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Graphical Evaluation of Blood-to-Brain Transfer Constants from Multiple-Time Uptake Data

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Summary: A theoretical model of blood–brain exchange is developed and a procedure is derived that can be used for graphing multiple-time tissue uptake data and determining whether a unidirectional transfer process was dominant during part or all of the experimental period. If the graph indicates unidirectionality of uptake, then an influx constant (K_1) can be calculated. The model is general, assumes linear transfer kinetics, and consists of a blood–plasma compartment, a reversible tissue region with an arbitrary number of compartments, and one or more irreversible tissue regions. The solution of the equations for this model shows that a graph of the ratio of the total tissue solute concentration at the times of sampling to the plasma concentration at the respective times (C_p) versus the ratio of the arterial plasma concentration–time integral to C_p should be drawn. If the data are consistent with this model, then this graph will yield a curve that eventually becomes linear, with a slope of K_1 and an ordinate intercept less than or equal to the vascular plus steady-state space of the reversible tissue region.
Key Words: Blood–brain transport—Multiple-time/graphical analysis—Transfer constants.

Most studies of solute transfer across the blood–brain barrier (BBB) employ the same general experimental procedure and derive one of three transfer numbers—the extraction fraction, the influx constant, or the efflux (washout) constant—from the data. The experimental procedure usually includes introducing the solute of interest (hereafter referred to as the test solute or material) into the bloodstream, taking blood samples throughout the experimental period, obtaining terminal brain and blood samples, and assaying the samples for the test solute. The calculation of the appropriate transfer number involves the assumption of a specific blood–tissue distribution model for the system and the insertion of the experimental data into the working equation of the assumed model.

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Abbreviation used: BBB, Blood–brain barrier.

Of the three transfer numbers listed above, the influx constant K_1 has been measured least to date. Yet, when properly determined, the influx constant reliably quantitates blood–brain transport for all but rapidly exchanging solutes and is the only transfer number which can be accurately determined for solutes that cross the BBB slowly. Moreover, a simple relationship between K_1, capillary blood flow, capillary surface area, and capillary permeability (the latter two variables are usually combined into a permeability–surface area or PS product) has been developed (Renkin, 1959; Crone, 1963) and can be used to convert the influx constant of the test solute to a PS product if blood flow is known (Patlak and Fenstermacher, 1975; Ohno et al., 1978; Fenstermacher et al., 1981).

The transfer constant for a particular solute across a membrane such as the frog skin or the toad urinary bladder is usually determined experimentally by measuring the unidirectional flux rate across the epithelium from a source solution of constant concentration. In this situation, K_1 is defined as the steady-state rate of solute flux across the membrane system divided by the test solute's concentration in the source solution (the term steady-state in this definition means that all fluxes and ve-

locities are constant with respect to time). Therefore, with respect to the BBB, K_i is defined as the steady-state rate of solute flux across the BBB complex from plasma (constant concentration) into brain extracellular fluid divided by the plasma concentration of the solute. The present paper illustrates how K_i can be found for any membrane system (including the BBB) when the source solution's (plasma) concentration is not constant.

For the calculation of an influx constant from blood–tissue distribution data, the net transfer of the test solute across the BBB is assumed to be unidirectional throughout the entire experimental period. This assumption means that all the test material that has crossed the BBB and entered the neural parenchyma during the course of an experiment is still present in the tissue at the time of brain sampling. Needless to say, this assumption best fits an experimental system in which the test material is held or bound within the parenchyma by a physiological or biochemical process. In addition, this assumption confines the estimation of the influx constant to experimental situations in which the unidirectional character of tissue uptake can be demonstrated.

Bradbury and Kleeman (1967) recognized that brain cells serve as a sink for potassium that has moved across brain capillaries and into brain extracellular fluid, and that this cellular sink would probably lead to a purely unidirectional transfer of tracer potassium across the BBB for several hours after intravenous administration. Therefore, these authors assumed a unidirectional uptake model for the blood-to-brain transfer of tracer potassium, established relatively constant blood levels of ^{42}K in their experimental animals, obtained brain samples at times ranging from 30 min to 3 h, and graphed the data from all of the experiments as the tissue/plasma concentration ratio of radioactivity versus the experimental duration. Linear plots were obtained for most brain regions, thus indicating the unidirectionality of the uptake process and the validity of the assumed model and the derived influx constants. Banos et al. (1973) employed the same assumption and model, as well as similar experimental and analytical techniques, in their study of amino acid uptake by the brain.

More recently, Go and Pratt (1975), Ohno et al. (1978), and Gjedde and Rasmussen (1980) have devised simpler ways of performing brain uptake experiments and analyzing the data for influx constants. The simplifications introduced by these authors include the administration of the test material by intravenous bolus, the employment of a single experimental time, and the calculation of the influx constant by an equation that was derived under the assumption of unidirectional flux during the experimental period. As will be presented in this paper, the injection of the test material by intravenous bolus is certainly an acceptable experimental and theoretical alternative to the more complex infusion schedules required for the constant blood level conditions used by Bradbury and Kleeman (1967) and by Banos et al. (1973). However, the employment of a single experimental period to determine the influx constant can lead to erroneous estimates if there is a significant brain-to-blood backflux during the experimental period and/or the measured tissue activity of the test solute is not corrected for the activity that has not moved completely across the BBB at the time of tissue sampling.

In the present paper, a theoretical model of blood–brain exchange is developed and used to analyze solute influx across the BBB. The model is general because no specific time course of arterial concentration is assumed and no particular arrangement or number of compartments in the system is presupposed. These compartments can be in series between plasma and brain extracellular fluid (resulting in a BBB complex) or in parallel to the BBB. The model does specify, however, that the movement of the test solute out of the last compartment(s) of the BBB complex and into brain extracellular fluid must be essentially "irreversible" (unidirectional) during the experimental period, and that this flux must dominate the blood–tissue distribution of the test solute for a finite period of time.

The equations that arise from the model indicate that the presence and duration of steady-state unidirectional influx can be assessed by plotting the uptake data from many experiments of differing durations (or multiple measurements in a single subject) in a particular way and determining whether the resulting curve has a linear portion. If a linear phase is found, then the net transfer process is effectively unidirectional for that specific period of time, and the influx constant can be evaluated from the slope of the line, just as Bradbury and Kleeman (1967) first described. In addition, the multiple-time/graphical approach eliminates the necessity of employing a vascular space marker and an intravascular distribution correction of the tissue data, provides physiological information about the distribution of the test material within the BBB complex, and can be used to assess the rate constant of an essentially irreversible metabolic process within the blood–brain system as well as a transport one. For example, the rate of glucose metabolism in the brain can be determined by making the appropriate plot of

2-deoxyglucose distribution data because the hexokinase reaction is essentially irreversible (Sokoloff et al., 1977).

THEORY

The following assumptions are made about the transport model.

1. There is a single source, the plasma (denoted by subscript "p"), for the test solute in the system.
2. The concentration of the test material in the plasma, C_p, may vary with time.
3. Relatively rapid exchange of the test solute can occur between plasma and a tissue region, which is made up of n compartments. The test solute can flow directly or indirectly from plasma into any of these compartments, move freely among these tissue compartments, and, in turn, flow back readily into the plasma. That is, the test solute transfer between and among the plasma and the compartments of this first region is reversible. This exchangeable tissue region is denoted by the subscript "r."
4. The test solute can enter a second tissue distribution region from the blood and/or the exchangeable tissue region; however, after entering this region, the test solute cannot leave. The second region is called the irreversible or bound region (denoted by the subscript "b") and consists of one or more parts that can be mathematically lumped together into a single compartment. Test material transfer into the bound region is functionally irreversible.
5. The test solute in the exchangeable region can leave this region only by going into either the plasma or the irreversible region.
6. The transfer of the test solute within this system obeys first-order kinetics, with the rate constant for its movement from the j^{th} to the i^{th} compartment being denoted as k_{ij}. (The validity of this assumption is enhanced by the employment of a radiolabeled form of the test solute present in tracer amounts.)
7. The test solute does not alter the system.
8. If the system metabolizes the test substance, this only occurs in the irreversible region and produces a metabolite that is trapped therein and is measurable.
9. The test solute is not initially present in either the exchangeable or the bound region.

Figure 1 illustrates this model system.

The necessary equations of this tracer distribution system are the standard ones (see Hearon, 1963). If t is the time, A is an $(n \times 1)$ vector of the amounts of model solute in each of the exchangeable compartments, K is the $(n \times n)$ matrix of the rate constants (k_{ij}), Q is the $(n \times 1)$ vector of the rate constants from the plasma to the exchangeable compartments (k_{ip}), and $C_p(t)$ is the tracer concentration in the plasma, then the following relationship describes tracer accumulation in the reversible compartment:

$$\frac{dA}{dt} = KA + QC_p(t) \tag{1}$$

If G is the $(n \times n)$ diagonal matrix of the rate constants from the exchangeable to the bound regions (k_{bi}), k_{bp} is the rate constant for the direct movement of material from the plasma to the trap, and $U_n' = (1 \ldots . 1)$, a $(1 \times n)$ vector, then the equation for the amount of material T in the irreversible region is

$$\frac{dT}{dt} = U_n' GA + k_{bp}C_p \tag{2}$$

The measurables of the experimental system are C_p and the total amount of the material in the tissue samples A_m. If V_p is the volume of the plasma in the tissue sampled, then

$$A_m = U_n'A + T + V_pC_p \tag{3}$$

The solution of Eq. 1 is (see Hearon, 1963)

$$A = e^{Kt}\int_0^t C_p\, e^{-K\tau}d\tau Q \tag{4}$$

Substituting Eq. 4 into Eq. 2 and solving the resultant equation yields

$$T = U_n'G\int_0^t e^{K\tau}\int_0^\tau C_p e^{K\Theta}d\Theta d\tau Q + k_{bp}\int_0^t C_p d\tau \tag{5}$$

The integration of Eq. 5 by parts, the insertion of Eqs. 3 and 4, and rearranging yields

$$A_m = (-U_n'GK^{-1}Q + k_{bp})\int_0^t C_p\, d\tau + U_n'(GK^{-1} + I)A + V_pC_p \tag{6}$$

If C_p is constant, A will approach a finite limit as $t \to \infty$, since the real parts of the eigenvalues of K are negative (Hearon, 1963). Thus, for the case when $t \to \infty$:

$$A_m\,(C_p = \text{constant}) = (-U_n'GK^{-1}Q + k_{bp})C_pt \tag{7}$$

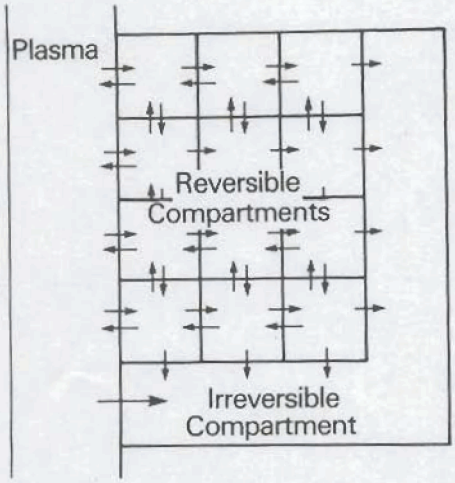

FIG. 1. Model of blood–brain exchange. The reversible region consists of n compartments, which freely communicate with the plasma; the test solute may move from the plasma to any of the compartments and vice versa. The irreversible regions, which are lumped together and shown as one region in this figure, communicate with the plasma and/or the reversible region; the solute can enter but cannot leave the irreversible region.

By definition, the rate of uptake for constant plasma level is given by $K_1 C_p$ in the limit as $t \to \infty$. Hence, Eq. 7 shows that

$$-U_n' G K^{-1} Q + k_{bp} = K_1 \tag{8}$$

Equation 6 may thus be written as

$$A_m = K_1 \int_0^t C_p d\tau + U_n'(K + G)K^{-1}A + V_p C_p \tag{9}$$

In order to evaluate A as a function of C_p, the following approach will be used. Let P be a $(n \times n)$ matrix which diagonalizes K, and let D be the $(n \times n)$ diagonal matrix whose elements are the eigenvalues of K. Thus,

$$PKP^{-1} = D \tag{10}$$

Therefore, Eq. 4 may be written as

$$A = P^{-1} e^{Dt} \int_0^t C_p e^{-D\tau} d\tau PQ \tag{11}$$

Consider the case where C_p is expressed in terms of a series of exponentials, i.e.,

$$C_p = \sum_1^m b_i e^{-\beta_i t} \tag{12}$$

where all of the β_i are real, non-negative, and arranged in decreasing order.

If Eq. 12 is substituted into Eq. 11 and the integration is carried out, then

$$A = P^{-1} \sum_1^m b_i (e^{-\beta_i I t} - e^{Dt})(D + \beta_i I)^{-1} PQ \tag{13}$$

We now assume that there exists a $q \leq m$ such that

$$\beta_q << |\text{real part of d}| \tag{14}$$

where d is the eigenvalue of K with the minimum absolute real component. If this relationship is satisfied, then there exists a t^* such that

$$\left.\begin{aligned} e^{-\beta_q t} &>> |e^{Dt}| \\ D + \beta_q I &\approx D \\ \sum_1^{q-1} b_i e^{-\beta_i t} &<< \sum_q^m b_i e^{-\beta_i t} \end{aligned}\right\} \quad t > t^* \tag{15}$$

The insertion of Eq. 15 into Eq. 13 and subsequent rearrangement produces

$$A_{t>t^*} = -K^{-1} Q C_p \tag{16}$$

If C_p is constant, inequality (Eq. 14) is trivially satisfied and Eq. 16 is valid for that case.

In the steady state, the "steady-state space" is, by definition, the ratio of the amount of material in an exchangeable region relative to a constant plasma level. Furthermore, the "space" of a region is the steady-state space for that region if all of the k_{bi} values are zero. Let A_0 be the $(n \times 1)$ vector of the amounts in the exchangeable region when all of the k_{bi} values are zero. Since the rate constant matrix for A_0 will be identical to that of A except for the absence of the k_{bi} values in the diagonal terms, then, in the steady state,

$$A = -K^{-1} Q C_p \tag{17}$$

$$A_0 = -(K + G)^{-1} Q C_p$$

Therefore,

$$-Q C_p = KA = (K + G)A_0 \tag{18}$$

Rearrangement of the second equality of Eq. 18 produces

$$(A - A_0) = K^{-1} G A_0 \tag{19}$$

Because the elements A_0 and G are positive but those of K^{-1} are negative (Hearon, 1963), Eq. 19 indicates that

$$A_0 \geq A \tag{20}$$

Therefore,

$$\text{(space of } A) \geq \text{(steady-state space of } A) = U_n' A / C_p \tag{21}$$

If inequality (Eq. 14) is valid, Eq. 16 shows that the amounts in each of the components of the exchangeable region will "follow" the plasma concentration after a sufficient length of time t^*. In this situation, the proportionality constant is the same as the steady-state space for that compartment.

In Eq. 9, $U_n'K$ is a row vector whose components are the K column sums, which are equal to $-(k_{pi} + k_{bi})$ (Hearon, 1963). Since the i^{th} component of $U_n'G$ is k_{bi}, the i^{th} element of $U_n'(K + G)$ is $-k_{pi}$. As has been discussed by Hearon (1963), A and K^{-1} consist of positive and negative elements, respectively. Therefore, the second term of Eq. 9 is positive and is less than $U_n'A$ alone, since $U_n'K$ is of the opposite sign to $U_n'G$.

Thus, from the discussion of the previous paragraph and Eq. 21, the second term on the righthand side of Eq. 9 yields, for $t > t^*$:

$$U_n'(K + G)K^{-1}A_{t>t^*} = V_0C_p \qquad (22)$$

$$0 \leq V_0 \leq (\text{steady-state space of } A) \leq (\text{space of } A)$$

where V_0 is a positive value described in relationship 22.

The insertion of Eq. 22 into Eq. 9 and rearranging the resultant equation yields

$$A_{m_{t>t^*}} = K_1 \int_0^t C_p d\tau + (V_0 + V_p)C_p \qquad (23)$$

The curve produced by plotting A_m/C_p vs. $\int_0^t C_p\, d\tau/C_p$ starts at the origin, varies in a manner dependent on the parameters of the system, and becomes a straight line with slope K_1 by $t = t^*$. The ordinate intercept of this straight line is positive and is smaller than the steady-state space of the exchangeable region plus the plasma space. If there is no irreversible region, then $K_1 = 0$ because $G = 0$; moreover, for $t > t^*$, this plot is a horizontal line whose ordinate intercept is equal to the equilibrium (that is, the steady-state case where all of the net fluxes are zero) space of the exchangeable region plus the plasma space.

DISCUSSION

The preceding theoretical treatment indicates a way to measure the classical unidirectional flux constant for *any* membrane system when the concentration of the test solute is not maintained constant in the source solution. It also shows that this approach can be applied to study solute flux across simple (single) membranes and across more complex (multiple-compartment) membrane systems. The following procedure demonstrates the method of assessing solute influx constants across the BBB complex and also serves as a model for the study of any membrane system. First, uptake experiments of varying duration are performed in a series of animals, or, multiple measurements over time are made in a single subject. The test solute can be administered in any manner, but the time course of the plasma concentration as well as the final concentrations in plasma and brain must be accurately measured in each animal or by serial measurements in a single subject. Then, all the data are combined by brain region, and plots of A_m/C_p vs. $\int_0^t C_p\, d\tau/C_p$ are made from each group of data. Finally, the forms of the resulting curves are analyzed for linearity (that is, for unidirectionality of uptake); if a linear phase or portion of the curve is discernible, then the influx constant is estimated from the slope of this line.

The multiple-time/graphical approach produces a relatively model-independent transfer constant. This results in a significant advantage for data analysis in comparison to compartmental analysis, since the results for any compartmental analysis are highly specific for the particular compartmental model chosen. An illustration of this problem is provided by Levin and Patlak (1972). These authors used two different compartmental models and produced two different sets of transfer numbers from their data.

The principal advantage of the multiple-time/graphical approach for the measurement of the influx constant is the opportunity it provides to assess the unidirectionality of the test solute's transport across the BBB and to validate the assumption of this unidirectionality. This approach also eliminates the employment of a marker—either a different isotopic form of the test solute or a different material—for the determination of the vascular space within the tissue sample and the correction of the measured tissue concentration for the amount of the test solute that was entrapped in this space at the end of the experimental period. In fact, the multiple-time/graphical procedure uses the test material itself to yield a lower limit of its rapidly reversible volume (namely, the vascular compartment, the BBB complex, and whichever extravascular compartments are in parallel with the BBB and rapidly exchange with the plasma).

As presented in the introduction, Bradbury and Kleeman (1967) first conceived of the multiple-time/graphical approach and applied it to their studies of tracer potassium exchange between blood and brain. In this study and the similar ones by Bradbury and co-workers (Wong and Bradbury, 1975; Sarna et al., 1977) and by Banos and col-

laborators (Banos et al., 1973, 1974; Bachelard et al., 1973), constant plasma levels of the various test solutes were maintained. This experimental condition allowed the simple plotting of the tissue/plasma concentration ratio versus time, the ready evaluation of the linearity of the uptake process, and the estimation of the influx constant. However, a time course of constant arterial concentration is hard to achieve experimentally. The infusion schedule needed to establish this condition can be obtained by procedures presented by Daniel et al. (1975) and Patlak and Pettigrew (1976), but the derived infusion schedule is usually complex and on many occasions fails to produce the desired steady arterial concentration during the experimental period.

Simpler experimental and analytical procedures for measuring the influx constant were introduced by Go and Pratt (1975), Ohno et al. (1978), and Gjedde and Rasmussen (1980). The simplifications included the administration of the test solute by intravenous bolus, the selection of a single experimental time, and the use of a simple equation for the calculation of the influx constant. However, these modifications introduced new problems, such as the choice of the specific experimental period, the need to correct the tissue data for the amount of test material entrapped in the vascular compartment, and the validity of the assumed unidirectionality of uptake for the substances investigated and the time periods employed.

Go and Pratt (1975) attempted to deal with the second problem cleverly by making an adjustment of their ^{24}Na data by employing a different isotope of sodium (^{22}Na), injecting it 1 min before termination, and using ^{22}Na tissue data as the basis for the intravascular space correction (the actual procedure used an iterative technique that is outlined in their paper). Ohno et al. (1978) used 5- and 50-min ^{14}C-sucrose data and a computerized iterative procedure to evaluate an intravascular distribution space (regional blood volume) and applied this same correction factor to the tissue data for all four of their test solutes. Since it may take a finite period of time (i.e., greater than the 1- or 5-min period used by the above investigators) for the fluids within the reversible compartments to reach a steady state with the plasma, their correction of the measured tissue level for "intravascular" radioactivity may be erroneously low. The usage of the multiple-time/graphical approach eliminates the correction of the tissue data for test solute within vascular and tissue compartments that rapidly and reversibly exchange with plasma.

In addition to eliminating a vascular space correction, the multiple-time/graphical approach produces information about the distribution of the test solute within the BBB complex and reversible compartments in parallel with the BBB. The ordinate intercept obtained by extrapolating the linear portion of the uptake curve is equal to or less than the apparent steady-state distribution volume of the solute in all the reversible and exchangeable compartments, including the blood space of the tissue. A number of studies have indicated that the rapid distribution spaces of extracellular solutes in the blood–brain system may be something more than the plasma space. For instance, Sisson and Oldendorf (1971), using the approach of Bradbury and Kleeman (1967), found the following relationship between the rapidly exchanging spaces of four solutes: plasma globulin (probably transferrin) $\simeq$ ^{14}C-dextran (molecular weight, 60,000–90,000) $<$ ^{14}C-inulin (nominal molecular weight, 5,000–5,500) $<$ ^{3}H-mannitol (molecular weight, 182). Further discussion of this space for both extracellularly and intracellularly distributing solutes is given in the following paper (Blasberg et al., 1983).

Gjedde (1981) properly combined the procedure of intravenous bolus injection and several experimental times with graphical analysis of the resultant data. He derived influx constants for D-glucose and other saccharides from the slopes of his linear plots and considered the ordinate intercepts to be equal to the plasma volume (Eq. 5 in Gjedde, 1981). The latter interpretation arose from Gjedde's view of the BBB as a single membrane of zero volume between capillary blood and brain extracellular fluid (Fig. 8 in Gjedde, 1981).

In contrast to Gjedde (1981), we have considered a more general BBB. Our derivation and model of blood–brain transfer consider the possibility of multiple compartments that rapidly equilibrate with plasma and each other, and that can be in parallel to, as well as in series with, overall blood-to-brain flux. The compartments in series (between plasma and brain extracellular fluid) constitute the BBB complex, and the influx constant determined by graphical analysis represents transport across the entire BBB complex.

There are at least two situations in which the multiple-time/graphical method is clearly inappropriate. The first is if the biological state of the animals in a series changes over the time intervals used in the study. The second is if the animal model is not reproducible from individual to individual. For the latter case, the single-time technique of Go and Pratt (1975), Ohno et al. (1978), or Gjedde and Rasmussen (1980) must be used. We have, for example,

used the single-time approach in our investigations of blood–tissue transfer in several brain tumor models (Blasberg et al., 1980, 1981).

In summary, graphical analysis of brain uptake data from experiments of varying times not only allows the evaluation of the unidirectionality of transfer and of the influx constant, but also eliminates the vascular space correction of other analytical approaches and the model dependency of compartmental analysis. In addition, the multiple-time/graphical approach produces information about the size and exchange rate of the compartments that rapidly and reversibly exchange with plasma. In the following paper (Blasberg et al., 1983), this generalized graphical method is applied to brain uptake data obtained from multiple-time experiments with α-aminoisobutyric acid, *N*-methyl-α-aminoisobutyric acid, and diethylenetriaminepentaacetic acid. Furthermore, the multiple-time/graphical approach can be used to assess the rate constant of any type of irreversible process within any organ system, provided that this process sets the net uptake rate over a significant, well-defined portion of time. The selection of an appropriate test solute is, of course, essential. Finally, the multiple-time/graphical approach is highly applicable to the analysis of data obtained by positron emission tomography because a series of timed data for graphical analysis can be obtained from a *single* subject.

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