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Measurements of Amino Acid Transport in Internally Dialyzed Giant Axons

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Summary. It is shown that the axoplasmic composition of acidic and neutral amino acids can be controlled effectively by the method of internal dialysis. Direct assay for specific binding and measurement of diffusion coefficients in axoplasm show that there is no significant binding or compartmentalization of amino acids. The dependence of amino acid efflux on substrate concentration can be measured under well-defined, true steady-state conditions. The taurine efflux-concentration relation in the *Myxicola* giant axon conforms to a second-order Hill equation. This fact is consistent with either a cooperative process or a mechanism in which membrane translocation is not the rate-controlling step. The effluxes of taurine and glycine from squid axon are an order of magnitude smaller than in *Myxicola*. The efflux-concentration relations are essentially linear up to 200 mM substrate concentration. This result may be produced by specific transporters which have very high asymmetry, or by simple diffusive leak in the absence of specific transporters.

Key Words amino acid · transport · giant axon · squid axon · efflux · taurine · glycine

Introduction

The method of internal dialysis of giant axons (Brinley & Mullins, 1967) may provide a means for a thorough, quantitative study of the kinetics of amino acid transport. The principle advantages of the method are that the compositions of the intracellular and extracellular media can be controlled independently, as can the membrane potential, and that influx or efflux can be measured in a true steady state. The method has been used extensively for study of inorganic ion transport, but it has only recently been utilized for the study of organic solute transport (Baker & Carruthers, 1981; Carruthers, 1983). No studies of amino acid transport by means of internal dialysis have been reported. The method should be effective for amino acid studies on theoretical grounds (Horn, 1983), but direct experimental verification is required.

Studies of amino acid efflux in other preparations are uncommon because it is difficult to control both the intracellular amino acid composition and any other factors which may influence the transport simultaneously. Most reported work is devoted to influx measurements because better control of experimental conditions is possible. However, there may also be poor control of the intracellular composition with influx, so that results may be subject to unknown trans effects. Even the membrane vesicle preparations have technical problems for studies of kinetic mechanisms (Kessler & Semenza, 1983; Turner, 1983; Hopfer, 1984). The internally dialyzed giant axon may serve as an important alternative preparation which can be especially useful when control of intracellular conditions is critical or when efflux is of primary interest.

Taurine is present at high concentration in the axoplasm of the giant axons of squid (Deffner & Hafter, 1959) and *Myxicola* (Gilbert, 1975; Horn, 1981). Studies with intact *Myxicola* axons (Horn, 1981) showed that taurine is not metabolized, and that taurine may be transported by a Na-dependent mechanism of low affinity which has the unusual property of trans inhibition of efflux by sodium. It is unlikely that any other native amino acids share the taurine transport system (Azari et al., 1979). Taurine is thus a useful model substrate for study of Na-dependent transport free of possible complications due to metabolism or competitive inhibition by other amino acids.

This communication establishes the validity of the internal dialysis technique for study of amino acid transport in the giant axons of *Myxicola* and squid, and presents flux-concentration relations for efflux. Taurine efflux in *Myxicola* has an unusual property, showing a second-order dependence on the taurine concentration. Squid axon, on the other hand, apparently does not transport taurine or glycine out of the cell at a significant rate. Most of the efflux from squid either occurs by simple diffusion

or "leak," or by means of highly asymmetric transporters, under the experimental conditions used in this work.

Materials and Methods

Animals

Myxicola were obtained from Marine Research Assoc., Lord's Cove, Deer Island, New Brunswick. Squid (*L. pealli*) were obtained at the Marine Biological Laboratory, Woods Hole, Mass.

Solutions

Normal artificial sea water (ASW) was composed as follows (mM): 433 NaCl, 10 KCl, 25 $MgCl_2$, 25 $MgSO_4$, 10 $CaCl_2$, 0.1 EDTA, and 5 HEPES (N-2-hydroxy ethyl piperazine-N'-2-ethanesulfonic acid). pH was adjusted to 7.6. Osmolality, determined by freezing-point depression, was 940 mOsm for *Myxicola* and 960 mOsm for squid. When the Na concentration was reduced, either Li or N-methyl-D-glucamine (NMG) replaced Na.

Internal dialysis solutions for *Myxicola* were composed as follows (mM): K-cysteate (220-X), Na-cysteate (X), K-aspartate (100), glycine (215-Y), taurine (Y), EGTA (1), $MgSO_4$ (3), phenol red (1), MgATP (1.5) and MOPS buffer (3-(N-morpholino) propanesulfonic acid) (10). pH was 7.2 and osmolality was 890 mOsm. Internal Na was varied by equivalent exchange with K. Taurine was varied by equivalent exchange with glycine.

Internal dialysis solutions for squid were composed as follows (mM): K-aspartate (320), NMG-glutamate (100-X), Na-glutamate (X), glycine (200-Y), taurine (Y), EGTA (1), $MgSO_4$ (5), MgATP (5), phenol red (1) and MOPS (10). pH was 7.4 and osmolality was 955 mOsm. Na was varied by equivalent substitution by NMG. Taurine was varied by equivalent substitution by glycine.

Amino Acid Analysis

Measurement of amino acid concentrations in dialysis solutions and tissues was made by means of an ion exchange column with *o*-phthaldialdehyde as detection reagent. The amino acid analyzer and the sample preparation techniques have been described (Horn, 1981).

Amino Acid Binding

Binding was assayed either directly with isolated axoplasm, or by measuring axial diffusion coefficients in the axoplasm of intact axons. Only *Myxicola* axons were used. The direct binding assays involved soaking isolated axoplasm at 4°C in a dialysis solution labeled with (^{3}H) taurine and (^{14}C) sucrose for 30 to 120 min. At the end of the soak the plasm was rinsed for 15 sec in ice-cold ASW to remove excess soak solution, then extracted with perchloric acid and assayed for radioactivity by dual-label counting procedures. The percent bound was calculated as the percent difference between the (^{3}H) taurine and (^{14}C) sucrose spaces assuming the sucrose distributes passively in the axoplasmic water.

Axial diffusion of solutes was determined by injecting the (^{3}H) or (^{14}C)-labeled solute over one-half the axon (Horn, 1981) and then allowing the solute to diffuse axially at 10°C for 5 to 6 hr. The injected zone was always 1.5 cm long, which is effectively an infinite length for the expected diffusion rates and time spans. The outside of the axon was washed continuously with ASW to prevent isotope from leaving and then re-entering the axon. At the end of the diffusion period the axon was tied off into injected and uninjected portions and the amount of radioactivity measured in each portion. The total amount of isotope injected was determined from the sum of the radioactivities in each region, and concentration of injectate was determined from the total amount injected and the length of injected axon. Total losses of solute across the membrane should be negligible because the fluxes are so low (Horn, 1981).

Assuming diffusion in a semi-infinite medium, with $x = 0$ corresponding to the boundary between injected and uninjected axon, the total amount of radioactivity diffusing into the uninjected axon can be calculated by integration of Eq. (12) of Carslaw and Jaeger (1959) between zero and infinity. Solving the result for the diffusion coefficient gives:

$$D = (1/t)\,[(M/0.5642)\,(L/Q)]^2$$

where t = total diffusion time, L = length of injection zone, M = amount of radioactivity that diffused into the uninjected zone, and Q = total amount of radioactivity injected. The greatest error in this calculation is likely to result from inaccurate tie-off of the axon at $x = 0$. Assuming this point is in error by as much as ± 500 μm, or one axon diameter, the corresponding error introduced into the calculation of D for this worst case can be calculated to be about $\pm 20\%$. This error is eliminated completely by measuring the diffusion of two solutes simultaneously and calculating the ratio of diffusion coefficients:

$$D_1/D_2 = [(M_1/M_2)\,(Q_2/Q_1)]^2.$$

In many experiments this was done with sucrose as the second solute, assuming that it diffuses freely in cell water.

Flux Measurement

Details of the experimental chamber have been described (Horn, 1981). Efflux of an amino acid was determined from the amount of radioactivity leaving the axon per unit time, the known internal specific activity, and axon geometry. Either 5- or 10-min efflux samples were used. All internal solutions had the same activity (DPM/ml). All samples were corrected for quench, which is especially large for ^{3}H samples. All ^{3}H- and ^{14}C-labeled solutes were obtained from New England Nuclear, Boston.

(^{3}H)-Taurine was used for all flux measurements with *Myxicola*. Direct assay of efflux wash solutions from some experiments (not all) on the amino acid analyzer showed that more than 90% of the ^{3}H leaving the axon can be recovered from the taurine peak of the analyzer, a result similar to that for injected axons (Horn, 1981). The (^{3}H) taurine stock used for squid work was supplied from the same lot as used for *Myxicola*. When received, the material was several years old. It was discovered that squid dialysis solutions prepared with the stock were contaminated with about 0.1% ^3H_2O, which resulted in very high apparent taurine fluxes in squid. Comparison of fluxes in the same squid

Table 1. Comparison of mole fractions of amino acids in dialyzed axoplasm (plasm) with those in the dialysis solution (DF)[a]

Dialysis medium number		$CySO_3H$	Tau	Asp	Ser	Glu	Gly	Ala	% Water
1	DF	0.581	0	0	0	0	0.419	0	
	Plasm	0.573	0.004	0.004	0	0	0.417	0.004	72
2	DF	0.321	0.094	0.211	0	0	0.374	0	
	Plasm	0.311	0.105	0.219	0	0	0.365	0	80
3	DF	0.376	0.174	0.175	0	0	0.275	0	
	Plasm	0.364	0.180	0.155	0	0	0.275	0	79
4	DF	0.313	0.224	0.176	0	0	0.287	0	
	Plasm	0.303	0.236	0.181	0	0	0.280	0	84
None	Plasm	0.229	0.141	0.165	0.011	0.022	0.421	0.011	87

[a] Any amino acid present at less than 1 mM was not detected. Normal undialyzed axoplasm composition taken from Horn (1981). Percent water was calculated (not measured) assuming glycine always equilibrated. The normal value is taken from Gilbert (1975). Total amino acid concentration in dialysis solution was 560 mM. Standard amino acid abbreviations, ($CySO_3H$) cysteic acid, (Tau) taurine.

axon measured simultaneously with (^{14}C)- and (^{3}H)-labeled taurine showed that the (^{14}C) taurine flux estimates were at least one order of magnitude smaller. Thus only (^{14}C)-labeled amino acids were used in squid work. All efflux samples were acidified with HCl to pH 1.5 and vortexed for 30 sec to remove $^{14}CO_2$. It was assumed that the remaining sample activity represented the original labeled material.

Internal Dialysis

The method was developed by Brinley and Mullins (1967, 1974). A rigorous theoretical analysis which defines the limits of applicability and error sources has been given (Horn, 1983). Most experiments with *Myxicola* were made with 95 μm ID × 140 μm OD cellulose acetate tubing (FRL Industries, Albany, N.Y.) which was rendered porous over 1 cm of its length by hydrolysis with 50 mM NaOH for 24 hr at room temperature. All other experiments were made with an improved dialysis tube which has about a twofold higher solute permeability as measured with phenol red, and a very low hydraulic permeability (10^{-7} cc/cm^2 sec/cm H_2O, measured at 50 cm H_2O). A typical pressure in the dialysis tube during an experiment is 10 cm H_2O. The dialysis tube is constructed with commercial dialysis tubing of the Cuprophan type which is available in assorted sizes (my 120 μm ID × 150 μm OD material was a generous gift from ENKA, AG, Barmen, West Germany). Short pieces of the dry tubing can be joined to suitably sized glass capillaries (OD 1 to 10 μm less than the dialysis tube ID) by means of DeKhotinsky cement, where the length of porous tubing can be controlled easily. The cement is important because the joints must withstand considerable stress when wet due to swelling of the dialysis tubing. A dialysis tube can be prepared in 15 min and reused many times.

Results

Myxicola axons were dialyzed at 2.2 μl/min for 1 hr at 10°C with solutions of different amino acid compositions. At the end of the period the dialysis tube was removed and the dialyzed zone of the axon (demarcated by phenol red) was tied off. Dialyzed axoplasm was removed and assayed for amino acids (Table 1). The compositions are given as mole fractions because the water content of the axoplasm was unknown. The amino acid analyzer is accurate to ±5%. Additional error could be contributed by contamination of the axoplasm by undialyzed axoplasm drawn into the dialyzed zone upon removal of the tubing, and by the contribution of poorly dialyzed end regions at the boundary between dialyzed and undialyzed zones. In no case does an axoplasm composition differ from the dialysis fluid by more than 10%.

Table 2. Estimates of the amount of taurine absorbed by isolated axoplasm, soaked in dialysis solution, which exceeds sucrose absorption (percent Tau bound)[a]

Dialysis solution composition (mM)	Mean apparent Tau bound (%)	SEM	*n*
100 Na, 100 Tau	6.5	0.6	11
100 Na, 10 Tau	7.0	1.7	4
0 Na, 100 Tau	−3.8	3.0	8
0 Na, 10 Tau	4.9	0.8	5

[a] Only the Na and Tau concentrations of the dialysis solutions were varied. Accuracy of the dual-label count separations was ± 5%, which defines the lowest detectable limit for percent bound measurements.

The results of direct taurine binding assays made with isolated *Myxicola* axoplasm are given in Table 2. The accuracy of the dual-label count separation was ±5%. Table 3 presents results of the diffusion coefficient measurements, where the

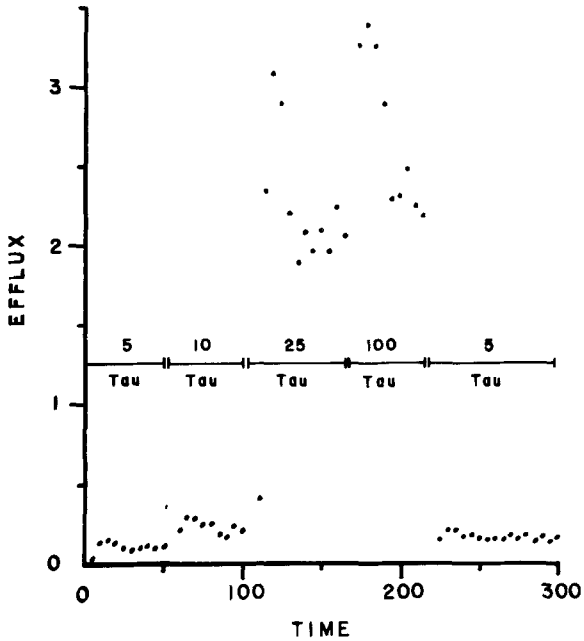

Fig. 1. Taurine efflux (pmol/cm² sec) from a 530 μm, internally dialyzed *Myxicola* axon at 10°C. Internal Tau concentration specified in figure. Internal Na was 100 mM. Membrane potential was −66 mV. Each point is a 5-min sample

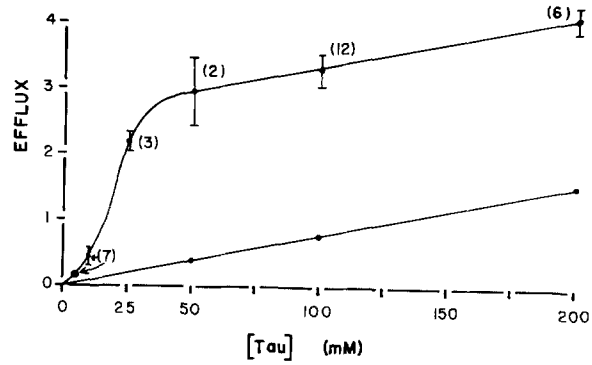

Fig. 2. Plot of total taurine efflux (pmol/cm² sec) against internal Tau concentration for 100 mM internal Na and 0 Tau, normal ASW. T = 10°C. Membrane potentials of all axons were between −60 and −66 mV. Vertical bars represent ± 2 SEM, with number of axons specified for each point. The straight line is assumed to represent passive leak

results are compared with predicted diffusivities based on the Wilkie-Chang correlation (Wilkie & Chang, 1955). In all cases the measured coefficients are 50 to 70% of the predicted values. Such reductions might be due to solute binding. However, account must be taken of the steric hindrance to diffusion caused by the macromolecules of the axoplasmic gel (Lauffer, 1961). The ratios of solute diffusivities should not be the same in axoplasm and in simple aqueous solution if there is significant specific binding in axoplasm, but my measurements of diffusivity ratios in axoplasm show no major deviations from the ratios predicted for simple solutions.

Figure 1 shows a typical *Myxicola* efflux dialysis experiment in which the internal taurine concentration was varied. The large transient jumps in the apparent flux following some dialysis solution changes are calculation artifacts caused by assuming an instantaneous change of axoplasm specific activity. The decay of the transient is determined by the true rate of equilibration of axoplasm with dialysis fluid. In all experiments equilibration required less than 20 min. Flux estimates at each taurine concentration were obtained from the apparent steady-state values, usually the mean of the final 4 or 5 data points.

Flux data for *Myxicola* axons were accumulated for the conditions of 100 mM internal Na, 0 external taurine, a constant membrane potential of −60 to −65 mV and constant temperature of 10°C. Figure 2 is an efflux-concentration plot for taurine in *Myxicola*. The straight line is the least-squares fit to the last three points of the curve and is taken to represent the simple diffusive "leak" of taurine. The corresponding leak permeability is 7.8×10^{-9} cm/sec. When the flux data are corrected for apparent leak the resulting Lineweaver-Burke plot is nonlinear (Fig. 3), with a V_{max} of 2.56 pmol/cm² sec and a $K_{1/2}$ of 18.7 mM. The leak correction has only a quantitative effect on the curve. Assuming the leak-corrected taurine efflux is mediated by a single transport system the data might be described by a Hill equation. Linear regression of the data, assuming a Hill coefficient of 2, gives a straight line with a V_{max} of 2.59 pmol/cm² sec, a $K_{1/2}$ of 18.5 mM and a correlation coefficient r^2 = 0.9998. Because this correlation is so good, and in lieu of a specific model, further analysis was not attempted.

In contrast to *Myxicola*, taurine effluxes are an order of magnitude smaller in squid (589 ± 33 fmol/cm² sec at 200 mM taurine in five axons). Efflux of taurine into taurine-free ASW can be described by a straight line through the origin over the taurine concentration range of 1 to 200 mM. This is true at two internal Na concentrations. The least-squares regression relations are:

$$\begin{aligned} 10\ \text{mM Na}_i \quad & J = 5.37 + 2.99{*}C \\ & r^2 = 0.9782\ (n = 25) \\ 100\ \text{mM Na}_i \quad & J = 6.77 + 2.94{*}C \\ & r^2 = 0.9905\ (n = 20) \end{aligned}$$

where the flux J has units of (fmol/cm² sec), the concentration C has units of (mM), and r is the cor-

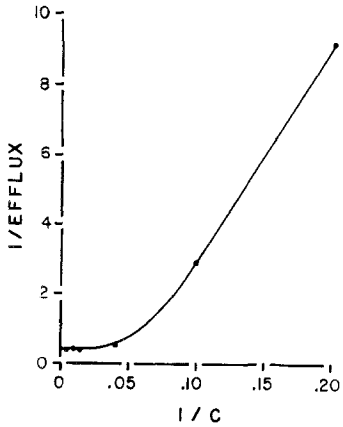

Fig. 3. Lineweaver-Burke plot for the leak-corrected taurine efflux. Plot derived from data in Fig. 2

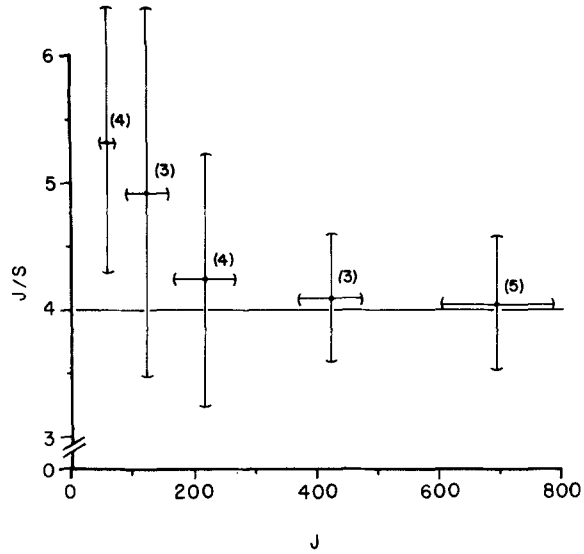

Fig. 4. Eadie-Hofstee plot for glycine efflux from squid axons at 10°C. Internal Na 100 mM; 0 Gly, normal ASW. Membrane potentials of all axons ranged from −60 to −65 mV. Bars represent ± 2 SEM with number of axons specified for each point. Horizontal line at J/S = 4 is apparent asymptote. J/S (cm/sec × 10^9), J (fmol/cm² sec)

relation coefficient. Student t-tests support the hypotheses that the intercepts are zero and the slopes are equal. Paired experiments (three axons) showed no effect of internal Na on efflux, consistent with the unpaired results. The slope of the line for the pooled data, converted to permeability units, is 2.97 × 10^{-9} cm/sec.

The taurine flux magnitudes are so low in squid that it is conceivable the taurine escapes the axon mainly via cut branches or damaged axolemma. Indeed, these fluxes could arise from a single 25-μm branch 1 mm long or from about three 15-μm branches each 1 mm long. Long, cut branches were present in 13 axons. There was no difference in flux magnitudes among these axons. The membrane potentials ranged between −60 and −65 mV in all axons, and E_m changed by no more than +5 mV over 4 to 6 hr of dialysis. Two of these branched axons were depolarized from −61 to −3 mV by means of 222 mM K-sea water (K substituted for Na) within 5 min, maintained at −3 mV for 20 min, and repolarized to −59 mV within 5 min of return to normal ASW. Damaged or weak axons, or axons with short branches, would not respond this way. It is also possible that an unknown inhibitor was present in the (^{14}C)-taurine stock solution. Effluxes measured in four axons at a specific activity 5 times lower than usual were equal to those measured at the higher specific activity at both 1 and 200 mM taurine.

Taurine efflux in squid can be increased reversibly by a factor of 1.8 ± 0.4 (13 axons) by removal of external Na. However, the flux increase in Na-free ASW did not reach a steady state after 40 min and recovery of the initial flux took about 20 min upon return to normal ASW. This result should be contrasted with the very rapid K-depolarizations, in which the taurine efflux increased reversibly by a factor of 1.2 (2 axons). Substituting 100 mM taurine for 50 mM NaCl in sea water also caused a 1.2-fold flux increase (three axons) which reached a steady state within 10 min and reversed as rapidly upon return to normal ASW.

Because the taurine results in squid are so unusual a second amino acid was tested. Glycine was selected because its transport has been studied more extensively than any other amino acid in undialyzed squid axon and because glycine fluxes in squid are the largest amino acid fluxes reported (Caldwell & Lea, 1978). Glycine efflux was measured at several internal Na concentrations and for internal glycine concentrations between 10 and 200 mM.

Figure 4 is an Eadie-Hofstee plot of glycine efflux data at 10°C. The largest efflux measured was about 700 fmol/cm² sec. The Eadie-Hofstee curve appears to be asymptotic to a J/S value of 4 × 10^{-9} cm/sec. This asymptote might be interpreted as the simple diffusion permeability of glycine. Clearly, most of the efflux occurs via this linear route. However, there does appear to be at least a small amount of mediated transport.

Two axons were dialyzed with 160 mM glycine and 0, 10 and 100 mM Na. There was no direct effect of internal Na on glycine efflux. Removal of external Na caused a rapid reversible, 1.3-fold efflux increase (two axons). Substitution of 100 mM glycine for 50 mM NaCl in ASW, when internal glycine was

Table 3. Solute diffusivities and diffusivity ratios (solute to sucrose) measured simultaneously in axoplasm by dual-label procedures, reported as the mean ± SEM with number of axons in parentheses[a]

Solute	Predicted diffusivity D_s ($cm^2/sec \times 10^6$)	Measured D_s ($cm^2/sec \times 10^6$)	Predicted ratio (D_s/D_{suc})	Measured ratio (D_s/D_{suc})
Glycine	10.6	4.8 ± 0.7 (4)	2.04	1.93 ± 0.15 (4)
Taurine	6.1	4.4 ± 0.3 (14)	1.79	1.97 ± 0.09 (8)
Aspartate	5.9	3.5 ± 0.6 (6)	1.73	1.53 ± 0.06 (6)
Sucrose	3.4	2.3 ± 0.2 (18)		

[a] Predicted values assume binary diffusion in simple, dilute aqueous solution. The predicted values are based on an empirical correlation which is accurate to ± 10% (Wilkie & Chang, 1955).

170 mM, caused a rapid reversible 1.3-fold efflux increase (one axon), while K-depolarization to −2 mV caused a rapid reversible 1.2-fold efflux increase (one axon).

Discussion

The present results are the first direct check of the efficacy of the method of internal dialysis for controlling the small organic solute composition of axoplasm. The method can control the axoplasmic concentrations of organic anions and zwitterions in the 75 to 170 molecular weight range. There is free exchange and simple equilibration between dialysis fluid and axoplasm. Taken together, the measurements for internal dialysis, direct taurine binding assays, and axoplasmic diffusion comprise a strong body of evidence against the possibility that there is significant specific binding or compartmentalization of amino acids in the axoplasm of giant axons. This conclusion applies to the axon only and may not be true for specialized regions such as the nerve terminal or the soma (Kontro et al., 1980).

Taurine efflux from *Myxicola* giant axons occurs by at least one saturable, mediated process, and by an apparent diffusive "leak" with a very low permeability. The sigmoid kinetics are not consistent with any combination of parallel simple carriers plus linear leak. Significant deviations from Michaelis-Menten kinetics for organic solutes such as sugars and amino acids is unusual, with the intestinal glucose transporter perhaps being the best-known example (Kessler & Semenza, 1983). It is premature to propose a mechanism for the taurine transport system although we may ascertain some constraints. It appears to be Na-dependent (Horn, 1981; *unpublished* dialysis work). The second-order kinetics require either that more than one taurine per transport cycle is bound to the carrier, or that the rate-determining step of the transport is binding of taurine to the carrier rather than membrane translocation. The apparent V_{max} for efflux is also fairly small, which may mean that the density of transport systems is low, but also may mean that the preferred orientation of the transporter is outward. Efflux can be doubled by removal of external Na (Horn, 1981), which is consistent with a preferred outward orientation stabilized by external Na.

The large quantitative difference between *Myxicola* and squid axon taurine fluxes can not be explained. Specific transport systems are conserved among cells of all species of higher animals (Christensen, 1984). If taurine is specifically transported in the squid axon it should be via the same basic system as in *Myxicola*. However, some systems are strongly regulated and may not be seen in a particular cell under normal conditions (Christensen, 1984). It also is not known whether kinetic parameter magnitudes, or even the kinetic behavior, of a system are conserved along with solute selectivity. Subtle changes of boundary conditions, even for the same cell, may alter the rate-controlling step of a transport system and profoundly affect kinetic behavior (Sanders et al., 1984). Species differences in transport kinetics may only reflect different modes of the same basic system.

The extremely small effluxes observed in the squid axon might be attributed solely to a metabolic turnover of less than 0.1% per hour. Metabolism can not be specifically excluded by these experiments. Since Caldwell and Lea (1978) concluded that 85% of the isotope leaving a squid axon injected with ^{14}C-glycine at 20°C is present in glycine, my results at 10°C in dialyzed axons are unlikely to be affected seriously by metabolism. Taurine is metabolized very poorly by most animal cells (Jacobsen & Smith, 1968). Most measurable taurine catabolism observed in animal tissues is due to bacteria (Shimamoto & Berk, 1979; Fellman et al., 1980).

The results of Hoskin et al. (1975) indicate that $^{14}CO_2$ is not derived from uniformly labeled (^{14}C) taurine in squid axon. Therefore the risk of metabolic artifacts with (^{14}C) taurine is small. Even if both solutes were catabolized equally it seems unlikely that the metabolic rates would be essentially linear functions of concentrations up to 200 mM.

If most of the efflux measured was due to metabolites of the amino acids then a necessary conclusion is that the squid axon membrane is virtually impermeable to amino acids. Impermeability is not consistent with an apparent sucrose permeability of 1.6×10^{-8} cm/sec (Mullins, 1966) in undialyzed axons, nor with a 3-0-methyl glucose permeability in dialyzed axons at 20°C which may be as high as 2×10^{-8} cm/sec (estimated from Figs. 4, 5, 8 & 10 in Baker and Carruthers, 1981). Impermeability is also inconsistent with the apparent mediated uptakes of glycine, glutamate, and several other amino acids measured in squid axons (Baker & Potashner, 1973; Caldwell & Lea, 1978). A passive carrier system must be able to transport in both directions if the conditions are right.

Unknown inhibitors might explain the very small flux magnitudes and the linear flux-concentration relations. The presence of contaminants can not be excluded absolutely. However, fluxes measured at ^{14}C specific activities which differed by a factor of 5 were the same. Furthermore, the effluxes estimated in this work (at 10°C) are of the same order of magnitude as amino acid influxes and effluxes measured by Baker and Potashner (1973) and Caldwell and Lea (1978) in undialyzed axons (at 20°C). The glycine influx concentration relation obtained by Caldwell and Lea is similar to the efflux relation of this work, especially with respect to the very large component of the flux which might be taken as simple diffusion. Finally, taurine and glycine do not compete significantly in any other cell preparation.

The ratio of apparent squid axon leak permeabilities (glycine to taurine) is about 1.35 while the square root of the molecular weight ratio (taurine to glycine) is 1.29. This is good agreement in view of the measurement errors and is consistent with a simple diffusive efflux of the amino acids through nonspecific "leak" channels. However, the apparent permeabilities for both glycine and taurine are an order of magnitude smaller than expected if the sucrose permeability is scaled by molecular weight, and are about twofold smaller than the 3-0-methyl glucose permeability when they should be larger. Simple diffusive "leak" should not show sensitivity to the external Na concentration, nor be sensitive to the membrane potential or extracellular amino acid concentration. Thus while the results seem consistent with simple diffusive "leak," the conflict with the sucrose and 3-0-methyl glucose observations, the small trans effects, and the influx data of other laboratories point to an alternative.

It is possible that the taurine and glycine transporters in squid axon belong to a low-affinity class which, under net efflux conditions, can give apparently linear flux-concentration relations. Taurine uptake in Ehrlich ascites cells (Christensen & Liang, 1966), urea uptake in red cells (Brahm, 1983), and net Cl efflux via the red cell Band 3 transporter (Fröhlich, 1984) are known specific transport systems which give approximately linear kinetics over a broad substrate concentration range. The transport of glycine and taurine by squid axon need not be so simple, however. The transporters might show large asymmetries such as those seen in the small intestine Na, D-glucose cotransporter (Kessler & Semenza, 1983). Such systems can work effectively to conserve intracellular substrate at a relatively low energy expense.

The concept of an asymmetric transport system is not new although, traditionally, symmetric systems are usually assumed. Further work with dialyzed axons should determine whether asymmetric systems are present in these cells. It shall be interesting to compare influx with efflux and to examine the effects of transport inhibitors. A finding of passive, asymmetric systems in giant axons would raise the questions of their general occurrence and importance for cell homeostasis.

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