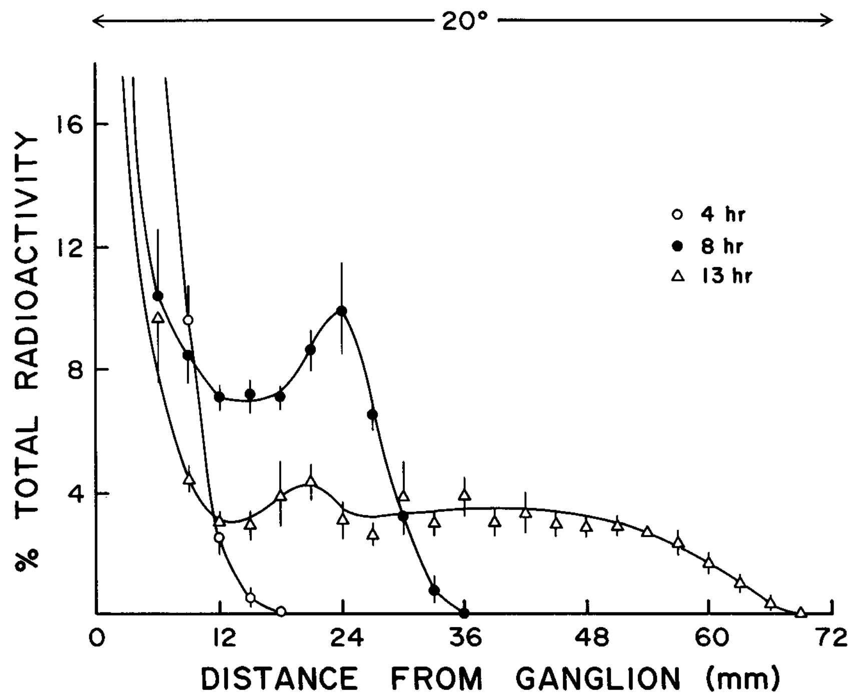

Figure 2. Waves of radiolabeled protein undergoing rapid axonal transport in postganglionic sympathetic fibers. To minimize variation between preparations a relative labeling index for each segment was calculated by expressing radioactivity as a percentage of the total in the wave (see "Materials and Methods"). Means and standard errors of these values are shown for the groups of nerves incubated at 20°C and examined 4, 8, and 13 hr after injection of the S9 sympathetic ganglion with [^{35}S]methionine. Note that, even with constant actual radioactivity, the labeling index of a given segment will fall with longer incubation time because of continued export of labeled material from the ganglion into the axons.

Data analysis. Background labeling was defined as the mean radioactivity in five segments of the preparation distal to the wavefront (typically averaging 25 cpm/segment). In order to facilitate comparison among experiments, the radioactivity in the separate segments of each nerve was expressed, after subtraction of the background, as a percentage of the total radioactivity distal to the ganglion:

$$\text{Relative labeling index} = \left(\frac{\text{cpm/segment} - \text{background cpm}}{\sum_{1}^{n}(\text{cpm/segment} - \text{background cpm})}\right) \times 100$$

The position of the front of the wave of transported radioactivity was determined by finding the most distal nerve segment that had more than twice the background radioactivity *and* had a relative labeling index of at least 0.25. When front positions were determined independently by a second observer ignorant of the experimental conditions, the two determinations were always in good agreement.

For statistical analysis, mean values and standard errors of the means were calculated. The *t* test was used as a measure of the statistical significance of differences between paired means. Analysis of covariance (Snedecor and Cochran, 1967) was used to determine whether there were significant differences between calculated velocities (which were based upon the regression of location at different times). Regression coefficients were calculated by the method of least squares, using a Hewlett Packard Model 85 microcomputer.

Results

The initial experiments were designed to determine the maximal velocity of axonal transport of [^{35}S]methionine-labeled proteins in sympathetic axons under resting conditions at 20°C. This temperature was chosen since it is the midpoint of the range over which transport velocity in the frog sciatic nerve is smoothly related to temperature. After the S9 sympathetic ganglia were injected with [^{35}S]methionine, the isolated ganglia and nerves were incubated at 20°C for 4 to 13 hr, as described under "Materials and Methods." The resulting profiles of radiolabeled protein are shown in Figure 2. In each experiment, the position of the front of the wave of labeled protein was determined as described above, and the regression of front position on time was calculated. The fit of the regression line was excellent (Fig. 3); the slope indicated a maximal transport velocity of 5.7 ± 0.3 mm/hr at 20°C.

A lag between injection of labeled precursor and onset of transport is evident from the data of Figure 3. This delay, which presumably reflects the interval needed to synthesize new protein and package it in a form suitable

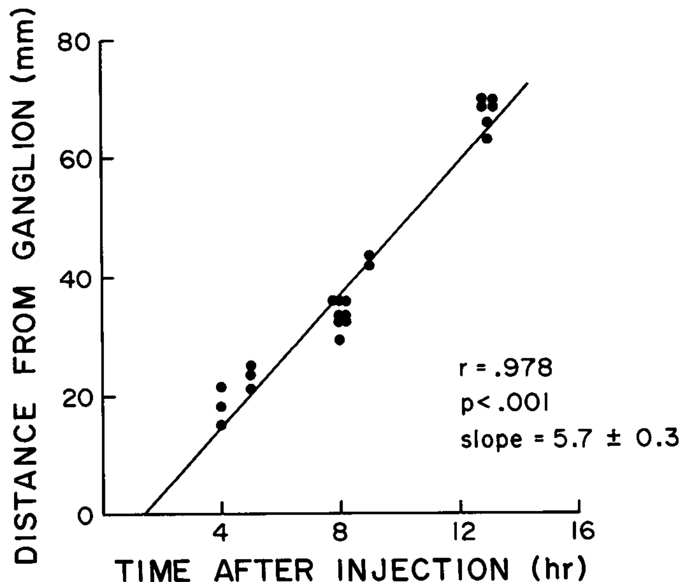

Figure 3. Velocity of axonal transport in "resting" sympathetic nerve fibers. The position of the fronts of the waves of labeled protein (estimated as described under "Materials, and Methods") are plotted as a function of time after injection of the S9 ganglion with radioactive amino acid. Temperature was maintained throughout at 20°C. Each *point* represents data from one nerve. The regression line was fitted by the method of least squares. Its slope indicates a velocity of 5.7 mm/hr. The positive intercept on the x axis indicates a lag of approximately 90 min before onset of transport.

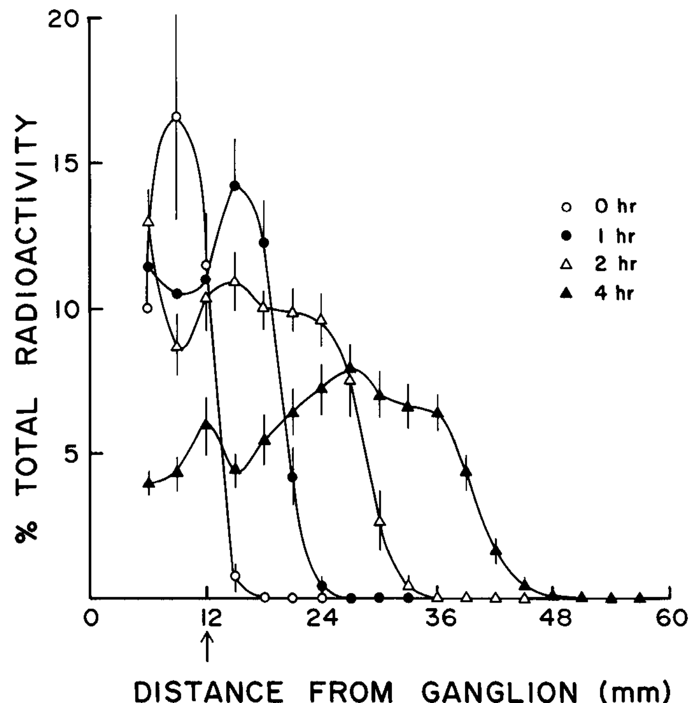

Figure 4. Downflow of labeled protein in sympathetic nerve fibers rewarmed after 4 hr of local cooling. The *arrow* marks the midpoint of an approximately 3-mm region cooled to 4°C. Proximal to this point (to the *left*) the nerve was always at 28°C, while distally it was always at 20°C. The profiles of the waves of radiolabeled protein are shown for the nerves examined 0, 1, 2, and 4 hr after the cooled segment was rewarmed to 20°C.

for transport, can be estimated from the intersection of the regression line with the time axis. In these experiments, the lag time was 90 min.

The next procedures were designed to increase the flux of material into the distal segments of the nerve and thus raise the local concentration of this material. After injection of the S9 ganglion with [^{35}S]methionine, the sciatic nerves were placed in three-compartment chambers, and the central region was cooled to 5°C. Since axonal transport in bullfrog nerve is reversibly blocked at this temperature (Edström and Hanson, 1973), these conditions can be used for a stop-flow experiment in which moving materials are allowed to accumulate at the proximal border of the cooled zone and are then released by rewarming of the nerve. To increase concentration by a large amount, the cold block was maintained for 4 hr. Proximal to the cooled zone, the nerve was kept at 28°C; distal to the zone the temperature was 20°C. By the end of the first phase of the experiment, little radioactivity had penetrated the cold block (Fig. 4). The temperature of the cooled zone was then raised to 20°C, and the accumulated radioactivity rapidly resumed its distal migration (Fig. 4). During the first 2 hr after rewarming, the wavefront moved almost exactly 18 mm (Fig. 5) which corresponds to a velocity of 9 mm/hr (more than 50% above the base line velocity). Displacement of the front during the next 2 hr was about 12.5 mm (Fig. 5), corresponding to transport at 6.2 mm/hr (only slightly above base line). A comparison of the regressions by analysis of covariance (Snedecor and Cochran, 1967) showed that the acceleration during the first 2 hr after rewarming was statistically significant (Table I). These results imply that cooling and rewarming of bullfrog sympathetic axons led to a transient but substantial rise in the velocity of transport of at least some labeled protein.

In an attempt to define more precisely the relationship between concentration and velocity, a series of experiments with small step gradients of temperature were carried out. From previous measurements of its dependence on temperature between 6° and 30°C (Edström and Hanson, 1973) we assumed that transport velocity in bullfrog sympathetic nerve would obey the equation

$$V = 0.93\, e^{(0.095)T}$$

where V is in millimeters per hour and T is in degrees Centigrade. The equation predicts that transport at 28°C will be twice as fast as at 20°C. One therefore expects that the flux of material from a proximal piece of nerve at 28°C into an adjoining distal one at 20°C will be twice the initial flux across the distal border of the latter. These conditions will therefore lead to an increase in the concentration of transported material in the distal segment. With no change in transport velocity, the increase in concentration at equilibrium should be 2-fold. If transport in the distal region were accelerated, however, the increase would be less.

One hour after injection of the ganglion with [^{35}S]methionine, nerves were placed in two-compartment chambers so that the first 9 mm distal to the ganglion could be incubated at 28°C while the rest of the nerve

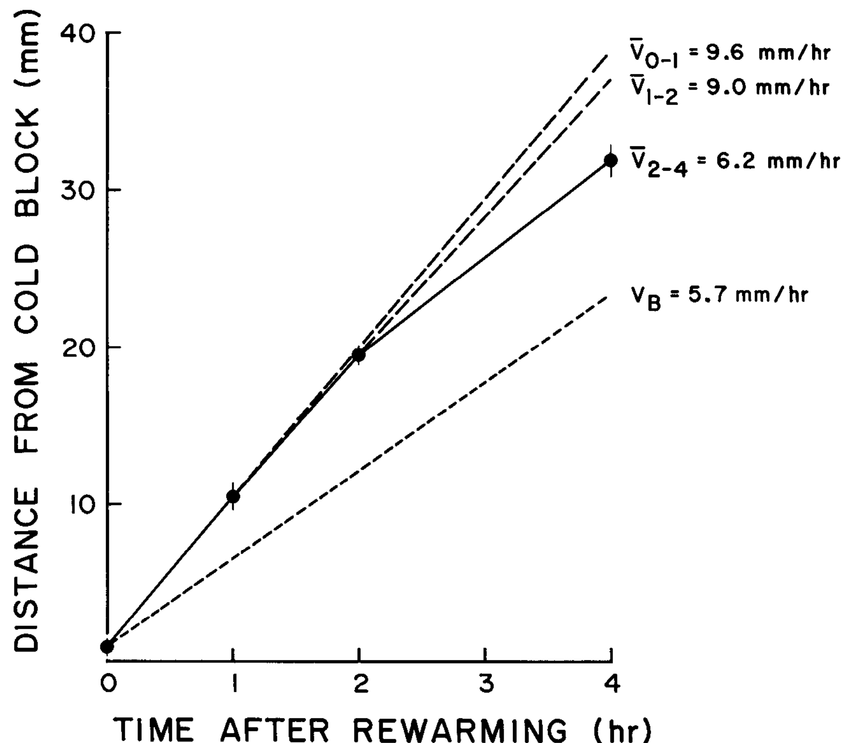

Figure 5. Time dependence of transport velocity in rewarmed nerves. Mean positions of the fronts of the waves of labeled protein are plotted versus time of rewarming. The slopes of the lines indicate velocities. The *dashed lines* (*top*) extrapolate displacement at the rates maintained during the 0- to 1-hr and the 1- to 2-hr intervals ($\bar{V}_{0-1}$ and $\bar{V}_{1-2}$). The *small-dashed line* (*bottom*) shows the displacement expected from a steady velocity of 5.7 mm/hr (V_B, the base line rate).

TABLE I

Comparison of the velocity of transport under base line conditions with velocity during the first 2 hr after rewarming of locally cooled nerves

Analysis of covariance was as described by Snedecor and Cochran (1967).

Source of Variance	Total		Regression Coefficient	Residual		
	df	Sum of Squares		*df*	Sum of Squares	Mean Square
Within Groups						
Base line transport (4–13 hr)	21	7595	5.7	20	342.0	17.1
Cooling-rewarming	21	1327	9.4	20	82.5	4.1
				40	424.5	10.6
Pooled	42	8922	5.9	41	605.0	14.6
	Difference between slopes			1	180.5	180.5
Comparison of slopes:	$F = 180.5/10.6 = 17.0$ ($df = 1$ & 40) $p < 0.001$					

was kept at 20°C. The preparations were then incubated for an additional 2.5 or 6.5 hr. The results show that the wave of labeled protein crossed the temperature boundary into the cooler distal region during the first 3.5 hr after injection (Fig. 6*B*). The mean transport velocity of the fastest moving proteins during the next 4 hr was calculated from the difference between the positions of the wavefront at the end of the two incubation periods. According to this calculation, the velocity of transport through the distal region was 6.2 ± 0.12 mm/hr. This represents a 10% increase over the velocity measured at the same temperature under resting conditions, but the increase was not statistically significant.

If transport velocity *were* positively related to concentration of transported material, one might be able to detect a slowing of transport when the concentration fell. To test this possibility, nerves were incubated in a "reversed" temperature gradient, in which the proximal region was cooled to 15°C while the distal region was held at the usual 20°C. From the temperature-velocity equation it was anticipated that the flux of material into the nerve segments of the distal region would be about half the initial efflux. With no compensating changes in velocity, one could therefore expect the local concentration of material available for transport to fall by 50%.

For these experiments, the nerves were transferred to the incubation chamber within 15 min after the S9 ganglia were injected with [^{35}S]methionine. The subsequent incubation was for 6 hr or 10 hr. As shown in Figure 6*A*, the shorter incubation was sufficient for the

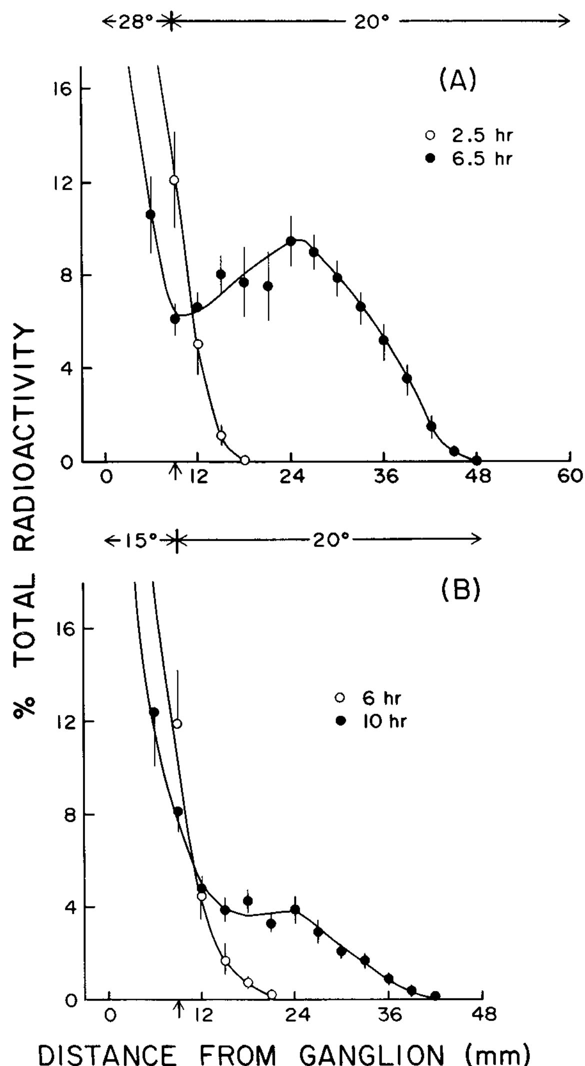

Figure 6. Effect of temperature gradients on transport velocity. *A*, Negative temperature gradient: proximal region at 28°C, distal region at 20°C. Expected velocity in the proximal region was twice that in the distal, with resulting increase in concentration of transported protein distal to the temperature boundary. *B*, Positive temperature gradient: proximal region at 15°C, distal region at 20°C. Expected velocity in the proximal region was half that in the distal, with resulting decrease in concentration of transported protein distal to the temperature boundary.

wave of transported label to cross the temperature boundary, even though transport from the ganglion was fairly slow. During the ensuing 4 hr in the 20°C test region, the transport velocity averaged 4.7 ± 0.75 mm/hr. This velocity is 18% slower than the baseline velocity and 25% slower than the velocity recorded in the presence of a 28° to 20°C temperature gradient. The difference from baseline is not statistically significant, but the difference between the velocities produced by the two types of temperature gradient did appear to be real. The front positions after the shorter incubations were both about 6 mm distal to the temperature boundary (Fig. 6). However, 4 hr later, statistically significant amounts of transported label appeared 42 mm from the boundary in the nerves warmed proximally to 28°C. By contrast, in the nerves cooled proximally to 15°C, the same additional period of incubation brought significant amounts of label no farther than 33 mm from the boundary. Comparison of Figure 6, *A* and *B*, shows the clear-cut displacement in the final profiles of radioactivity.

Discussion

The results of our experiments strongly suggest that the maximal velocity of rapid axonal transport in frog sympathetic nerve does depend on the concentration of transported material. Nevertheless, it is legitimate to ask whether this finding could represent an artifact of the experimental method.

One type of artifact that can be definitively rejected is a spread of temperature from the proximal to the distal chamber of the incubation apparatus. Direct monitoring by thermistor probes showed that the temperature in the distal test region remained at a uniform 20°C right up to the border of the outflow zone (i.e., the chamber boundary).

A second, subtler artifact that deserves to be considered is bias in the estimated position of the wavefront. In particular, one might be concerned about the possibility of a systematic tendency to choose more distal positions for the wavefront of radiolabel when the amount of label in the wave was increased, and vice versa. This possibility strikes us as unlikely for the following reason. The spontaneous variation in the total amount of transported radioactivity from preparation to preparation was up to 100-fold, owing to differences in the placement of injections and in the metabolic status and anatomical arrangement of the sympathetic ganglia. This source of random error should have overwhelmed any systematic error attributable to the 2- to 4-fold variations in radioactivity among the different experimental groups. Despite the large spontaneous variation in labeling, the standard deviations of wavefront location in each experimental group were small (averaging 1.9 mm). Furthermore, within groups there was virtually no relation between the total amount of radioactivity and the calculated velocity of transport. For example, in the experiments on base line velocity the coefficient of correlation of these variables was $r = 0.04$ ($n = 22, p > 0.5$). We concluded that our calculated transport velocities were not biased toward high values in experiments where the concentration of transported label was locally increased.

Our results are consistent with those obtained by Goldberg et al. (1976, 1978) in their studies of the transport of [^{3}H]serotonin in the giant cerebral neuron of *Aplysia californica.* Both sets of results indicate that transport velocity may increase up to 50% when the local concentration of transported material rises to a value that is twice normal or somewhat greater. In addition these results agree in indicating that velocity may fall when the concentration of material is reduced. This agreement is significant since the techniques used by Goldberg and co-workers to raise and lower the concentration of serotonin-containing vesicles (i.e., ligation of

collaterals and poisoning of protein synthesis, respectively) were radically different from those used in our experiments. A further consideration favoring the generality of these results is the evolutionary gulf between the giant invertebrate axon and the vertebrate sympathetic neuron.

On the other hand, if transport velocity depends universally on the concentration of available material, it is difficult to explain the failure of several deliberate attempts to detect this relationship. At first thought, it seems that a poor signal-to-noise ratio might have obscured an acceleration in transport of dopamine β-hydroxylase when rabbit nerves were subjected to temperature gradients (Brimijoin, 1979). The radioactivity in a wave of isotopically labeled protein is usually many times greater than background, whereas the peak concentration of enzyme activity in a stop-flow experiment is rarely even twice that of the "base line." The displacement of a wave of labeled protein can therefore be determined much more precisely. Nevertheless, the demonstrated precision of measurements of the transport velocity of dopamine β-hydroxylase and other proteins by "stop-flow" is certainly high enough to reveal a 50% acceleration.

This problem will not be resolved until the transport of dopamine β-hydroxylase and that of radiolabeled proteins are compared in a single neural system. Our unpublished data suggest that the transport of dopamine β-hydroxylase in sympathetic neurons of the frog is less dependent on concentration than is that of radiolabeled protein in general. If these data should be supported by further study, one would be forced to consider the possibility that the concentration dependence of transport is different for different types of organelle.

Two other reports raise questions about the relationship between concentration of material and velocity of transport. O'Brien and Snyder (1982) used a multiple proportional counter for real-time analysis of the transport of [^{35}S]methionine-labeled protein in sensory axons of the bullfrog sciatic nerve. Despite the application of a cold block followed by rewarming, the velocity of the fastest moving label was calculated to be only 6.1 mm/hr at 23°C, in good agreement with other estimates of base line velocity at this temperature. However, there are reasons to think that the concentration of transported protein was not greatly elevated during the main observation period. First, the phase of cooling was as brief as 2 hr, and the ganglia together with proximal nerve were removed from the preparations 2 hr after rewarming. These conditions would tend to limit the amount of protein delivered distally. Second, measurements were not begun until about 2 hr after rewarming, which is long enough for the pulse of moving material to have dissipated. Our own data (Fig. 4) show a return to base line velocity shortly after 2 hr of rewarming. Therefore, the results of O'Brien and Snyder (1982) do not rule out the kind of transient, concentration-dependent acceleration that we have seen. In fact, a closer look at Figure 2 of O'Brien and Snyder (1982) suggests additional evidence *for* such acceleration. The delay between rewarming the nerves and transferring them to the proportional counter apparently allowed the labeled protein to reach the most proximal counter window. Since the distance traversed was about 20 mm and the time taken was about 2 hr, a rough estimate of the initial transport velocity would be 10 mm/hr, which is about 50% faster than the velocity measured subsequently.

Most recently, Bisby (1983) has also addressed the issue of concentration dependence in the system for rapid transport. His experiments involved injection of rat sensory ganglia with [^{3}H]leucine, local cooling of the sciatic nerve *in vivo*, and subsequent rewarming. The results provide no sign of faster than normal transport, even during the first hour after release of the cold block (Bisby, 1983). The explanation might lie in the fact that mammalian nerves at body temperature are already operating near the upper limit of their range of possible transport velocities. However, it is worth pointing out that local cooling leaves a depleted zone distal to the temperature boundary, which must be filled up when the nerve is rewarmed. It was to hasten this phase and to ensure maximal local concentration of transported protein that we kept the proximal part of our nerves 8°C warmer than the distal one, both during and after the cooling period.

Clearly, more experiments are needed to define the conditions under which changes in the concentration of material available for transport increase or decrease the transport velocity. Meanwhile it is appropriate to begin considering what this phenomenon implies for the mechanism of transport.

There are some difficulties in incorporating concentration dependence of transport velocity into the "microstream" theory, according to which local shearing forces propel fluid streams through the axon, carrying suspended matter along (Gross, 1975). However, the present results can be accommodated if one is willing to assume that the stationary compartment is saturable, so that any increase in the total concentration of material is disproportionately greater in the moving compartment.

Alternatively, if one believes that rapid transport reflects a specific interaction between the transported material and the "machinery" of transport, one can make certain inferences about the nature of this interaction. As Goldberg et al. (1978) point out, a positive concentration dependence of transport velocity suggests positive cooperativity in the association of transported organelles with the "transport-track." Mackey et al. (1981) have generated an explicit mathematical model of this effect. An independent mathematical approach (Rubinow and Blum, 1980) fount it necessary to invoke positive cooperativity even to reproduce the profiles of the waves of radiolabeled protein generated in "resting" nerves.

Although many models of axonal transport can encompass positive cooperativity, the physical basis for such cooperativity is not yet obvious. It is certainly conceivable that the association of one vesicle with a moving transport filament (e.g., as envisaged by Ochs, 1982) somehow favors the binding of a second vesicle. It is also possible that certain rapidly transported organelles have a *mutual* affinity. If particles were to move as aggregates, the binding of any one member to the transport track would ensure the transport of the whole. The result might

indeed be a positive concentration dependence of transport velocity.

Continued study of the kinetics of rapid axonal transport under a variety of conditions in which the availability of transported materials is perturbed in specific ways offers the potential for elaborating and testing theories of the mechanism of transport.

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