LETTER TO THE EDITOR

Dependence of Fast Axonal Transport on the Local Concentration of Organelles

Dear Sir:

In a recent paper published in the *Biophysical Journal,* Rubinow and Blum (1980) developed a mathematical model for analyzing rapid axonal transport. In this model, n particles are assumed to move distally along the axon when they become associated with a carrier at an average velocity, v. Association between particles and the carriers is reversible and free particles can only move by diffusion. Partial differential equations were presented to describe the concentration of particles and the particle-carrier complex with respect to time and position along the axon. Rubinow and Blum found that they could solve these equations using a traveling wave solution (i.e., having the population of particles move as a wave along the axon at a constant velocity, a) only if the "interaction between particles and carriers exhibits positive cooperativity," (i.e., if $n > 1$).

Although not noted by Rubinow and Blum (1980), the prediction of cooperativity has previous experimental support from studies of the movement of serotonergic vesicles along axons of the giant cerebral neuron (GCN), an identified cell of *Aplysia californica* (Goldberg et al., 1976; 1978). In these experiments, the kinetics of vesicle transport were examined under conditions in which the local concentration of serotonergic vesicles within the axon was altered. To summarize their experimental results, Goldberg et al. (1976; 1978) concluded that the velocity of axonal transport is positively dependent on the local concentration of vesicles (except at very high concentrations, where the dependence is negative), and suggested a model for transport in which the serotonergic vesicle is translocated along the axon in an intermittent fashion, either moving in association with a track, or stationary when free in the axoplasm. They suggested that "the association of a vesicle with the track is enhanced by the local presence of other vesicles so that the vesicle finds an association site more rapidly, or, once associated, dissociates less readily. At very high vesicle concentrations, though, vesicles outnumber the association sites and it becomes more time consuming for a free vesicle to find an available association site. Therefore, at all concentrations it is the fraction of time spent in the moving state

$$\frac{t_{\text{moving}}}{t_{\text{moving}} + t_{\text{stationary}}}$$

that is sensitive to concentration; the velocity in the moving state, v_m, remains constant over the entire range of concentration."

Because the two physicial models appear to be quite similar, we have attempted here to combine experimental data from our studies in *Aplysia* neurons with the mathematical description of Rubinow and Blum (1980) to see if our experimental data could be fit by the kinetics predicted by computer curves generated with their formulations.

Thus, given the reaction

$$np + (m_o - c) \underset{k_-^n}{\overset{k_+^n}{\rightleftharpoons}} c \tag{1}$$

(where p is the free vesicle concentration, m_o, the total concentration of tracks, and c is the vesicle-track complex in which n vesicles reversibly bind in an all-or-none fashion to free tracks $[m_o - c]$ to form a

vesicle-track complex), what is the relation between total vesicle concentration and the velocity of axonal transport for a given value of n?

Rubinow and Blum (1980) described fluxes in particle and carrier-particle complex with two differential equations that make use of the wave velocity a

$$(v - a)c' = k_+^n p^n (m_o - c) - k_-^n c \tag{2}$$

and

$$Dp' = n(v - a)\, c - ap \tag{3}$$

where D is the diffusion coefficient for the particles. They noted that the value of a should be subject to the reaction rates (k_+, k_-), as well as to m_o, the total concentration of carrier.

In order to apply these equations to the experimental results from *Aplysia* (Goldberg et al., 1976; 1978) we derived a relation between a and p_t, the total concentration of vesicles. We assume that the experiments were carried out under steady-state conditions, and therefore that c' and p' are equal to 0. Eqs. 2 and 3 can then be transformed:

$$c = \frac{K^n p^n m_o}{1 + K^n p^n} \tag{4}$$

$$p = n\left(\frac{v}{a} - 1\right) c \tag{5}$$

where K^n is the equilibrium constant $(k_+/k_-)^n$ Letting the free vesicle concentration equal total vesicles less the vesicles associated with the tracks,

$$p = p_t - nc \tag{6}$$

Eq. 5 becomes

$$p_t - nc = n\left(\frac{v}{a} - 1\right) c \tag{7}$$

and

$$\frac{a}{v} = \frac{nc}{p_t}. \tag{8}$$

Thus, the ratio of the wave velocity (or the velocity of axonal transport) to the velocity of the carrier is equal to the fraction of vesicles bound. (Note the similarlity of this equation to the equation

$$\frac{v_o}{v_m} = \frac{t_{\text{moving}}}{t_{\text{moving}} + t_{\text{stationary}}} \tag{8a}$$

proposed by Goldberg et al. [1978] relating the ratio of the velocity of axonal transport [v_o] to the velocity of the moving vesicle [v_m] as a function of the partitioning of vesicles between moving and stationary states.)

To determine the relation between relative transport velocity as a function of total vesicle concentration and the stoichiometry (n) of the reaction between vesicles and the tracks we must solve Eq. 4 for c, and use that value to calculate (a/v) in Eq. 8 for trial values of n. The resulting curves of (a/v) vs. p_t for each value of n can then be compared to the data obtained experimentally. But before applying the equations, we require estimates for the concentration of the tracks in the axon, m_o, and for the equilibrium constant for the association of vesicles to the tracks. These parameters have not yet been measured experimentally, but reasonable estimates can be proposed.

We would suggest that the concentration of tracks is of the same order as the concentration of microtubules in the axon of GCN: this concentration can be obtained experimentally from electron micrographs (Shkolnik and Schwartz, 1980), and is $5/\mu m^3$. (By choosing to work with this value we do not wish to imply that the actual carriers are microtubules. Rather, we suggest that the carrier is likely to be present in the axon at a similar concentration. Thus the concentration of neurofilaments is somewhat greater; the concentration of microfilaments is less certain, because polymerized actin is not well preserved by the fixatives used for electron microscopy.) For the equilibrium constant, K, we suggest 0.13, a value equivalent to the reciprocal of the normal vesicle concentration in the axon ($7.6/\mu m^3$), a value that also can be obtained by counting in electron micrographs. The justification for choosing to work with this value of K is that generally it has been observed that binding constants correspond approximately to the intracellular concentration of ligand normally present. Thus, enzymatic reactions within cells are most sensitive to regulation by substrate in the concentration range equal to the Michaelis constant (Cleland, 1967).

We therefore set m_o equal to $5/\mu m^3$ and K, 0.13. Eq. 4 was rearranged to a polynomial expression for c and solved numerically by computer using Dekker's algorithm for values of p_t corresponding to the range of our experiments. The solution was restricted to the domain $0 < c < m_o$. The value of c was then substituted into Eq. 8 to solve for (a/v). The resulting computer curves are shown in Fig. 1. Note that only values of $n > 1$ yielded curves that show the same general relation between p_t and (a/v) as that obtained experimentally. Thus, application of Rubinow and Blum's mathematical model to the experimental results requires the condition of cooperativity in binding of the vesicles. Moreover, only a choice of $n = 2$ or 4 gave solutions over the entire range of p_t tested, with $n = 4$ best approximating the experimental data. The algorithm will predict the existence of a solution if the root is within the domain

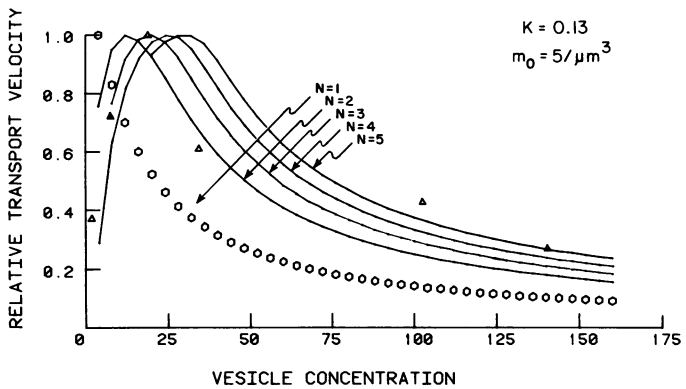

FIGURE 1 The dependence of the velocity of fast axonal transport on local vesicle concentration: a comparison of velocities obtained from experimental measurements in the axon of the giant cerebral neuron of *Aplysia* and of computer curves generated using Eqs. 4 and 8. See text. All experimental values were taken from the experiments of Goldberg et al. (1976, 1978) who measured the movement of vesicles containing ^{3}H-serotonin under a variety of conditions resulting in altered vesicle concentrations (Δ); the rate in the unperturbed axon is represented by ▲. The subnormal vesicle concentration was obtained by treating the GCN with anisomycin, an inhibitor of protein synthesis (for the determination of transport velocity, the coefficient of variation (c.v.) = 18% in $N = 9$ independent experiments) (Goldberg et al., 1978). The point of maximum velocity resulted from cutting one branch of GCN's bifurcating axon, thus diverting extra vesicles into the other branch (c.v. = 8%, $N = 4$) (Goldberg et al., 1976). The three points of greatest vesicle concentration were obtained by allowing vesicles to accumulate, at a cooled region of the axon where transport had ceased, for 1 hr (c.v. = 8%, $N = 5$); for 2 h (c.v. = 8%, $N = 4$); and for 4 h ($N = 1$) (Goldberg et al., 1978). After choosing a value of $n = 1, 2, 3, 4, 5$, we obtained the computer curves by solving Eq. 4 for a given value of p_t, using that solution to solve Eq. 8 for a/v. The ordinate is normalized either to the maximum value of a/v obtained for each computer curve, or to the maximum transport velocity measured experimentally. The unit of the abscissa is the number per cubic micrometer.

of c bracketed by the limits 0 and m_o; this is true even though the convergence criteria are fairly stringent. For $n = 3$ and 5, no roots were found to exist when p_t is small, and consequently no results were plotted for those values of p_t.

Because the values for m_o and K are uncertain, we next selected values for these constants over a fourfold range, in order to generate a family of computer curves, choosing $n = 4$ for all conditions (Fig. 2). For all values of these constants, we again found that the general shape of the curves is in good agreement with the experimental data. The best approximation, however, is with the set of constants originally chosen (Fig. 2 *B*).

We are quick to point out the limitations of our results, namely the many assumptions on which our approach is based, including (*a*) the nature of the binding reaction; (*b*) the use of the steady-state approximation; (*c*) uncertainty of values for K and m_o; and (*d*) the possible inaccuracy of the experimental data. In addition, it has been intuitively difficult to appreciate why the rate of axonal transport is dependent on vesicle concentration. Perhaps it is primarily a regulatory device for focusing vesicles: the system behaves as if individual vesicles take longer jumps under crowded conditions and shorter jumps when they are alone: if by chance an exceptional vesicle pulls ahead of the crowd, it will slow down; if sluggard vesicles accumulate, they tend to speed up.

There is little macroscopic or molecular information to explain cooperativity in vesicle binding. We suggest the possibility that cooperativity may arise from a snow-plough model for transport: passage of

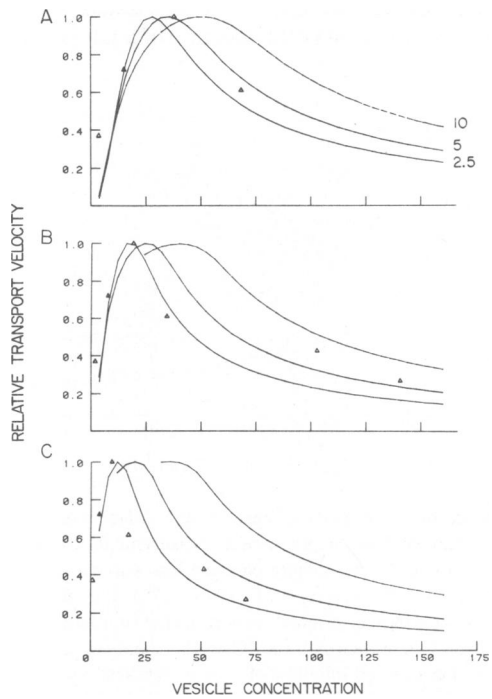

FIGURE 2 The dependence of the normalized relative transport velocity of fast axonal transport on local vesicle concentration: a comparison of the velocities obtained from experimental measurements with computer curves, letting $n = 4$ and allowing the constants p_t and m_o to vary independently. Experimental values were estimated as described in the legend to Fig. 1. We generated the computer curves selecting three values of K: $K = 0.26$ (*A*); $K = 0.13$ (*B*); and $K = 0.065$ (*C*). For each of these values of K, we selected three values of m_o (2.5, 5, and $10/\mu m^3$). Using these values of K and m_o, we solved Eqs. 4 and 8 as described in the legend to Fig. 1. In *C* the experimental points at the two highest concentrations of vesicles are beyond the end of the abscissa. The unit of the abscissa in the number per cubic micrometer.

one vesicle may change the environment in the axon transiently, for example, by altering local viscosity or by brushing aside the dense cytoskeletal network that appears to be present throughout the axon (Ellisman and Porter, 1980). Alternatively, the tracks, composed of monomeric units like tubulin or actin, may be easily disordered when unoccupied. Although not actually depolymerized, the disordered polymer would then become easily blocked, causing single vesicles to fall off. Successful passage of one vesicle might stabilize the track, facilitating the passage of additional vesicles.

It is encouraging that a mathematical model arrived at independently makes the same prediction about the nature of vesicle interaction with tracks and yields a similar functional relationship between vesicle concentration and transport velocity as an experimental approach. The results obtained here illustrate once again the utility of wedding mathematical models to experimental data.

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We were saddened to learn of the death of Dr. Sol I. Rubinow on 22 February 1981 during the preparation of the manuscript.

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