*Biochem. J.* (1979) **180**, 677–679
*Printed in Great Britain*



# Effects of some Chlorinated Sugar Derivatives on the Hexose Transport System of the Blood/Brain Barrier

By JILL E. CREMER and VINCENT J. CUNNINGHAM
*MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, U.K.*

(*Received* 12 *March* 1979)

The inhibition of D-glucose transport into brain by several hexose analogues has been investigated in adult anaesthetized rats. D-Glucose was transported with apparent $V_{max.} = 1.22\,\mu$mol/g per min, $K_m = 11.12$ mM and $K_d = 0.008$ ml/g per min. 6-Chloro-6-deoxyglucose was transported with corresponding values of $V_{max.} = 1.33\,\mu$mol/g per min, $K_m = 5.5$ mM and $K_d = 0.0155$ ml/g per min and inhibited D-glucose transport with apparent $K_i = 3.01$ mM. 6-Chloro-6-deoxymannose, 6-chloro-6-deoxygalactose and 6-tosyl-6-deoxygalactose also inhibited D-glucose transport, but 6-chloro-6-deoxyfructose was without effect. The results were consistent with a model for glucose transport at the blood/brain interface that involves a hydrophobic site on the transport protein at or near the 6-position of bound glucose.

There is strong evidence that the entry of glucose from blood to brain is mediated by a facilitated transport process (Crone, 1965). The unidirectional influx of glucose has been shown to be partly saturable, stereospecific and competitively inhibited by other hexoses (Betz *et al.*, 1973; Pardridge & Oldendorf, 1975). Most proposed models to account for these properties include a mobile carrier protein with at least one binding site for the transport substrate (see Deves & Krupka, 1978) and the present paper will refer to a 'carrier' for hexoses. Because of the presence of tight junctions between adjacent capillary endothelial cells of central-nervous tissue (Reese & Karnovsky, 1967), the passage of water-soluble substrates has to be through these cells and the carrier proteins are likely to be localized within their plasma membranes.

The structural requirements for substrate transport and its inhibition are of interest for an understanding of the mechanisms involved. We have recently tested several sugar analogues, including 6-chloro-6-deoxyglucose. This chlorinated sugar has been shown to have an anti-fertility action in male rats (Ford & Waites, 1978), similar to the effect of α-chlorohydrin (3-chloropropane-1,2-diol; Ford *et al.*, 1977; Brown-Woodman *et al.*, 1978). We now show that 6-chloro-6-deoxyglucose enters the brain from blood by a partly saturable transport process and, in addition, inhibits the transport of D-glucose. We have also estimated apparent inhibitor constants for several other chlorinated sugar derivatives.

## Experimental

### *Measurement of transport*

The influx of D-glucose and 6-chloro-6-deoxyglucose was measured in adult Wistar rats by using the intracarotid-artery-injection technique described by Oldendorf (1971) and Cremer *et al.* (1976). Briefly, animals were anaesthetized with pentobarbital, the right common carotid artery exposed and a 0.2 ml bolus, containing $^{14}$C-labelled sugar and $^3H_2O$ in Ringer's solution buffered to pH 7.5 with 10 mM-Hepes {2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulphonic acid}, was injected rapidly. After 10 s, the animals were decapitated, the right cerebral hemisphere removed and its radioactive isotope content measured. The extraction of sugar relative to water was estimated and converted into a rate of unidirectional influx ($v_t$, μmol/g per min) by using a cerebral-blood-flow value of 0.58 ml/g per min for anaesthetized rats (Pardridge & Oldendorf, 1975). For inhibition studies, the injection solutions contained a small constant amount of D-[2-$^{14}$C]-glucose (about 0.2 mM) mixed with increasing concentrations of unlabelled sugar derivative.

### *Estimation of kinetic parameters*

The variation of the rate of influx ($v_t$) with substrate concentration ([S]) in the injection mixture may be described in terms of two components, one saturable and one non-saturable. The former is defined by a Michaelis constant ($K_m$) and a maximum velocity ($V_{max.}$) and the latter by an apparent diffusion constant ($K_d$) (Betz *et al.*, 1973; Bachelard *et al.*, 1973; Pardridge & Oldendorf, 1975). The relationship is:

$$v_t = \frac{V_{max.}[S]}{[S]+K_m} + K_d[S] \qquad (1)$$

Estimates of $V_{max.}$, $K_m$ and $K_d$ were obtained from weighted best fits to data as described by Cunningham & Sarna (1979).

Inhibition data were interpreted in terms of a simple competitive model, assuming that the inhibitor affected the saturable component of inflow as follows:

$$v_t = \frac{V_{max.}[S]}{[S]+K_m(1+[I]/K_i)} + K_d[S] \quad (2)$$

where [I] is inhibitor concentration and $K_i$ an apparent inhibitor constant. Estimates of those parameters pertaining to D-glucose ($V_{max.}$, $K_m$ and $K_d$) were obtained as described above and inserted into eqn. (2). $K_i$ values for the various sugar derivatives were then estimated from the variation of $v_t$ (for D-glucose) at a fixed concentration of D-glucose in the presence of a range of inhibitor concentrations.

## Results and Discussion

The rate of inflow across the blood/brain barrier of 6-chloro-6-deoxyglucose at various concentrations is shown in Fig. 1. At all concentrations tested the rate was higher than that for D-glucose. The Michaelis constant for the saturable inflow transport of 6-chloro-6-deoxyglucose is about half that for D-glucose and the apparent diffusion constant for non-saturable inflow is higher (Table 1). The maximum rate of inflow on the saturable carrier is very similar for both sugars (Table 1). Separate measurements established that the rate of inflow of D-glucose was inhibited by 6-chloro-6-deoxyglucose and the apparent $K_i$ for this effect is given in Table 1. The estimated $K_i$ for the inhibition of D-glucose transport by the analogue was close to, but not identical with, the estimated $K_m$ for the saturable transport of the analogue itself. These observations suggest that the same transport system is involved in each case, with the analogue having a higher affinity for the saturable carrier than the physiological substrate D-glucose.

6-Chloro derivatives of mannose and galactose were also tested for inhibition of D-glucose transport and the results are presented in Table 1. Oldendorf (1971) and Pardridge & Oldendorf (1975) have shown that the parent sugars mannose and galactose are transported across the blood/brain barrier and inhibit the transport of D-glucose. Relevant values taken from

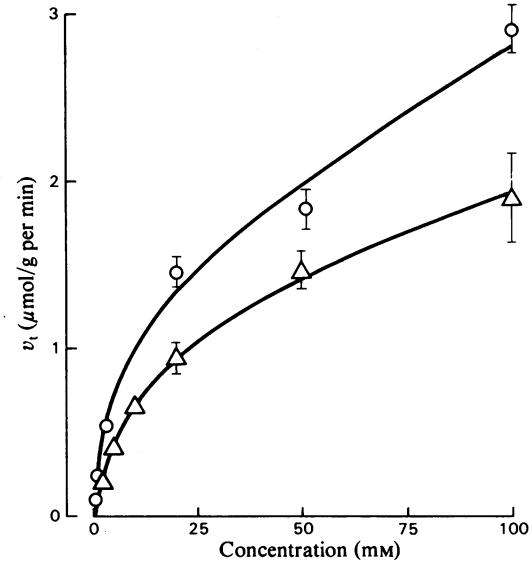


Fig. 1. *Inflow velocity of* D-*glucose and 6-chloro-6-deoxyglucose across rat blood/brain barrier*
Results are given as means ± S.E.M., except at low concentrations, when only the mean is shown for clarity. Symbols: △, D-glucose; ○, 6-chloro-6-deoxyglucose. Concentration is that of the substrate in the injected bolus (see the text).

Table 1. *Transport and inhibition constants for* D-*glucose and hexose analogues across the blood/brain barrier*
Results are presented as means ± S.E.M. for the number of observations (*n*) shown. Kinetic constants were obtained from best fits to eqns. (1) and (2) (see the text) over a range of substrate or inhibitor concentrations up to 100 mM. Inhibition was measured against a D-glucose concentration of about 0.2 mM. Exceptions: *data obtained at one concentration (20 or 50 mM) only; **data taken from Pardridge & Oldendorf (1975).

| Substrate | $K_m$ (mM) | $V_{max.}$ (μmol/g per min) | $K_d$ (ml/g per min) | *n* |
|---|---|---|---|---|
| D-Glucose | 11.12 ± 2.76 | 1.22 ± 0.26 | 0.0083 ± 0.0030 | 24 |
| 6-Chloro-6-deoxyglucose | 5.53 ± 0.85 | 1.33 ± 0.17 | 0.0155 ± 0.0025 | 22 |

| Inhibitor | $K_i$ (mM) | *n* |
|---|---|---|
| 6-Chloro-6-deoxyglucose | 3.01 ± 0.26 | 34 |
| 6-Chloro-6-deoxymannose | 4.27 ± 0.24 | 16 |
| 6-Chloro-6-deoxygalactose | 9.47 ± 1.51 | 16 |
| 6-Tosyl-6-deoxygalactose | 7.72* | 4 |
| D-Mannose | 21** | |
| D-Galactose | 40** | |
| 1-*O*-Methyl-6-chloro-6-deoxypyranoside | No effect* | |
| 6-Chloro-6-deoxyfructose | No effect* | |

these papers are also given in Table 1. As with glucose, substitution of a chloro group at the 6-position leads to a decrease in the apparent $K_i$. In addition introduction of a tosyl group in the 6-position of galactose decreases the apparent $K_i$ against D-glucose transport. These results are consistent with a model for glucose transport at the blood/brain interface (Betz *et al.*, 1976) that involves a hydrophobic site on the transport protein near the 6-position of bound glucose. This model resembles that for the glucose–carrier complex in the human erythrocyte discussed by Barnett *et al.* (1973). Further substitution at the 1-position to give 1-*O*-methyl-6-chloro-6-deoxypyranoside removes the inhibition against D-glucose transport completely (Table 1), which may be attributed to steric hindrance.

As mentioned in the introduction, 6-chloro-6-deoxyglucose is of interest as having an anti-fertility action in male rats. 6-Chloro-6-deoxyfructose also has an anti-fertility action (Ford & Waites, 1978). When tested against glucose transport into brain, it was without effect (Table 1).

We thank Professor G. M. H. Waites and Dr. W. C. L. Ford, Department of Biochemistry and Physiology, University of Reading, Reading, Berks., U.K., who are supported by grant number 75311 from the World Health Organization and Dr. D. J. Snodin, Tate and Lyle Ltd., for generous gifts of sugar derivatives. We also express our thanks to Mrs. H. M. Teal and Mr. T. Lister for their skilled assistance.

## References

Bachelard, H. S., Daniel, P. M., Love, E. R. & Pratt, O. E. (1973) *Proc. R. Soc. London Ser. B* **183**, 71–82

Barnett, J. E. G., Holman, G. D. & Munday, K. A. (1973) *Biochem. J.* **131**, 211–221

Betz, A. L., Gilboe, D. D., Yudilevich, D. L. & Drewes, L. R. (1973) *Am. J. Physiol.* **225**, 586–592

Betz, A. L., Gilboe, D. D. & Drewes, L. R. (1976) *Adv. Exp. Med. Biol.* **69**, 133–149

Brown-Woodman, P. D. C., Mohri, H., Mohri, T., Suter, D. & White, I. G. (1978) *Biochem. J.* **170**, 23–27

Cremer, J. E., Braun, L. D. & Oldendorf, W. H. (1976) *Biochim. Biophys. Acta* **448**, 633–637

Crone, C. (1965) *J. Physiol. (London)* **181**, 103–113

Cunningham, V. J. & Sarna, G. S. (1979) *J. Neurochem.* in the press

Deves, R. & Krupka, R. M. (1978) *Biochim. Biophys. Acta* **513**, 156–172

Ford, W. C. L. & Waites, G. M. H. (1978) *J. Reprod. Fertil.* **52**, 153–157

Ford, W. C. L., Harrison, A. & Waites, G. M. H. (1977) *J. Reprod. Fertil.* **51**, 105–109

Oldendorf, W. H. (1971) *Am. J. Physiol.* **221**, 1629–1639

Pardridge, W. M. & Oldendorf, W. H. (1975) *Biochim. Biophys. Acta* **382**, 377–392

Reese, T. S. & Karnovsky, M. J. (1967) *J. Cell Biol.* **34**, 207–217