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D-Glucose transport in erythrocytes and synaptosomes— A comparison of the effects of three centrally acting drugs

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The passage of D-glucose from blood to brain is mediated by a facilitated diffusion system [1–4]. Similar transport systems for D-glucose are considered to exist in brain cortex slices [5] and in synaptosomes [6–8,*]. The resemblance between the D-glucose transport systems associated with nerve tissue and those in the human erythrocyte [9] has prompted some authors to use red cells as models for determining the effects of centrally acting drugs upon the nerve facilitated systems [10–12].

The method employed in the red cell studies has generally been based on the light scattering technique of Ørskov [13] which was later applied by Sen and Widdas [14]. It is a simple and rapid method having advantages over nerve tissue studies because of the availability and stability of red cell preparations. However, no studies have yet shown that drug effects on the red cell transport system are paralleled by similar effects on the nerve tissue systems.

This study compares the effects of three centrally acting drugs, chlorpromazine (CPZ), mescaline and secobarbital, on the D-glucose transport systems of the rat brain cortex synaptosome and of the human erythrocyte. In the synaptosome studies, the analogue 3-deoxy-3-fluoro-D-glucose (3-FG) was used. Previous work with this sugar has established the nature and kinetics of its transport across the synaptosome as distinct from its subsequent metabolism.*

The effects of chlorpromazine on the human erythrocyte D-glucose transport system were not studied in this work. Baker and Rogers [11, 12] have reported previously in this area.

Secobarbital, chlorpromazine hydrochloride and tritiated 3-deoxy-3-fluoro-D-glucose were the respective gifts of Dr. R. J. Thibert, Dr. G. Wood and Dr. N. F. Taylor of the Chemistry Department of the University of Windsor, Windsor, Ontario, Canada. Mescaline hydrochloride was the gift of R. A. Graham, Aldrich Scientific Services, Tunney's Pasture, Ottawa, Ontario.

Synaptosome preparations, incubation techniques and phosphorylation studies have been documented in a previous publication.* Essentially, synaptosomes were incubated with [3-^{3}H]-3-FG (sp. act. 1.25 μCi/m-mole) and additions. Activity uptake was measured after Millipore filtration.

For the erythrocyte studies fresh blood was obtained from the Windsor Red Cross Society and was stored at 4° for up to 2 weeks. Red cells were separated from 10 ml of whole blood by centrifugation (3000 rev/min for 10 min at room temperature). The plasma and the top layer of cells were removed by aspiration. The red cells were then suspended in buffered saline [1% (w/v) NaCl – 4 mM sodium phosphate, pH 7.4] and shaken gently for 10 min. This was followed by recentrifugation and two more washings in buffered saline to ensure the removal of endogenous D-glucose from the cells. Finally the red cells were resuspended in 10 ml of 0.09 M phosphate buffer, pH 6.8.

Studies of the D-glucose transport were carried out using the light scattering technique outlined by Sen and Widdas [14]. Red cells were preloaded by incubating with 100 mM D-glucose for 30 min at 37°. Fifty μl of this preloaded preparation was mixed with 2.5 ml of saline buffer solution containing up to 12 mM D-glucose and varying concentrations of each drug.

The D-glucose exit from the preloaded cells is accompanied by a loss of water and a consequent shrinkage of the cell. This results in an increase in absorption because of changes in the refractive index of the cells. The process, in this instance, was followed in a Beckmann Acta MVI UV/vis. recording spectrophotometer. The increase in absorption was followed at 37°.

Synaptosomal phosphorylation of 3-FG occurs up to 13 per cent of the total uptake for 0.01 to 2 mM incubations. Phosphorylation of 3-FG is rate limiting for transport, and at low 3-FG concentrations modulation of the phosphorylation step can influence the total amount of recorded 3-FG uptake.* This feature provides a convenient technique for distinguishing between additions which alter the transport step and those which alter phosphorylation. Table 1 shows the influence of the three drugs on these separate processes.

Chlorpromazine at a 10^{-5} M concentration causes a 27 per cent decrease in the amount of 3-FG taken up. This, in part, seems to be related to the influence of the drug upon hexokinase where the phosphorylation is reduced by 7 per cent from the control. Chlorpromazine is apparently inhibiting both the transport and phosphorylation steps. An attempt at measuring the K_i value for transport inhibition by a Dixon plot analysis was unsuccessful since the inhibition does not follow classical Michaelis–Menten kinetics. There is a wide and erratic scatter of points in the 1.0 to 6.0 $\times$ 10^{-5} M region (Fig. 1). Above this concentration of chlorpromazine points showed poor reproducibility. This erratic behavior may reflect the influence of the drug on the hexokinase step, although

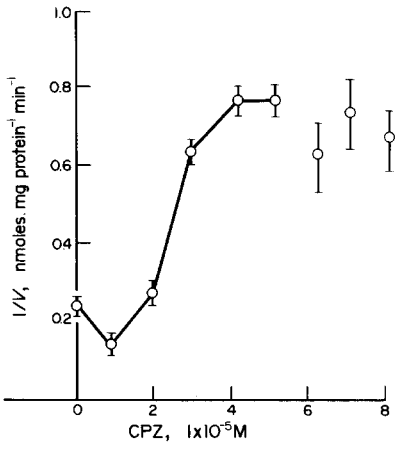

Fig. 1. Effects of chlorpromazine on the uptake of 0.1 mM [3-^{3}H]-3-FG by rat cortex synaptosomes. Each point represents the mean of three determinations. Above 6 $\times$ 10^{-5} M CPZ, standard errors of the means are considerable (barred lines). Incubation techniques have been described previously (see Materials and Methods).

* D. M. Halton, N. F. Taylor and D. P. Lopes, manuscript submitted for publication.

Table 1. Influence of three centrally acting drugs on the uptake and phosphorylation of 0.75 mM 3-FG into synaptosomes *

	% Relative uptake of 3-FG	% of transported 3-FG that phosphorylates in 3 min
No addition	100	9
Chlorpromazine hydrochloride (10^{-5} M)	83	2
Mescaline hydrochloride (10^{-3} M)	93	8
Sodium secobarbital (10^{-3} M)	127	17

* Results are the average of three determinations. All results were obtained from a single synaptosome preparation.

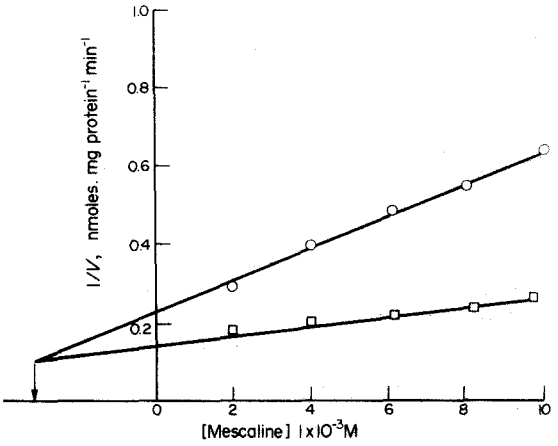

Fig. 2. Inhibition of [3-^{3}H]-3-FG uptake in synaptosomes by mescaline hydrochloride. Key: (O——O) 0.1 mM 3-FG; and (□——□) 0.5 mM 3-FG. Incubation techniques have been described previously (see Materials and Methods).

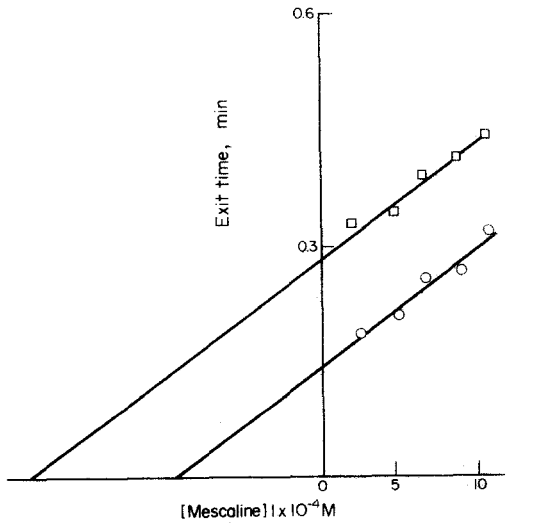

Fig. 3. Inhibitor plot to give the half-saturation constant, ϕ_i, for the competitive inhibition of erythrocyte D-glucose transport by mescaline hydrochloride. Analysis of inhibitor action by the light scattering technique differs from the classical Dixon plots that are used in enzymology or in radiolabeling techniques for transport studies (see references in text). Key: (O——O) 2 mM external D-glucose; and (□——□) 7 mM external D-glucose.

Baker and Rogers [11] have studied the effects of chlorpromazine on glucose efflux from the red cell, where the effects on hexokinase need not be considered, and have found a similar variability in their results. Their data indicated that a non-competitive type inhibition was occurring. A number of authors have observed chlorpromazine-induced morphological changes [15, 16] and membrane expansion [17, 18] and these features may be responsible for the subsequent effects observed on the glucose transport system.

Mescaline hydrochloride was a weak competitive inhibitor of 3-FG transport into synaptosomes, having a K_i of 3.2 mM (Fig. 2). The drug did not apparently alter the phosphorylation step. This same drug was also a weak competitive inhibitor of D-glucose efflux from the red cell with an inhibitor constant ϕ_i of 1.4 mM (Fig. 3), as determined by established methods involving a special type of inhibitor plot analysis [14, 19].

Secobarbital was unique in its ability to stimulate 3-FG uptake into synaptosomes by almost 30 per cent. This effect seems largely due to the ability of the drug to increase the amount of analogue which is phosphorylated. The result does not preclude the possibility that the drug is also affecting transport system. Secobarbital could not be shown to have an influence upon glucose efflux from the red cell. The half saturation constant, ϕ_g, for glucose efflux from the red cell was recorded at 4.1 mM. This value did not change significantly in the presence of 10^{-3} M secobarbital.

Other studies, using pentobarbital, have reported that glucose transport across the blood–brain barrier (BBB) is not altered remarkably by this drug [20–22]. Phillips and Coxon [23], however, observed that *in vitro* pentobarbital increased 2-deoxy-D-glucose transport into brain slices.

Such stimulation effects by barbiturates may, in part, represent a rationale for the anti-convulsant activity of these drugs in coma conditions arising from nerve tissue D-glucose deprivation [24].

Differences in the roles played by hexokinase in the subsequent metabolism of D-glucose transported across the blood cell, the BBB and neuronal tissue possibly account for the variance observed in the effects of barbiturates on these three systems.

Hexokinase activity in neuronal tissue could be playing a key role in the regulation of the high affinity transport system. A high hexokinase activity would mean that the facilitated transport system might never equilibriate since phosphorylation would rapidly remove the internal D-glucose. With a low hexokinase activity the facilitated system could approach equilibrium and, at this point, further entry of the substrate would be determined by the rate of phosphorylation.

In summary, two of the three drugs used in this study, chlorpromazine and mescaline, had similar influences on the D-glucose transport systems of rat cortex synaptosomes and of human erythrocytes. Study of the latter system, thus, can be useful in indicating the probable central action of a drug, but, as can be seen from the secobarbital result, the ineffectiveness of a drug on the human erythrocyte transport system does not rule out the possibility that it may exert an influence on the synaptosome system.

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Effect of 2′-deoxycoformycin on the biologic half-life of 9-β-D-arabinofuranosyladenine 5′-triphosphate in CHO cells

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Ara-A* is a purine nucleoside analogue that has potent antiviral activity [1, 2] and is also a clinically promising antitumor agent [3]. It is phosphorylated intracellularly to the 5′-triphosphate, ara-ATP, and exerts its major action as a competitive inhibitor of DNA polymerase [4, 5]. In addition, ara-ATP is incorporated into DNA [6–8] and inhibits ribonucleotide reductase [9].

The rapid deamination of ara-A by ADA to the relatively inactive ara-Hx severely limits the effectiveness of this drug [10–12]. Inhibitors of ADA that potentiate the action of ara-A have been developed recently. EHNA [13] increased the lethality of ara-A to mouse fibroblasts by 20-fold [14], whereas dCF, a more potent inhibitor of ADA [15], significantly enhanced the toxicity of ara-A to the mouse leukemia L1210, both *in vitro* [16] and *in vivo* [17–19]. dCF is also an inhibitor of adenylic deaminase from erythrocytes [20].

We have studied the biochemical basis for the increased activity of ara-A in the presence of deaminase inhibitors [21, 22]. Higher ara-ATP concentrations were achieved in cells treated with ara-A in the presence of a deaminase inhibitor than were achieved in those cells treated with ara-A alone, both *in vitro* [23, 24] and *in vivo* [22, 25]. The experiments described here extend these observations by investigating the effects of the inhibition of ADA and possibly adenylic deaminase on the biologic half-life of ara-ATP in CHO cells (the time necessary for the decay of one-half the initial concentration of ara-ATP). It seemed likely that, after utilization of ara-ATP as a phosphate donor by a wide variety of phosphate transferring enzymes or cellular phosphatases, the adenine moiety of the analogue might become susceptible to deamination at either the monophosphate [14] or the nucleoside [10] level. Such deamination might lessen the lethality of the incubation and would effectively eliminate the possibility of phosphorylative salvage to ara-ATP. Inclusion of dCF would be expected to block these major routes of deaminative detoxification [20, 26] and should provide an opportunity to evaluate the importance of these catabolic pathways in the maintenance of cellular ara-ATP levels.

All chemicals used were reagent grade. Ara-A was a product of Pfanstiehl Laboratories (Waukegan, IL) and obtained through the NCI. [2-^{3}H]ara-A (18.7 Ci/m-mole) was purchased from New England Nuclear Corp. (Boston, MA). It was 98.7 per cent pure [^{3}H]ara-A, as determined by thin-layer chromatography. dCF was produced by Parke, Davis & Co. (Detroit, MI) and obtained through the NCI.

CHO cells were maintained in monolayer cultures in McCoy's modified 5a medium and supplemented with 20% horse serum (Grand Island Biological Co., Grand Island,

* Abbreviations: ADA, adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4); ara-A, 9-β-D-arabinofuranosyladenine; ara-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; ara-Hx, 9-β-D-arabinofuranosylhypoxanthine; CHO cells, Chinese hamster ovary cells; dCF, (R)-3-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol, deoxycoformycin, covidarabine; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)-adenine; h.p.l.c., high pressure liquid chromatography; and PCA, perchloric acid.