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D-GLUCOSAMINE UPTAKE BY RAT BRAIN SYNAPTOSOMES

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Summary

Uptake of D-glucosamine by rat brain synaptosomes occurs via a saturable transport process (K_m 2.1 mM, V 3.0 nmol/mg per min) which was clearly distinguishable from simple diffusion. This transport process is highly sensitive to cytochalasin ($K_i = 7 \cdot 10^{-5}$ mM). D-Glucose competitively inhibits D-glucosamine uptake with a K_i value of $8 \cdot 10^{-1}$ mM.

D-Glucosamine has been shown to be incorporated into glycoprotein components of synaptic nerve endings both in vivo and in vitro [1—4]. It might be expected that the level of intrasynaptosomal D-glucosamine or its derivatives would profoundly influence its incorporation into glycoproteins, and that this level would be dependent upon D-glucosamine transport across the synaptosomal membrane. Although synaptosomal uptake of some carbohydrates has been previously reported [5—7], information on D-glucosamine transport is lacking. This present report describes synaptosomal transport of D-glucosamine by a carrier-dependent process which is inhibited competitively by D-glucose and cytochalasin B.

Synaptosomes were prepared from cerebral cortices of adult Sprague-Dawley rat brains according to the method of Kurokawa et al. [8] (yield 3–5 mg synaptosomal protein per g wet weight of cortex) and suspended at 4°C in a medium containing 10 mM Tris·HCl (pH 7.4), 15 mM $MgCl_2$, 146 mM NaCl and 4 mM KCl. Incubations were carried out in duplicate at 37°C for 2 min in the above medium with 0.5 mg synaptosomal protein, determined by the method of Lowry et al. [9], and 1 μCi of D-[1-^{14}C]glucosamine hydrochloride (specific activity 56.6 Ci/mol) in a final volume of 1 ml. The incubation mixture also contained various concentrations of unlabeled D-glucosamine with or without D-glucose or

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cytochalasin B. Cytochalasin B was dissolved in 50% Me_2 SO. The final concentration of Me_2 SO in the incubation mixture was 0.25%. At this concentration, Me_2 SO had no inhibitory effect on D-glucosamine uptake. The incubations were stopped by adding 4 ml of ice-cold medium and the synaptosomal particles were collected on Millipore filters, washed with cold medium and prepared for scintillation counting as described previously [10].

Preliminary experiments indicated that D-glucosamine uptake was linear with time up to 3 min over the range of substrate concentrations used. Uptake was also a linear function of synaptosomal protein concentration up to a concentration of 0.75 mg/ml. The rate of D-glucosamine uptake was not changed by replacing NaCl with sucrose of equal osmolarity. When the synaptosome-containing filters were washed with cold distilled water less than 0.01% of the radioactivity was retained on the filter. This sensitivity to osmotic shock indicates that D-glucosamine was accumulated in pools which were contained within intact membranes. The amount of radioactivity remaining after this treatment was subtracted from the amount of radioactivity in the untreated samples. Heat-treated synaptosomes (100°C for 1 min) did not accumulate any appreciable radioactivity.

The component of D-glucosamine uptake arising from simple diffusion was determined using L-[1-^{14}C]glucose. The rate of L-glucose uptake, which measures simple diffusion in sugar transport experiments [11], was found to be concentration dependent and non-saturable (Fig. 1). At a substrate concentration of 1 mM the rate of diffusion was very small compared to the rate of transport, whereas at 10 mM it accounted for approximately 40%

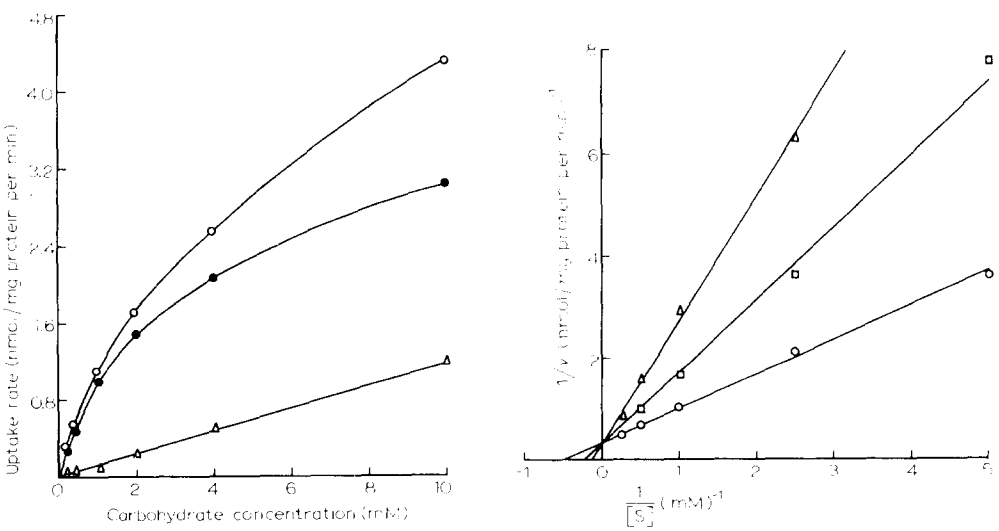

Fig. 1. Rate of uptake of D-[1-^{14}C]glucosamine and L-[1-^{14}C]glucose as a function of substrate concentration. ○—○, D-glucosamine uptake; △—△, L-glucose uptake; ●—●, D-glucosamine uptake, corrected for simple diffusion. Incubations were carried out for 2 min with 0.5 mg synaptosomal protein.

Fig. 2. Lineweaver-Burk plots of D-[1-^{14}C]glucosamine uptake in the absence and presence of 1 mM D-glucose or 0.2 μM cytochalasin B. All values shown had been corrected for simple diffusion. ○—○, D-glucosamine alone; □—□, D-glucosamine + D-glucose; △—△, D-glucosamine + cytochalasin B.

of the observed transport rate. Therefore a correction was made for diffusion at substrate concentrations above 1 mM. The rate of D-glucosamine uptake (after correction for simple diffusion) obeyed Michaelis-Menten kinetics (Figs. 1 and 2). The apparent affinity constant (K_m for D-glucosamine transport was obtained from the Lineweaver-Burk plot (Fig. 2) at substrate concentrations varying from 0.2 to 10 mM. The K_m and V were found to be 2.1 mM and 3.0 nmol/mg per min, respectively. D-Glucose inhibited D-glucosamine uptake competitively with an apparent inhibitor constant (K_i) of $8 \cdot 10^{-1}$ mM (Fig. 2). Cytochalasin B also inhibited synaptosomal D-glucosamine transport in a competitive manner (Fig. 2). The K_i value for the inhibition was $7 \cdot 10^{-5}$ mM. The results of the effect of various concentrations of cytochalasin B on the inhibition of D-glucosamine transport is shown in Fig. 3. The I_{50} value (the concentration of cytochalasin B which inhibited D-glucosamine transport by 50%) at 0.1 mM. substrate concentration was 0.1 μM.

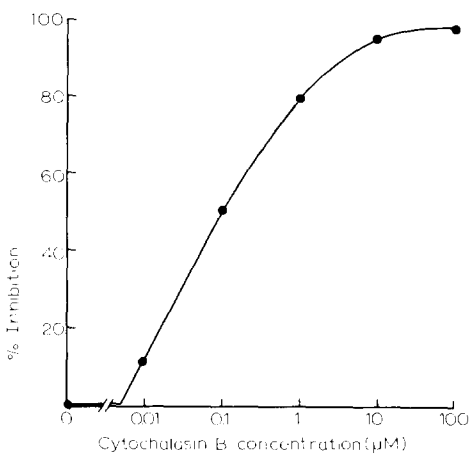

Fig. 3. Inhibition of D-[1-^{14}C]glucosamine uptake as a function of cytochalasin B concentration.

The fact that D-glucosamine uptake is competitively inhibited by D-glucose indicates that D-glucosamine shares a common transport system with D-glucose, but has a lower affinity for the transport site. Although cytochalasin B has been shown to be a powerful competitive inhibitor of the facilitated model of sugar transport in cultured cells [12--14], its effect on synaptosomal carbohydrate uptake has not been reported. The present study clearly shows that cytochalasin B is also a potent competitive inhibitor of D-glucosamine transport in synaptosomes, and thus provides strong evidence that D-glucosamine is transported into isolated synaptosomal particles via a facilitated diffusion system.

Festoff et al. [4] have demonstrated that the incorporation of glucosamine into synaptosomal proteins is sensitive to external Na^+ and K^+ concentration. However, we did not observe such an effect of these ions on synaptosomal uptake of glucosamine. This would suggest that the Na^+-K^+ dependency that they observed was not related to the initial uptake process.

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