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INOSITOL UPTAKE BY CULTURED ISOLATED RAT SCHWANN CELLS

Stanton Segal, Shing Mei Hwang, Janet Stern, and David Pleasure

Division of Biochemical Development and Molecular Diseases,
Division of Neurology Research, Children's Hospital of Philadelphia,
Departments of Pediatrics and Neurology,
University of Pennsylvania School of Medicine, Phila, PA

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The uptake of radiolabeled myo-inositol by Schwann cells isolated from the sciatic nerve of 2-4 day old rats was found to occur by a saturable, sodium-dependent phlorizin-inhibited mechanism with an estimated K_m of 30μM. The system was inhibited by galactose and glucose but not by galactitol. At high concentrations of myo-inositol, a diffusion-like process appeared to be functional. The characteristics of the saturable system are very similar to those of myo-inositol uptake by the endoneural fascicle preparation of sciatic nerve.

Free myo-inositol is an important component of both the central and peripheral nervous system where its concentration is almost one hundred-fold that of plasma (1,2). In peripheral nerve of rats fed a high galactose diet (3) or made diabetic with streptozoticin, (1,2,4) the free myo-inositol concentration is considerably diminished and in both experimental conditions motor nerve conduction is below normal (1,5,6). Oral administration of myo-inositol improves motor nerve conduction in diabetic animals (1,6). The mechanism of the reduction of peripheral nerve myo-inositol in galactose toxicity and diabetes has been of great interest, especially the possibility that there is an effect of these conditions on transport of the cyclitol from plasma. Studies in vitro using endoneural fascicles of sciatic nerve from rabbits (7) and rats (8) have demonstrated the existence of a saturable, sodium-dependent mechanism for the uptake of myo-inositol which is inhibited by glucose and impaired in diabetic tissue. These results have indicated that a portion of myo-inositol present in peripheral nerve is likely to be derived from extracellular fluid and suggest the diminution of myo-inositol content of diabetic or galactose toxic animal tissue could result from the interference of transport by elevated levels of the hexoses.

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Endoneural fascicles contain a variety of cell-types including Schwann cells, fibroblasts, endothelial cells and axons. While the exact cellular localization of the myo-inositol transport mechanism is not known, much of the free myo-inositol in peripheral nerve is believed to exist in Schwann cells (9) and this suggests that these cells may be the locus of the observed uptake process. We recently developed a rapid method for the preparation of relatively large numbers of Schwann cells from newborn rat sciatic nerves (10). Cells prepared in this fashion have been found to have a saturable, sodium dependent and phlorizin-inhibitable mechanism for the accumulation of myo-inositol.

METHODS OF PROCEDURE

Schwann cells were prepared from the sciatic nerves of 2-4 day old Sprague-Dawley rats as previously described, (10) and were suspended in Eagle's MEM containing 10% (v/v) fetal calf serum. The cells were seeded at a density of 50,000 per 15 mm round, poly-D-lysine-coated coverslip, and the coverslips were incubated in 24 well Linbro plates at 37°C. Indirect immunofluorescence studies of these cells using cell-type specific antisera (10) indicated more than 95% were Schwann cells, the remainder fibroblasts. Three hours after seeding, the coverslips were rinsed twice in a modified Krebs-Ringer buffer which contained 135 mM NaCl, 4.6 mM KCl, 1.2 mM $CaCl_2$, 1 mM sodium phosphate, 1.3 mM $MgSO_4$ and 5 mM N-2-hydroxyethylpiperazine-2-ethane-sulfonic acid (HEPES), pH 7.4.

Uptake experiments were carried out at 37°C, and were initiated by replacing the medium with 0.3 ml of modified Krebs-Ringer buffer containing 10 microcuries of [2-^{3}H]myo-inositol (Amersham), 5 microcuries of L-[1-^{14}C]-glucose per ml (Amersham), and added myo-inositol, D-glucose, D-galactose, phloretin or phlorizin. In experiments testing sodium dependence of myo-inositol uptake, choline chloride was substituted for the sodium chloride in the modified Krebs-Ringer buffer. Uptake was terminated by removing the coverslip from the Linbro well and passing it rapidly through a series of 6 wells containing ice cold 140 mM NaCl. The coverslip was blotted on absorbent paper between washes and then dried on filter paper. Each dried coverslip was added to 5 ml of a scintillation cocktail which contained ethanol/OCS (New England Nuclear) 2.8/7, v/v and radioactivity was determined using a Packard liquid scintillation counter. The L-[1-^{14}C]-glucose uptake results were used to correct the [2-^{3}H]-myo-inositol data for non-specific uptake and for incomplete exchange of the extracellular fluid during the washing procedure. Poly-D-lysine-coated coverslips without cells were run with each uptake experiment in order to correct for binding of [2-^{3}H]myo-inositol to the coverslips.

The dependence of uptake on concentration of myo-inositol was analyzed in two ways. The first was by linear regression using the Hofstee transformation (V versus V/S). Data obtained over a range of myo-inositol concentrations between 3 and 103 μM were used to arrive at a least squares linear fit. The second method of analysis was applied to results obtained with myo-inositol concentrations between 3 μM and 2 mM and involved a non-linear regression employing the general kinetic equation:

$$V = \frac{V_{max}\,[S]}{K_m + [S]} + K\,[S]$$

K is a correction factor for myo-inositol radioactivity which could not be removed by washing or for myo-inositol taken up by a very low affinity system difficult to characterize. The best fit was determined using an HP 85 computer and a direct grid search method described previously (11) and employed by us previously (12).

RESULTS AND DISCUSSION

In medium containing 3 μM myo-inositol and 136 mM sodium, uptake of the cyclitol by the Schwann cells was linear for 30 minutes (Figure 1). When myo-inositol concentration was raised to 23 μM, uptake was greater and appeared to deviate from linearity after 15 minutes. Substitution of choline for all but 1 mM sodium ion demonstrated that myo-inositol uptake was partially dependent upon the presence of sodium ions in the medium (Figure 1). The

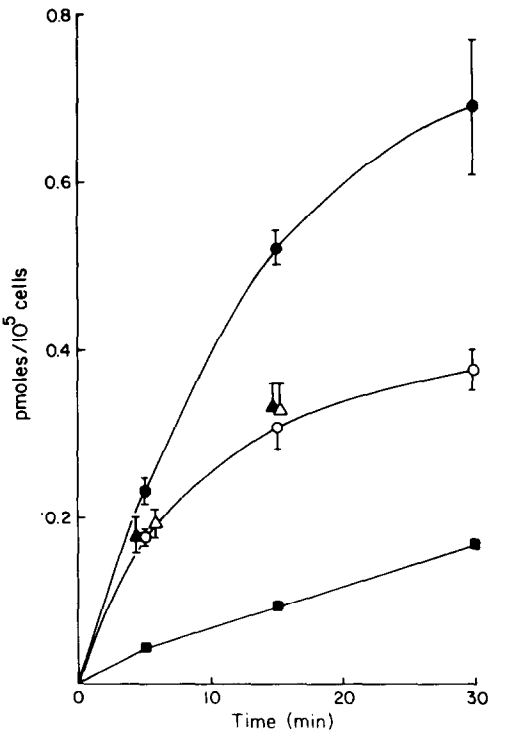

Fig. 1. [^{3}H] Inositol uptake by Schwann cells vs. time. Schwann cells, 2-3 μg protein applied per cover slip, were incubated at 37°C in modified Krebs-Ringer buffer, pH 7.4, with radioactive myo-inositol as described in Methods. Uptake date of 23 μM inositol in 135 mM sodium buffer (●—●) are the averages of 12-20 determinations; there were 4-12 determinations for 1 mM Na buffer (○—○), 8 determinations for the addition of 0.2 mM phlorizin (▲—▲) and 8-12 determinations for the addition of 0.2 mM phloretin (△—△). Uptake data at 3mM myo-inositol (■—■) are the averages of 16 determinations. V is expressed as picomoles per 100,000 cells. Each value is the mean ± SEM. Where no SEM is shown, it is within the size of the point.

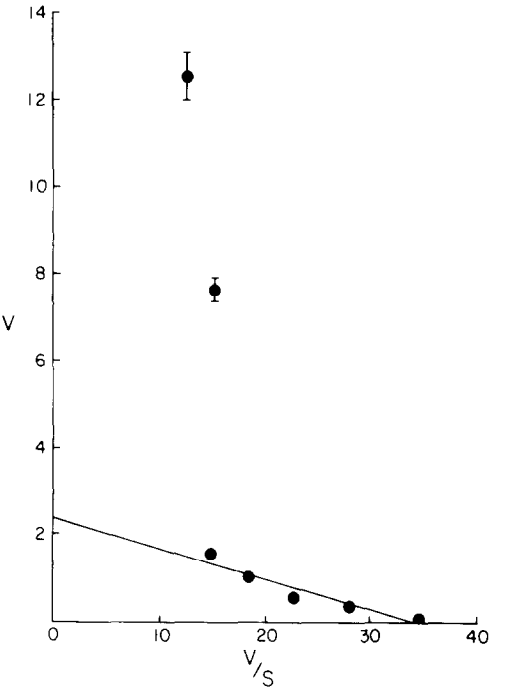

Fig. 2. Hofstee plot of velocity vs. velocity over substrate concentration. Schwann cells, 2-3 μg protein applied per cover slip, were incubated for 15 min. at 37°C in modified Krebs-Ringer buffer, pH 7.4 with [^{3}H] myo-inositol and sufficient unlabeled inositol to give the desired concentrations. Each value is the mean ± S.E. of 8 to 20 determinations. V is expressed as picomoles per 100,000 cells and S as mM.

cyclitol uptake was inhibited by 0.2 mM phloretin or 0.2 mM phlorizin (Figure 1) to about the same level found in the low sodium medium.

Uptake of [2-^{3}H]-myo-inositol at 15 minutes by the Schwann cells was studied over a concentration range of myo-inositol from 3 to 2003 μM in sodium containing buffer. Hofstee plots of the results showed saturation kinetics only in a myo-inositol range between 3 and 103 μM. (Figure 2). At higher concentrations of myo-inositol, the uptake was non-saturable. From the linear portions of the Hofstee plots, apparent K_m for myo-inositol uptake was 70 μM (SE ± 10 μM) and apparent V_{max} was 2.4 picomoles of myo-inositol/100,000 cells/15 minutes. When the myo-inositol uptake data over the full range of concentrations was analyzed using the non-linear computer method, the apparent K_m for myo-inositol was 30 μM, apparent V_{max} was 0.5 picomoles for myo-inositol/100,000 cells/15 minutes, and K of equation 1 was 11.9. The latter

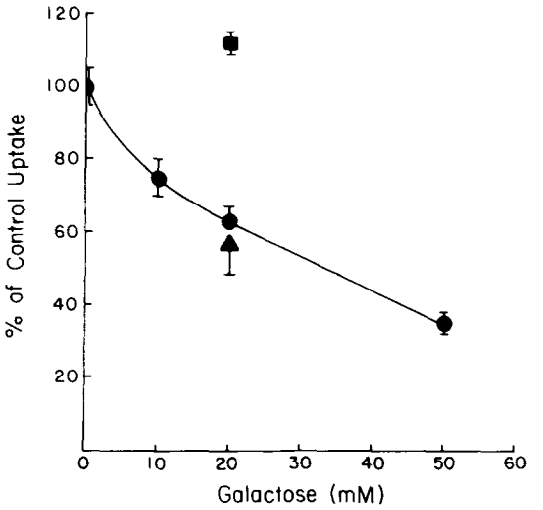

Fig. 3. Effect of galactose on myo-inositol uptake. Myo-inositol concentration was 3 μM, with incubation at 37° for 30 min. (●—●) indicates incubation in the presence of galactose; (▲) glucose and (■) galactitol. Data are the means ± SEM of 4 to 10 determinations.

may represent a diffusion system or a system with a very high K_m which could correspond to the sodium independent, phlorizin insensitive component of uptake observed in Figure 1.

Because of our interest in galactose toxicity we examined the effect of this hexose on uptake of 3μM myo-inositol. The results shown in Figure 3 indicate that high medium galactose concentrations inhibit myo-inositol uptake. At 20 mM galactose there was a 33% inhibition which was not observed with the sugar alcohol, galactitol. D-glucose at the same concentration caused a comparable inhibition. The inhibition by glucose is similar to that reported in the rabbit (7) and rat endoneural preparations (8). Indeed, in the studies by Green and Lattimer (7) glucose was shown to be a competitive inhibitor of myo-inositol uptake with a Ki of 38 mM. Galactose effects, however, were not examined in the endoneural fascicle. The lack of effect of galactitol on myo-inositol uptake by Schwann cells parallels the lack of effect of sorbitol observed in the endoneural rat experiment (8) and mannitol in rabbit tissue (7). The sodium dependent phlorizin sensitive myo-inositol uptake system of rat Schwann cells has many characteristics in common with that found in rat renal brush border membranes (13).

The present study is the first in which uptake of a substrate or metabolite by isolated Schwann cells has been accomplished. It demonstrates that rat Schwann cells have a relatively high affinity uptake mechanism for myo-inositol. It resembles the system described in endoneural fascicle preparations of sciatic nerve and indicates that the myo-inositol uptake by this multicell type preparation can be attributed in part to the Schwann cell component. It is difficult to estimate the Schwann cell contribution to endoneural fascicle myo-inositol uptake, not only because there is no data in the number of Schwann cells in the endoneural preparation but also because the Schwann cells we've isolated are from very young rats and have been put through an isolation procedure. Whether axons in the endoneural preparation transport myo-inositol is not known. In this regard Warfield et al (14) have reported that isolated synaptosomes from rat brain do not contain a discernable transport mechanism other than diffusion for myo-inositol uptake.

The inhibition by galactose and glucose of myo-inositol uptake by Schwann cells may aid in explaining the decrease in myo-inositol content of peripheral nerve in galactose toxicity (3) as well as diabetes mellitus in rats (1,2,4). The presence of aldose reductase in Schwann cells which would lead to formation of sugar alcohols (15) could also be important in the biochemical and physiological dysfunction of peripheral nerve in these conditions. The fact that inositol administration can reverse the slow conduction time (1,6) and impaired Na^{+}-K^{-} adenosine triphosphatase activity in diabetic rat sciatic nerve (16) attests to the importance of myo-inositol to peripheral nerve function.

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