Transport of glucose across the blood-brain barrier: Reevaluation of the accelerative exchange diffusion

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In an earlier study of glucose transport across the blood-brain barrier it was concluded that the kinetic transport constants increase as the brain glucose concentration increases, a finding that was attributed to accelerative exchange diffusion (Betz et al. 1975). The conclusion, however, relied upon application of a commonly used simplified treatment of tracer extraction data. In this study it is demonstrated that the simplified treatment is applicable only in the case of zero brain glucose concentration, and a more general model for determination of the kinetic constants is developed. Re-analysis by this model of the data of Betz et al. (1975)—comprising a range of brain glucose concentrations—gave kinetic constants which did not vary significantly over a wide range of brain glucose concentrations. For brain glucose concentrations up to about 12 mmol l^{-1}, the kinetic constants obtained for glucose transport across the blood-brain barrier are

$K_m = 6.18 \pm 0.38$ mmol l^{-1}
$V_{max} = 1.65 \pm 0.06$ μmol g^{-1} min^{-1}

The transport of glucose between blood and brain across the blood-brain barrier occurs by a facilitated diffusion process (Crone 1960, 1965, Betz et al. 1973, Lund-Andersen 1979). Betz et al. (1975) reported a series of indicator diffusion studies, in which the affinity constant K_m apparently rose from 8 to 36 mmol l^{-1} as brain glucose concentration was increased from 2.4 to 14.4 mmol l^{-1}. The corresponding values for V_{max} increased from 1.6 to 3.8 μmol g^{-1} min^{-1}. In a recent review Lund-Andersen (1979) argued that the increase of the kinetic constants seemed to reflect conceptual errors in the kinetic analysis rather than a real change of the transport mechanism.

In the present paper we focus on problems encountered in the calculation of the kinetic constants and show that a commonly used procedure is correct only when the brain solute concentration is zero. A more generally applicable procedure is developed and used to reanalyze the original data of Betz et al. (1975), yielding significantly different results.

EXPERIMENTAL PROCEDURE

The experimental procedures were described by Betz et al. (1975); a short review is presented to provide the reader with some background.

22 isolated perfused mongrel dog brains were equilibrated by perfusion with blood which was either 6.11, 16.8, 26.3, 43.9 or 56.0 mmol l^{-1} in plasma glucose. This produced in whole brain water phase glucose levels of 2.4, 5.2, 8.2, 12.4 or 14.4 mmol l^{-1} respectively.

Following the initial equilibration, the perfusion was switched to a second oxygenator system containing a test glucose concentration that varied between 2 and 100
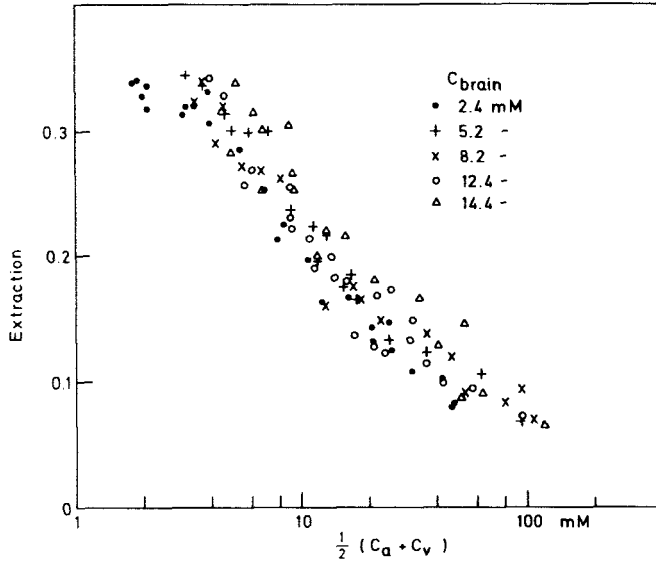
Fig. 1. Extraction of labelled glucose plotted versus the arithmetical mean of arterial and venous cold glucose concentration. All data from experiments at five different brain concentrations are shown. Data originate from Betz et al. (1975).

mmol l^{-1}. Thus in some instances the glucose concentration of the perfusate was lower than that of the brain. After 15 s at the test glucose concentration. a 50 μl bolus containing D−(6−^{3}H) glucose as the test molecule and ^{22}Na as the vascular marker was injected and the extraction of labelled glucose was determined from 30 venous samples obtained at 1 sec intervals. Arterial and venous blood samples were obtained and analyzed for unlabelled glucose and the blood flow rate was determined. After each injection, the perfusion was returned to the equilibration perfusate for 15 min prior to determining glucose extraction at another test concentration of glucose.

Thus for each injection the following data are available: the plasma flow rate F(ml g^{-1} min ^{1}), the extraction E* of labelled glucose and the concentrations of unlabelled glucose in arterial (C_a) and venous (C_v) blood. 17–25 individual injections were made at various plasma glucose levels for each brain glucose concentration.

THEORY

The model to be used for re-analysis of the data of Betz et al. (1975) is based on the following assumptions: (a) with respect to glucose transfer the blood-brain barrier (the capillary wall) can be considered as a single membrane containing symmetric glucose carriers of Michaelis-Menten type and having, in parallel with these, pathways for non-facilitated diffusion, (b) backflux of labelled glucose *from brain to blood may be neglected*, (c) the concentration of unlabelled glucose in brain, C_{br}, is constant (during the 30 s perfusion with test perfusate). (d) the brain is homogeneous with respect to capillary dimensions. blood flow. carrier density in the capillaries and concentration

of unlabelled glucose, (e) all plasma elements pass through a capillary with the same velocity.

In view of the situation existing during perfusion with test perfusate it is further assumed that the perfusion rate and the glucose concentration in the perfusate are constant.

Derivation of equations

Besides C_{br} the following symbols will be used. The plasma concentrations of unlabelled glucose will be termed C_a at the arterial end of a capillary, C_v at the venous end, and C_c at a general position in the capillary. A * suffix is used to denote the corresponding concentrations of labelled glucose. $\bar{C}_c$ will be used as a symbol for the arithmetic mean of the arterial and venous concentrations of unlabelled glucose (i.e. for $(C_a+C_v)/2$). F will be used for the rate of plasma flow through the whole brain, and τ for the transit time of plasma in capillaries. PS_{nf} denotes the PS-product of all brain capillaries for glucose transfer by non-facilitated diffusion. E* denotes (overall) extraction of labelled glucose, i.e. $(C_a^*-C_v^*)/C_a^*$, and E_{nf}^* the extraction by non-facilitated diffusion. V_{max} and K_m are defined in the conventional way, V_{max} applying to all brain capillaries.

On the above assumptions the differential equation for the rate of plasma to brain passage of unlabelled glucose in a fractional element, dl/l, of the capillaries containing plasma with C_c will be

$$F\tau \frac{dl}{l} \frac{dC_c}{dt} = \left[-\left(V_{max}\frac{dl}{l}\right)\frac{C_c}{K_m+C_c} \right]$$
$$+ \left[\left(V_{max}\frac{dl}{l}\right)\frac{C_{br}}{K_m+C_{br}} \right]$$
$$+ \left[PS_{nf}\frac{dl}{l}(C_{br}-C_c) \right] \qquad (1)$$

Table 1. *Comparison of kinetic parameters for glucose transport into brain*

Brain glucose concentration (mM)	2.4	5.2	8.2	12.4	14.4
Values of K_m (mM)					
Direct fit to eq. (3)	8.45±0.80	11.52±1.44	17.83±2.34	28.8±6.30	37.7±13.5
Direct fit to eq. (2 c)	5.70±0.53	5.44±0.82	7.39±1.18	5.95±1.14	10.43±2.21
Values of V_{max} (μmol min^{-1} g^{-1})					
Direct fit to eq. (3)	1.61±0.10	1.87±0.15	2.21±0.21	2.68±0.44	3.83±1.10
Direct fit to eq. (2 c)	1.53±0.09	1.60±0.11	1.80±0.16	1.62±0.13	2.71±0.32

In this equation the left-hand side represents the rate of increase in unlabelled glucose content in the plasma of the considered fractional element of the capillaries. On the right-hand side the first term is the rate of carrier-mediated loss of unlabelled glucose from the element; the second term is the corresponding rate of carrier-mediated gain by influx from brain; the third term is the net rate of gain of unlabelled glucose in the element by non-facilitated diffusion.

From eq. (1) a corresponding differential equation for transfer of labelled glucose can be obtained by replacing dC_c with dC_c^* and multiplying on the right side the first term with C_c^*/C_c, the second term with C_{br}^*/C_{br}, C_{br} in the third term with C_{br}^*/C_{br}, and C_c in the third term with C_c^*/C_c. Since, corresponding to the assumption of no backflux of labelled glucose, C_{br}^* must be considered (essentially) zero one obtains

$$(F\tau)\frac{dC_c^*}{dt} = -V_{max}\frac{C_c^*}{K_m+C_c}-PS_{nf}C_c^* \qquad (2)$$

or

$$(F\tau)\frac{dC_c^*}{C_c^*} = \left(-V_{max}\frac{1}{K_m+C_c}-PS_{nf}\right)dt$$

which by integration from C_a^* to C_v^* and from 0 to τ gives

$$(F\tau)\ln\frac{C_v^*}{C_a^*} \simeq -V_{max}\int_0^\tau\frac{dt}{K_m+C_c}-PS_{nf}\cdot\tau \qquad (2\,a)$$

To make use of this equation the integral must be replaced by a term which—apart from K_m—contains only measurable variables. Calculations of glucose profiles, mentioned below, have shown that the term $\tau/(K_m+\bar{C}_c)$ approximates the integral to within a few per cent. Replacing the integral by this term, replacing C_v^*/C_a^* by $1-E^*$ and eliminating τ gives

$$-F\ln(1-E^*) \simeq \frac{V_{max}}{K_m+\bar{C}_c}+PS_{nf} \qquad (2\,b)$$

Now for a homogeneous system with no tissue-to-blood backflux the relationship between the PS-product and the extraction for a substance passing the capillary wall merely by non-carrier mediated transport has been developed by Crone (1963). Using our chosen symbols this relationship is found from Eq. (2 b) with $V_{max}=0$:

$$PS_{nf} = -F\ln(1-E_{nf}^*)$$

which for small values of E_{nf}^* can be modified to

$$PS_{nf} \simeq F E_{nf}^* \cdot$$

Inserting this in eq. (2 b) and multiplying by $\bar{C}_c$ gives

$$-F\bar{C}_c(\ln(1-E^*)+E_{nf}^*) \simeq \frac{V_{max}}{K_m+\bar{C}_c}\bar{C}_c \qquad (2\,c)$$

For comparison the equation used by Betz et al. (1975) for determination of V_{max} and K_m was:

$$F C_a(E^*-E_{nf}^*) = \frac{V_{max}}{K_m+\bar{C}_c}\bar{C}_c \qquad (3)$$

K_m and V_{max} were calculated from both eq. (2 c) and eq. (3). In both cases E_{nf}^* was taken as 0.036, since Betz et al. (1973) found that the—presumably non-facilitated—extraction of fructose to be 0.036±0.003 (SEM, n=17).

Evidently considerable differences between the values obtained are to be expected if C_a differs considerably from $(C_a+C_v)/2$ and/or E^* is greater than 0.2–0.3. Calculations were made by a direct fit method which for all data corresponding to a particular level of brain glucose concentration minimized the sum of squares of deviations between the value of the left-hand side ('experimental velocity') and the value of the right-hand side ('theoretical velocity') of the two equations. Weighting factors to the data values were calculated as described by Betz et al. (1973). With proper weighting, this type of fitting gives estimates and standard errors for K_m and V_{max} and also statistical information to a χ^2-test. One can thus get a probalistic measure of how well the equations describe the experimental data.

4.2. Calculation of glucose profiles

In order to evaluate how the concentrations of labelled and unlabelled glucose in plasma vary with time during its passage through a capillary eqs. (1) and (2) were normalized with respect to transit time ($t'=t/\tau$) and numerically integrated from $t'=0$ to $t'=1$. When the concentrations were expressed relative to K_m the only parameter in the equations was $V_{max}/(K_mF)$. The purpose of these calculations was to get some qualitative impression of the variation of glucose concentrations during capillary passage. Therefore, the non-facilitated diffusion term was disregarded in the calculations.

RESULTS

Fig. 1 shows the observed extraction of labelled glucose as a function of the mean of the arterial and
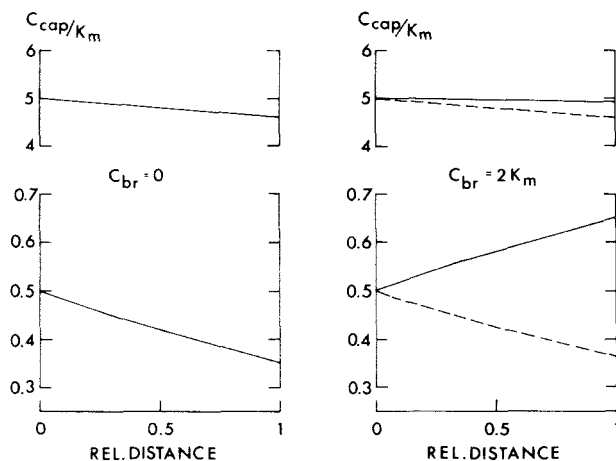
Fig. 2. The variation of capillary concentrations of cold and labelled glucose with distance from arterial to venous end of the capillary. The ordinate is the ratio of glucose concentration to K_m for the cold concentration, shown as full lines. The labelled glucose, shown as dashed lines, is in arbitrary units but scaled to coincide with cold concentration at the arterial end. The left panel shows the situation for zero brain concentration with same variation of cold and labelled glucose (coincidence of solid and dashed lines). The right panel is for a brain glucose level of $2 K_m$. In both panels are shown results for perfusate (arterial) concentrations of 0.5 and 5 times K_m. The calculations are performed with $V_{max}/(K_m F)=0.5$.

venous test glucose concentrations for 5 different brain glucose concentrations. As might be expected if transcapillary glucose transfer occurs (mainly) by a saturable process the extraction decreases with increasing capillary glucose concentration.

The kinetic constants for glucose transfer across the blood-brain barrier calculated from fits to eqs. (2c) and (3) are given in Table 1. It is evident that the V_{max} and K_m values obtained from the fits to eq. (3) increase with increasing brain glucose concentrations. By contrast the V_{max} and K_m values obtained from fits to eq. (2c) are similar for the 4 lowest levels of brain glucose concentration, but somewhat higher for the highest brain glucose concentration.

For each of the 5 sets of data the ability of eq. (2c) to fit the measurements was examined by the χ^2-test. The data corresponding to the 4 lowest levels of brain glucose concentration were found to be well represented by this equation (p =0.4–0.6), while for those corresponding to the highest brain concentration a significant lack of fit was found (p about 0.01). This will be commented on in the discussion. Taking K_m and V_{max} to be independent of brain glucose concentration up to 12.4 mmol l–1 we may pool all data from the first four groups of

experiments to obtain the following values for the kinetic constants:

$$K_m = 6.18 \pm 0.38 \text{ mmol } l^{-1}$$
$$V_{max} = 1.65 \pm 0.06 \, \mu\text{mol g}^{-1} \text{ min}^{-1}.$$

Fig. 2 shows some sample calculations of glucose concentration profiles along a capillary. The calculations were based on the values of K_m and V_{max} given above and a typical flow of 0.5 ml g^{-1} min^{-1} —corresponding to a value of $V_{max}/(K_m F)=0.5$. Profiles are given for brain concentration equal to zero (left panel) and to $2 K_m$ (right panel) and for test perfusate concentrations equal to 0.5 K_m and to 5 K_m. The full lines gives the plasma concentration of unlabelled glucose as a fraction of K_m. The dashed lines show the profiles of labelled glucose using arbitrary units so as to make the (relative) concentration at the arterial end coincide with the concentration of cold glucose. With zero brain glucose concentration the dashed lines obviously coincide with the fully drawn lines and the specific activity remains constant during the capillary transit. From the right panel it appears that the profiles for labelled and unlabelled glucose differ with the specific activity of the plasma glucose decreasing along the capillary (the relative alteration in the

specific activity is represented by the alteration in the ratio between the ordinate value for the dashed line and the ordinate value of the corresponding fully drawn line). The concentration of unlabelled glucose at non-zero brain concentration in the right panel may be thought of as composed of two fractions. The first fraction is the concentration of perfusate still remaining in the capillary, given by the dashed curve. The second fraction is caused by influx from the brain and is given as the difference between the solid and dashed lines.

DISCUSSION

The present paper has focused upon the calculation of kinetic transport parameters from data obtained by aid of the indicator diffusion technique. This method and its formalism was originally developed by Crone (1963) to study exchange of labelled substances which pass the capillary walls by non-facilitated diffusion. For a labelled substance entering a tissue *de novo* with the plasma of the arterial blood and showing an initial extraction, E^*, the rate of unidirectional plasma to tissue passage of labelled substance (v^*) was taken to be

$$v^* = F\,C_a^*\,E^*$$

on the assumption that the early samples of venous blood used for calculation of E^* contained (essentially) no labelled substance which had entered into the tissue and then returned to the plasma.

If the tissue did not a priori contain unlabelled molecules, the initial extraction of unlabelled substance entering with the arterial plasma would also be E^*, and with the above reservation for 'back-flux' the rate of unidirectional plasma to tissue passage of unlabelled substance would be given by

$$v = F\,C_a\,E^*$$

If, however, unlabelled molecules are present beforehand in the tissue (interstices) such molecules will pass into the plasma traversing the capillaries and produce a fall in the specific activity of the substance in the plasma. Since for any length element of the capillaries the ratio between the unidirectional plasma to tissue passages of labelled and unlabelled substance will be equal to the existing specific activity (C_c^*/C_c) the rate of unidirectional passage of unlabelled substance will be underestimated by using the velocity $FC_a\,E^*$ (cf. Fig. 2).

When the pre-existing concentration of unlabelled glucose in the brain (interstices) is small relative to the concentration in the inflowing plasma the underestimates will be only small. This will often—but not always—be the case when the indicator diffusion method is used without altering the glucose concentration in the inflowing plasma appreciably by the introduction of the labelled glucose. However, in the experiments of Betz et al. (1975) cold glucose was in many cases present in brain (water) in concentrations considerably exceeding those of the test perfusate. Consequently the applied calculation procedure (eq. 3) lead to erroneous results and conclusions, viz. increasing values of K_m and V_{max} with increasing brain glucose levels.

The application of a procedure based on Eq. (2 c), which should largely eliminate errors of the said source, gave values for K_m and for V_{max} which were essentially the same within the range of brain glucose concentrations from 2.4 to 12.4 mmol l^{-1}. For experiments corresponding to brain glucose concentrations of about 14.4 mmol l^{-1} the calculated K_m and V_{max} values were somewhat higher. However, this determination is statistically improbable indicating that the analytical transport model employed here does not describe the data well.

Within the framework of the transport model used here it appears therefore reasonable to conclude that the experimental data of Betz et al. (1975) do not support the view that accelerative exchange diffusion is involved in the passage of glucose across the blood-brain barrier. This conclusion receives some support by the fact that the kinetic constants obtained by our recalculation (of data for dog brains) are quite close to those derived from steady-state methods applied to rat brains (Lund-Andersen 1979, Gjedde 1980).

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