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KINETICS OF BLOOD-BRAIN BARRIER TRANSPORT OF HEXOSES

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SUMMARY

1. The kinetics of transport of glucose and four other hexoses through the blood-brain barrier were studied with a tritiated-water reference technique in the anesthetized rat. Brain clearance of [^{14}C]hexose was measured 15 s after a single injection of the hexose and ^{3}HOH reference into the common carotid artery.

2. Saturation of brain clearance of [^{14}C]hexose conformed to Michaelis-Menten kinetics. Linear transformation of the uptake data yielded the K_m of carrier-mediated hexose transport: 2-deoxy-D-glucose 6 mM, D-glucose 9 mM, 3-*O*-methyl-D-glucose 10 mM, D-mannose 21 mM, and D-galactose 40 mM. A maximum transport velocity of 1.56 μmol/g per min was calculated and shown to be constant for all five hexoses.

3. The kinetics of ^{3}HOH and 3-*O*-methyl-D-[^{14}C]glucose efflux from brain to blood were studied with a modification of the water reference technique. An estimate of cerebral blood flow, 0.56 ml/g per min, was made from the efflux rate constant for ^{3}HOH, 0.61 min^{-1}. The fractional extraction of 3-*O*-methyl-D-[^{14}C]glucose uptake from blood was estimated from the efflux rate constant, 0.22 min^{-1}, for this sugar and found to be 0.25. This value approximated the fractional extraction of 3-*O*-methyl-D-[^{14}C]glucose uptake that was determined from influx studies (0.24). These results indicated that the bidirectional movement of glucose across the blood brain barrier was symmetrical, which suggested that barrier sugar transport is equilibrative and not active.

4. Blood-brain barrier sugar transport was shown to be reversibly inhibited by phloretin, yet no modulation of transport was demonstrable after 2 or 8 days of starvation. Finally, regional analysis (olfactory bulb, caudate-putamen nucleus,

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thalamus-hypothalamus, and inferior-superior colliculi) demonstrated that, in addition to blood-brain barrier permeability, brain clearance of glucose was a function of cerebral blood flow.

INTRODUCTION

The carrier-mediated transport of glucose across the blood-brain barrier has been demonstrated by several investigators since the early studies of Geiger [1]. The blood-brain barrier hexose carrier exhibits properties compatible with those of a mobile carrier including saturable uptake [2–13], stereospecificity [2, 8, 9], competitive inhibition with other hexoses [3–4, 9], and transport counter-flow [4, 6, 7]. The presence of a blood-brain barrier hexose carrier, therefore, is firmly established and provides the basis for McIlwain and Bachelard's [14] suggestion that the limiting factor in the cerebral utilization of glucose may be the rate of sugar transport across the blood-brain barrier. The preliminary step in assessing the role of glucose transport in the regulation of cerebral glycolysis is the characterization of the Michaelis-Menten kinetic parameters (K_m, V) that describe the carrier-mediated transport of sugar between blood and brain.

In the present investigation, the kinetics of glucose transport across the blood-brain barrier are described with the use of the water reference technique in the anesthetized rat. In addition to confirming the estimates of the K_m and V of glucose transport into brain made by other investigators, kinetic constants are reported for four other hexoses structurally related to glucose. The kinetics of tracer efflux from brain to blood are also described with a modification of the water reference technique that is similar to the local clearance method of Lassen and Trap-Jensen [16]. Efflux studies permit an estimation of cerebral blood flow and an examination of the symmetry of hexose flux across the blood-brain barrier. These results demonstrate the applicability of a relatively simple technique to the quantitative analysis of cerebral capillary permeability.

METHODS

Uptake studies

Wistar rats, 275–350 g of either sex and maintained on routine laboratory diet were anesthetized with an intraperitoneal injection of sodium pentobarbital. The common carotid artery was surgically exposed, the animal positioned in a guillotine, and the artery cannulated using a sharp 27-gauge (0.38 mm external diameter) needle. The needle did not occlude the vessel assuring free arterial flow throughout the procedure. The needle was left in the artery after injection of the test mixture (described below) to prevent bleeding. The temperature of the injected solution was 22–25 °C.

The test mixture contained approx. 1 μCi/ml of ^{14}C-labelled hexose and approx. 5 μCi/ml of tritiated water (a freely diffusible internal reference) mixed in 0.2 ml of Ringer's solution buffered to pH 7.4 with 10 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES). Injection of the 0.2-ml test solution was rapid and completed within 0.25 s. The artery became clear during the injection of the bolus.

The anesthetized rat was decapitated 15 s after the injection, a period sufficient to permit a single passage of the injected bolus through the brain microcirculation [15]. Any of the hexose remaining intravascular is cleared from brain by the time of decapitation and distributed to the entire rat. The cerebral hemisphere ipsilateral to the carotid injection was immediately subjected to simultaneous ^{14}C and ^{3}H liquid scintillation counting [15]. An aliquot of the injected mixture was similarly counted. The ratio of ^{14}C radioactivity (dpm) to ^{3}H radioactivity (dpm) in brain tissue divided by the same ratio in the injection mixture times 100 provides a brain uptake index (I_b) percentage. The calculation is as follows:

$$I_b = \frac{^{14}C/^{3}H\ (\text{brain})}{^{14}C/^{3}H\ (\text{mix})} \times 100$$

Rearranged algebraically the brain uptake index is equivalent to the fractional extraction of the ^{14}C-labelled test compound (E) relative to the fractional extraction of the ^{3}HOH reference (E_{HOH}):

$$I_b = \frac{^{14}C\ (\text{brain})/^{14}C\ (\text{mix})}{^{3}H\ (\text{brain})/^{3}H\ (\text{mix})} \times 100,$$

that is, $I_b = E/E_{HOH}$.

The use of an internal reference, therefore, provided an indirect measure of brain uptake as opposed to a direct measure of fractional extraction obtained by indicator dilution methods [23]. The brain uptake index is an index of the maximal fractional extraction and, therefore, of the rate of unidirectional influx, if efflux of the ^{14}C-labelled compound is shown to be small during the 15-s circulation period subsequent to carotid injection.

Once the brain uptake index for a tracer concentration of a ^{14}C-labelled hexose has been measured, the characteristics of carrier-mediated transfer, self-inhibition, cross-inhibition, and stereospecificity can be assessed. Self-inhibition or cross-inhibition of the brain uptake index for a tracer concentration of ^{14}C-labelled hexose may be examined by adding varied concentrations of unlabelled hexose to the injection mixture. Complete self-inhibition or cross-inhibition of the brain uptake index for a hexose which traverses the blood-brain barrier solely by a carrier-mediated mechanism was observed when the brain uptake index was depressed to 2 %, the brain uptake index of sucrose or inulin [9], by high substrate concentration in the injection mixture. The substrate concentration in the injection mixture was assumed to be the concentration delivered to the cerebral capillary bed since the injectate entered the cranium as a bolus. Paton [17] has shown that a rapid injection into the carotid artery is circulated as a bolus largely undiluted by plasma for at least one circulation.

Calculation of kinetic constants of hexose influx

The rate of influx (v) of glucose across the blood-brain barrier is defined by $v = (E)\ (v_f)\ (S)$, where E is the maximal fractional extraction of glucose influx, v_f is the rate of cerebral blood flow in the anesthetized rat, and S is arterial concentration, i.e. the total concentration of glucose in the injection mixture. Since the brain uptake index is an indirect measure of E, the brain uptake index is also an indirect measure of v, assuming cerebral blood flow is maintained constant; therefore, the K_m of sugar transport is readily calculated from one of several equivalent linear trans-

formations [18] of brain uptake index saturation data. The maximal velocity of transport (V), however, is not readily calculated from linear transformation because the brain uptake index is a dimensionless index of rate, not an absolute measure.

The V of sugar transport may be measured indirectly with the use of Crone's [19] equation derived for single-injection techniques,

$$P = -(v_f) \ln (1-E),$$

where P is the blood-brain barrier permeability constant for a given hexose. Since $E = (I_b)(E_{HOH})$, the brain uptake index may be converted to E and P values if E_{HOH} and cerebral blood flow in the barbiturate-anesthetized rat are known. Given the P and K_m values of hexose transport across the blood-brain barrier, the transport V may be computed from the relationship

$$P = V/K_m.$$

The permeability constant is equal to the V/K_m ratio because the Michaelis-Menten equation reduces to a first-order form, $v = V/K_m(\bar{S})$, when tracer fluxes are measured, e.g. $\bar{S} \ll K_m$. In this instance, carrier-mediated transport is kinetically equivalent to free diffusion described by the Fick equation, $v = P(\bar{S})$. The quantity $\bar{S}$ is the mean capillary concentration. Substitution of $P = V/K_m$ into Crone's equation yields $-\ln (1-E) = (V/v_f)(1/K_m)$. Therefore, a plot of $-\ln (1-E)$ versus the K_m reciprocal for a series of hexoses should be linear if the V of sugar transport is constant for each hexose and cerebral blood flow is maintained constant.

The E_{HOH} value was obtained from values for the maximal fractional extraction of water, 0.85 [8, 20]. This value was corrected for back-diffusion of the ^{3}HOH reference during the 15-s circulation period by quantitation of the rate of efflux of water across the blood-brain barrier (see Efflux studies). The rate of cerebral blood flow used in V calculations was also determined from the rate of efflux of the ^{3}HOH reference (see Efflux studies).

Efflux studies

The rate of exodus of the ^{3}HOH reference or ^{14}C-labelled hexose from brain to blood is readily measured with the water reference technique if the decapitation time, i.e. the time interval between carotid injection of the test mixture and decapitation, is prolonged up to 4 min beyond the usual time of 15 s used in uptake studies. Since the brain metabolism of any metabolizable substrate will distort the efflux kinetics, a non-metabolizable hexose, 3-*O*-methyl-D-glucose was used in the efflux studies. As the brain uptake index is a ratio of the fractional extraction of the test substance relative to the fractional extraction of the ^{3}HOH reference, the brain content of 3-*O*-methyl-D-[^{14}C]glucose at a given time following carotid injection may be determined from the brain uptake index and known brain content of the ^{3}HOH reference for each respective time interval. Brain uptake of the ^{3}HOH reference was determined from measurements of total radioactivity (dpm) injected into the common carotid and total radioactivity (dpm) retained by brain rostral to the midbrain [15]. Brain uptake of ^{3}HOH and 3-*O*-methyl-D-[^{14}C]glucose were measured for decapitation times of 15, 60, 120 and 240 s.

The rate of efflux of tracer concentrations of a substance follows first-order rate laws [21] and was quantitated by determination of the efflux rate constant, B,

in units of min^{-1}. The rate constant was determined from a logarithmic plot of the tracer efflux from brain versus time; analogous studies have been developed for muscle with the local clearance method [16]. Lassen and Trap-Jensen [16] define B in terms of blood flow (v_f), volume of distribution (V_d), and fractional extraction (E) of the tracer by blood from the tissue depot, i.e.

$$B = Ev_f/V_d.$$

Efflux studies complement uptake measurements in three ways: (i) the degree of back-diffusion of the ^{3}HOH reference or 3-*O*-methyl-D-[^{14}C]glucose during the 15-s circulation period used in uptake studies is quantitated; (ii) an estimation of cerebral blood flow may be made from the E, V_d, and B values for a freely diffusible substance, e.g. ^{3}HOH or ^{133}Xe [22]; and (iii) sugar penetrability of the brain side of the blood-brain barrier may be quantitated by estimation of the extraction fraction from B, V_d, and v_f for 3-*O*-methyl-D-[^{14}C]glucose. If the fractional extraction of 3-*O*-methyl-D-[^{14}C]glucose determined from efflux studies is equal to the fractional extraction of the hexose measured from uptake studies, then hexose transport across the blood-brain barrier is presumed to be symmetrical [23].

Sodium effects

The effects of sodium on glucose transport across the blood-brain barrier were assessed by stoichiometric substitution of the sodium chloride in the Ringer's injection solution with Tris · Cl so that the ionic strength and osmolarity of the injection mixture were maintained constant but the Na^+ concentration was reduced from a normal 140 mequiv/l to 79 mequiv/l or 10 mequiv/l. The effects of ouabain, a potent inhibitor of sodium dependent processes, was assessed by including an ouabain concentration of 1 mM or 10 mM in the injection mixture.

Phloretin reversibility

The reversibility of a pharmacologic inhibition of the blood-brain barrier hexose carrier (such as) with phloretin was quantitated by measuring the brain uptake index for D-[^{14}C]glucose at 2, 4 and 30 s after injection of 0.5 ml of a 2-mM phloretin solution. The phloretin was administered by a tuberculin syringe connected by a foot-long narrow polyethylene tubing to a 27-gauge needle. At the indicated times after the 2–3 s phloretin injection, the standard labelled glucose solution was injected through a second carotid puncture anterior to phloretin injection. The brain uptake index for the D-[^{14}C]glucose was then measured in the usual way after a 15-s circulation.

Starvation studies

Rats were fasted for a period of 2 or 8 days prior to sacrifice. Animals receive water ad libitum and are housed in cages with open-grid floors to minimize coprophagy.

Regional uptake analysis

The effect of cerebral blood flow on the brain uptake of a given compound may be examined with regional analysis of glucose uptake since cerebral blood flow varies widely between different brain regions [24]. The cerebral hemisphere ipsilateral

to the carotid injection was sub-divided into four brain regions prior to liquid scintillation counting. The regions analyzed were the caudate-putamen nucleus, inferior-superior colliculi, olfactory bulb, and the thalamus-hypothalamus. Since the brain uptake of the ^{3}HOH reference is largely flow-limited [20] and the cerebral blood flow is known to vary widely between different brain regions [24], the brain uptake of the ^{3}HOH reference varied accordingly. Therefore, each regional brain uptake index for D-[^{14}C]glucose was normalized by converting each brain uptake index to a regional distribution ratio (ml/g) by multiplying the brain uptake index by the known distribution ratio of the ^{3}HOH reference for each region. The regional ^{3}HOH distribution ratio at 15 s following carotid injection was calculated as follows:

$$\frac{\text{total brain, } ^3\text{H radioactivity (dpm)/g, brain}}{\text{total mix, } ^3\text{H radioactivity (dpm)/ml, mix}}$$

Materials

D-[^{14}C[Glucose, D-[^{14}C]galactose, D-[^{14}C]mannose, 3-*O*-methyl-D-[^{14}C]-glucose and ^{3}HOH were obtained from New England Nuclear Corp., Boston, Massachusetts. D-Glucose, D-galactose, D-mannose, and Tris · Cl were purchased from Sigma Chemical Co., St. Louis, Missouri. *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid was from Calbiochem, La Jolla, California. Ouabain and phloretin were from K and K Laboratories, Plainview, New York.

RESULTS AND DISCUSSION

Uptake studies

The self-inhibition, cross-inhibition with similar hexoses, and marked stereospecificity of brain uptake of glucose has been described with the use of the water standard technique and previously reported [9]. The brain uptake index for a tracer concentration (0.42 mM) of D-[^{14}C]glucose was shown to saturate from 32.6±2.8 % to 9.4±1.2 % as the injection concentration of unlabelled D-glucose was increased 200-fold to 80 mM [9]. These saturation studies have been repeated and extended in Fig. 1A. The brain uptake index for a tracer concentration (0.005 mM) of D-[^{14}C]-glucose was depressed from 34.1±2.1 % to 7.5±0.4 % as the concentration of unlabelled D-glucose is increased 30 000-fold to 160 mM.

In previous studies [9] the transport K_m was obtained by inspection of brain uptake index saturation data illustrated in Fig. 1A. The K_m was defined as the concentration of unlabelled glucose that depressed the brain uptake index to one-half the value observed for a tracer concentration of D-[^{14}C]glucose. However, the substantial fraction of brain uptake of glucose that is non-saturable introduces a considerable error to such a determination of the K_m. The non-saturable component of brain uptake was readily eliminated from the K_m calculation if the difference (I_{bm}) between the brain uptake index for a tracer concentration and the brain uptake index for each self-inhibiting concentration was plotted versus the concentration (S) of unlabelled glucose in the injection mixture. Such a plot is illustrated in Fig. 1B. An accurate measurement of the K_m was obtained (Fig. 2A) by substitution of the data in Fig. 1B into a linear transformation of a form analogous to an Eadie-Hofstee plot [18]. The slope of the line represents the K_m of glucose transport across the blood-brain barrier

and is equal to 9 mM (Table I). The reproducibility of these measurements may be judged by the S.D. values of Fig. 1A. A K_m of 9 mM for glucose transport across the blood-brain barrier compared favorably with similar values obtained in the rat, 7 mM [6, 11], dog, 8 mM [12], and sheep and rabbit, 6 mM [13]. Since the brain uptake index is an index of transport rate, not an absolute measure, the ordinate is dimensionless and the V of glucose influx cannot be obtained from the intercept of Fig. 2A.

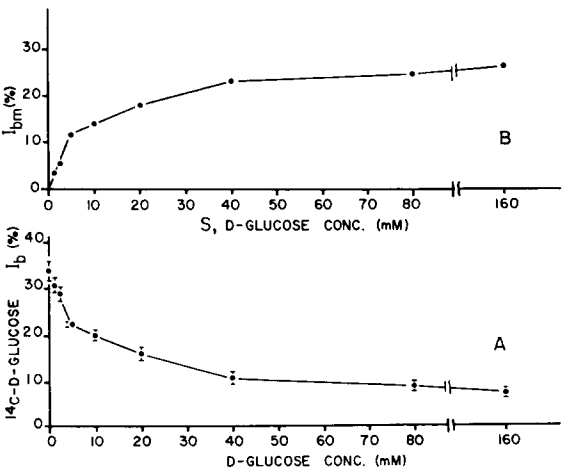

Fig. 1. (A) Saturation data for brain uptake of glucose. Ordinate = brain uptake index (I_b) for D-[^{14}C]glucose 15 s following rapid carotid injection. Abscissa = arterial glucose concentration. Means ± S.D. for each point based on data from three to five rats. (B) Saturation data expressed in form suitable for linear transformation. Ordinate = brain uptake index (I_{bm}) corrected for non-saturable component of uptake. Abscissa = arterial glucose concentration.

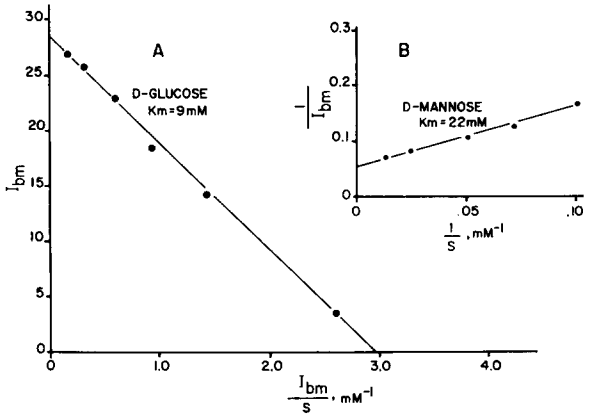

Fig. 2. (A) Linear transformation of glucose uptake saturation data that is analogous to an Eadie-Hofstee plot. Ordinate = brain uptake index (I_{bm}) corrected for non-saturable component of uptake. Abscissa = ratio of corrected brain uptake index to arterial glucose concentration. Slope equals K_m of glucose transport. Intercept is not equal to transport V since the ordinate is dimensionless. (B) Linear transformation of mannose saturation data that is analogous to a Lineweaver-Burk plot. Ordinate = reciprocal of D-mannose corrected brain uptake index (I_b). Abscissa = reciprocal of arterial mannose concentration. Transport K_m calculated from product of slope and intercept.

Although the cross-inhibition of a tracer concentration of D-[^{14}C]glucose by unlabelled D-mannose or D-galactose has been reported previously [9], the saturation data of the brain uptake of D-[^{14}C]mannose or D-[^{14}C]-galactose have not been reported and are listed in Table II. A double-reciprocal plot analogous to a Lineweaver-Burk plot [18] of the mannose saturation data is presented in Fig. 2B; a K_m of 22 mM (Table I) for D-mannose uptake is determined from the slope and intercept. A similar double reciprocal plot of the D-galactose saturation data in Table II yields a K_m of 42 mM (Table I).

The cross-inhibition of the brain uptake of a tracer concentration of D-[^{14}C]-glucose by unlabelled 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose has been reported previously in conjunction with the D-mannose or D-galactose cross-inhibition studies [9]. These data have been analyzed with double-reciprocal plots and the respective K_i values are reported in Table I. The accuracy of the K_i values may be judged from the S.D. of the cross-inhibition data reported previously [9]. The K_i determined from cross-inhibition studies is equivalent to the K_m determined from saturation data if (i) the cross-inhibition is competitive, and (ii) dual transport systems of different affini-

TABLE I

BLOOD-BRAIN BARRIER HEXOSE CARRIER AFFINITY CONSTANTS

K_m is determined from self-inhibition data: Figs 1 and 2 and Table II. K_i is determined from cross nhibition data: Table II [9].

Substrate	K_m (mM)	K_i (mM)
2-Deoxy-D-glucose	—	6.0
D-Glucose	9.0	-
3-*O*-methyl-D-glucose	—	10
D-Mannose	22	21
D-Galactose	42	40
Phlorizin	—	0.40
Phloretin	—	0.016

TABLE II

HEXOSE INHIBITION BY MANNOSE, GALACTOSE AND PHLORETIN

D-[^{14}C]Mannose self-inhibition		D-[^{14}C]Galactose self-inhibition		Phloretin cross-inhibition of D-[^{14}C]glucose	
S^1	I_b[4]	S^2	I_b[4]	S^3	I_b[4]
0.05	23.2 ±2.4	0.21	12.9 ±1.2	0	34.1 ±2.1
10	17.0 ±0.5	20	10.1 ±0.8	0.025	16.3 ±4.0
20	13.7 ±0.8	40	8.00±0.25	0.05	13.9 ±3.7
40	9.75±0.79	80	7.29±0.48	0.10	12.5 ±3.4
80	8.40±0.74			1.0	5.69±0.77

[1] S = concentration of unlabelled mannose in injection mixture, mM.
[2] S = concentration of unlabelled galactose in injection mixture, mM.
[3] S = concentration of unlabelled phloretin in injection mixture, mM.
[4] Percent mean brain uptake index (I_b ± S.D.) based on data from three to five rats.

ties are not operative [25]. Therefore the equivalence of the K_i and K_m for D-mannose and D-galactose uptake is indirect kinetic evidence for a competitive mechanism of the inhibition of the brain uptake of D-[^{14}C]glucose by these sugars. That is, the three hexoses all share the same carrier binding site. Betz and Gilboe [26] have recently presented direct kinetic evidence that the inhibition of brain uptake of glucose by 3-*O*-methyl-D-glucose is also competitive.

Although the V of glucose transport cannot be computed directly from the linear transformation data of Fig. 1, V was estimated from the brain uptake index and K_m as described in Methods. Since $E = (I_b)\ (E_{HOH})$, the brain uptake index for a given hexose may be converted to a corresponding extraction fraction if E_{HOH}, the fractional extraction of water 15 s subsequent to carotid injection, is known. Although E_{HOH} is not readily determined in the rat with tissue sampling techniques, the maximal fractional extraction of ^{3}HOH across the blood-brain barrier has been measured in the barbiturate-anesthetized dog [8] and in the barbiturate-anesthetized rhesus monkey [20]. In the former study, the maximal extraction of water is 85 % at an unspecified rate of cerebral blood flow and in the latter study, the value is approx. 85 % at the rate of cerebral blood flow reported for the barbiturate-anesthetized rat, 0.58 ml/g per min [20]. Since the rate constant of ^{3}HOH efflux from brain to blood is 0.61 min^{-1} (see Results: Efflux studies), then approx. 14 % of the ^{3}HOH reference taken up initially has effluxed from brain by the end of the 15-s circulation period. Therefore a correction for back-diffusion of the ^{3}HOH reference can be made which results in $E_{HOH} = 0.75$. The brain uptake index values reported here and in earlier investigations [9] may be converted to extraction fractions by multiplying each brain uptake index by 0.75. The resultant E values are 0.34 ± 0.03 for 2-deoxy-D-glucose, 0.26 ± 0.02 for glucose, 0.22 ± 0.02 for 3-*O*-methyl-D-glucose, 0.16 ± 0.01 for mannose, and 0.11 ± 0.002 for galactose. Computation of E from the brain uptake index slightly underestimates the maximal extraction since the brain uptake index was not corrected for the small fraction of hexose (less than 7 % of the initial extraction fraction in the case of 3-*O*-methyl-D-glucose, see Results: Efflux studies) that effluxes from brain during the 15-s circulation period.

Given the E and K_m values for each of the five hexoses, the V may be estimated using the relationship, $-\ln(1-E) = (V/v_f)\ (1/K_m)$ derived in Methods. The quantity $-\ln(1-E)$ for each hexose has been plotted against the K_m reciprocal for each of the five hexoses (Fig. 3). The relationship is linear indicating that the V of hexose transport is constant and independent of hexose structure. Since the slope of Fig. 3 is equal to V/v_f, the hexose V that is calculated from the slope and a cerebral blood flow of 0.56 ml/g per min (see Results: Efflux studies) is $V = 1.56$ μmol/g per min. The intercept of Fig. 3 is equivalent to $E = 0.04$ which approximates the fractional extraction of hexose from blood that is mediated by a non-saturable mechanism [12]. The non-saturable component of sugar uptake may be due to free diffusion or to a high capacity carrier-mediated mechanism.

The linear relationship in Fig. 3 is an important result because the demonstration of V constancy provides evidence for the kinetic mechanism of the blood-brain barrier hexose transport system. As emphasized by Christensen [25], constancy of transport V suggests the Michaelis-Menten equilibrium assumption is valid and shows that the rate-limiting step of transport, probably the movement of the carrier across the membrane, is independent of substrate structure.

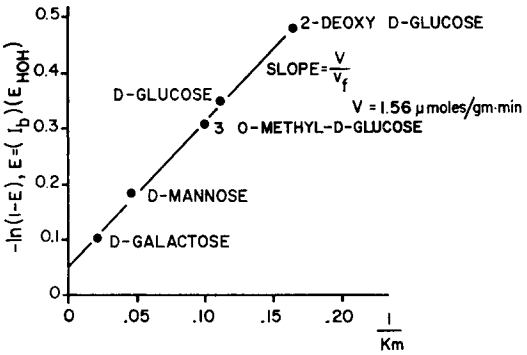

Fig. 3. Demonstration of constancy of hexose transport V. Ordinate = $\ln(1/1-E)$ for each hexose. Abscissa = reciprocal of transport K_m for each hexose. Slope = V/v_f. Intercept = fraction of hexose uptake due to non-saturable mechanism.

Efflux studies

The kinetics of hexose efflux from brain to blood were studied in order to assess the symmetry of blood-brain barrier sugar transport. The non-metabolizable sugar, 3-*O*-methyl-D-glucose, was employed since the rapid metabolism of utilizable sugars would distort the efflux kinetics. In Fig. 4A, the brain uptake index of 3-*O*-methyl-D-[^{14}C]glucose increased with time as the decapitation time was prolonged up to 4 min beyond the usual 15-s period used for influx studies. Since the brain uptake index is a measure of the flux of 3-*O*-methyl-D-[^{14}C]glucose relative to the flux of the ^{3}HOH reference, the brain uptake index paradoxically increases with time because, as demonstrated in Fig. 4B, the exodus of the ^{3}HOH proceeds at a rate approx. 3 times faster than the efflux of 3-*O*-methyl-D-[^{14}C]glucose. The efflux of ^{3}HOH from brain follows first-order rate laws with a rate constant (B) of 0.61 min^{-1} (Fig. 4B).

The efflux curve for ^{3}HOH in Fig. 4B was extrapolated back to zero to give an estimate of the maximal fractional extraction of the ^{3}HOH after injection into the common carotid artery; the value is approx. 0.08 which is about one-tenth the value reported when the bolus injection is made in the internal carotid artery [8, 20]. This result confirms earlier measurements [15] that only a small fraction of the injected bolus enters the rat internal carotid artery, the remainder being distributed to the external carotid.

Given the relationship for the efflux rate constant, $B = Ev_f/V_d$, used by Lassen and Trap-Jensen [16], the rate of cerebral blood flow, v_f, was calculated from B = 0.61 min^{-1}, a brain volume of distribution (V_d) for water of 0.78 ml/g in the rat [6], and an initial extraction fraction for water of 0.85 [8, 20]. The cerebral blood flow calculated is 0.56 ml/g per min which compares with estimates of 0.48 ml/g per min [28], 0.58 ml/g per min [27], and 0.75 ml/g per min [29]. The above calculation of cerebral blood flow assumes that the blood-brain barrier is equally permeable to water on the brain and blood sides of the barrier.

The 3-*O*-methyl-D-glucose efflux rate constant calculated from Fig. 4B is 0.22 min^{-1}. The reproducibility of this determination may be judged from the S.D. of the points in Fig. 4A. The volume of 3-*O*-methyl-D-glucose distribution in rat brain is 0.63 ml/g [6]. Based on V_d = 0.63 ml/g, B = 0.22 min^{-1}, and v_f = 0.56 ml/g

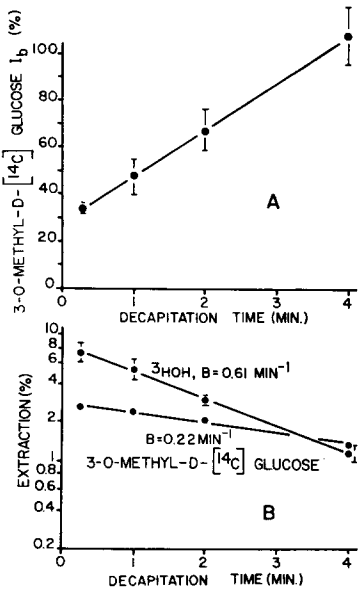

Fig. 4. (A) Efflux from brain to blood of a tracer concentration of 3-*O*-methyl-D-[^{14}C]glucose. Ordinate = brain uptake index (*I*) for 3-*O*-methyl-D-[^{14}C]glucose. Abscissa = time between carotid injection and decapitation. Mean±S.D. for each point based on data from three to five rats. (B) Ordinate = fractional extraction of 3-*O*-methyl-D-[^{14}C]glucose or ^{3}HOH following common carotid injection. Values for ^{3}HOH determined directly; mean±S.D. based on data from three to six rats; ^{3}HOH extraction fractions are 7.73±1.73 %, 5.16±0.97 %, 2.84±0.21 %, and 1.09±0.22 % for 15-, 60-, 120- and 240-s decapitation periods, respectively. Extraction fractions for 3-*O*-methyl-D-[^{14}C]glucose are determined indirectly from product of brain uptake index and ^{3}HOH extraction fractions for each of four points. Slope = rate constant (*B*) of ^{3}HOH or 3-*O*-methyl-D-[^{14}C]glucose efflux.

per min, the fractional extraction (*E*) of 3-*O*-methyl-D-glucose from brain calculated from efflux studies is 0.25. The *E* value for 3-*O*-methyl-D-glucose calculated from influx studies is 0.22±0.02; however, since $B = 0.22\ \text{min}^{-1}$, approx. 7 % of the initial 3-*O*-methyl-D-glucose cleared by brain returns to blood during the 15-s circulation time. The corrected *E* value for 3-*O*-methyl-D-glucose influx is 0.24, approximating the value calculated from the efflux studies, and indicates that the transport of 3-*O*-methyl-D-glucose across the blood-brain barrier is symmetrical [23]. The observation of sugar transport symmetry supports the equilibrative mode of glucose uptake by brain originally suggested by Crone [2] and later by Buschiazzo et al. [6].

Sodium independency

There is an apparent sodium independency of glucose transport across the blood-brain barrier (Table III). The brain uptake index for D-[^{14}C]glucose was not altered when Na^+ was stoichiometrically replaced with Tris buffer and the Na^+ concentration of the injection mixture reduced from a normal 140 mequiv/l to either 79 mequiv/l or 10 mequiv/l. Similarly the addition of ouabain, an inhibitor of many sodium transport systems, to the injection solution (at a concentration of 1 mM or 10 mM) had no effect on the brain uptake of glucose.

TABLE III

EFFECTS OF OUABAIN AND DECREASED SODIUM ON BRAIN UPTAKE OF GLUCOSE

Condition	Concentration[2]	D-Glucose I_b[3]
Sodium[1] (mequiv/l)	140	34.1±2.1
Sodium[1] (mequiv/l)	79	37.0±1.0
Sodium[1] (mequiv/l)	10	37.2±1.8
Ouabain (mM)	0	34.1±2.1
Ouabain (mM)	1	32.0±2.1
Ouabain (mM)	10	33.2±1.8

[1] Sodium chloride is replaced stoichiometrically with Tris/chloride.
[2] Concentration of either sodium or ouabain in the injection mixture.
[3] Brain uptake index (I_b) for tracer concentration (0.005 mM) of D-[^{14}C]glucose mean±S.D. based on data from three to five rats.

The sodium independency of blood-brain barrier sugar transport suggested by the data in Table III also supports an equilibrative mode of glucose movement into brain. For example, the classic example of active sugar transport is the intestinal epithelium where glucose movement is coupled to an ATP-driven sodium pump [31]. The K_m of 2-deoxy-D-glucose transport is elevated from 4 mM to 40 mM as the sodium concentration is depressed from 145 mM to 24 mM [31]. Although gut sugar transport is clearly sensitive to sodium changes that parallel the concentrations used in these studies, the present data do not exclude a very high affinity co-transport system of glucose and sodium with a K_m for sodium less than 10 mM.

Phloretin sensitivity

The cross-inhibition of the brain uptake of D-[^{14}C]glucose by increasing the concentration of an unlabelled pharmacologic agent such as phlorizin (in the injection solution) has been demonstrated with the water standard technique [9]. Phloretin, the aglycone of phlorizin, similarly cross-inhibits D-[^{14}C]glucose uptake (Table II). The K_i of phlorizin or of phloretin was determined from linear transformations of the cross-inhibition data (Table I). Based on the differences in K_i, the potency of phloretin as an inhibitor of glucose uptake is 25 times greater than phlorizin. This 25-fold potency of phloretin inhibition as compared to inhibition by the glycoside phlorizin, may be explained by the fact that phloretin has been shown to be a non-competitive or allosteric inhibitor of sugar transport, whereas phlorizin has been shown to be a competitive inhibitor [32]. For example, phloretin has been shown to inhibit both glucose and amino acid transport across intestinal epithelia, presumably by binding to a site mutual to the two transport systems [33]. Phloretin may also be an allosteric inhibitor of blood-brain barrier transport systems, since the agent is a potent inhibitor (Oldendorf, W. H. and Pardridge, W. M., unpublished observations) of the blood brain barrier monocarboxylic acid carrier [34].

Phloretin inhibition of the blood-brain barrier hexose carrier is reversible (Table IV). Any phloretin bound to the blood-brain barrier after carotid injection of 0.5 ml of a 2-mM phloretin solution is completely washed away by 30 s. The carrier-phloretin dissociation half-time is approx. 4 s (Table IV).

TABLE IV

TIME COURSE OF REVERSIBLE PHLORETIN INHIBITION OF BRAIN UPTAKE OF GLUCOSE

Post-phloretin[1] injection period (s)	D-[^{14}C]Glucose I_b[2]
Control	34.1±2.1
0[3]	5.69±0.77
2	14.3
4	20.5
30	33.8

[1] Time elapsed between injection of 0.5 ml of 2 mM phloretin and 0.2 ml of test solution containing 0.005 mM labelled glucose.

[2] Mean brain uptake index (I_b±S.D.) based on data from three rats for control and 0 s; data for 2, 4 and 30 s from one rat each.

[3] Simultaneous injection of labelled glucose and 1 mM phloretin.

Starvation effects

The effect of starvation on glucose uptake was assessed by measuring the brain uptake index for glucose after 2 and 8 days of fasting. The glucose brain uptake index was enhanced approx. 25% after either 2 or 8 days of starvation (Table V). In order to determine whether the enhanced uptake was due to increased influx of the labelled glucose or rather to a decreased rate of efflux secondary to greater metabolic sequestration, the brain uptake index of 3-*O*-methyl-D-[^{14}C]glucose was measured in the starved rats. The starvation-enhanced brain uptake of glucose can be attributed to a greater metabolic sequestration of the label, as there is no increase in the brain uptake of 3-*O*-methyl-D-[^{14}C]glucose with 2 or 8 days of starvation (Table V). The lack of an effect on sugar transport was confirmed by measuring the K_m of glucose influx and the rate constant of 3-*O*-methyl-D-glucose efflux. These data overlapped the results in Figs 1, 2 and 4, and there is no observable change in the K_m or B of sugar transport after 2 days of fasting (Table V). The increase in glucose brain uptake index with 2 or 8 days of fasting represents an increase in glucose extraction of 0.26–0.31 based on the relationship, $E = (I_b)(E_{HOH})$. The 0.05 difference is slightly greater but still approximates the fraction of brain glucose that is lost during a 15-s circulation period based on the efflux rate constant of 0.22 min^{-1} for 3-*O*-methyl-D-glucose. Therefore, cerebral glycolysis appears to be accelerated within 2 days of fasting to

TABLE V

EFFECTS OF STARVATION ON BRAIN UPTAKE OF GLUCOSE

Condition	D-[^{14}C]Glucose I_b[1]	3-*O*-methyl-D-[^{14}C]glucose I_b[1]	D-Glucose K_m (mM)	3-*O*-methyl-D-glucose B (min^{-1})
Fed	34.1±2.1	33.9±2.5	9	0.22
Fast, 2 days	42.7±4.0	32.2±4.0	9	0.22
Fast, 8 days	42.1±1.6	35.5±3.4	—	—

[1] Mean brain uptake index (I_b±S.D.) based on data from three to five rats.

TABLE VI

REGIONAL BRAIN UPTAKE INDEX FOR GLUCOSE AND 3-*O*-METHYL-D-GLUCOSE AND REGIONAL VOLUMES OF DISTRIBUTION FOR WATER, GLUCOSE AND 3-*O*-METHYL-D-GLUCOSE IN RELATIONSHIP TO REGIONAL RATES OF CEREBRAL BLOOD FLOW

Region	Regional v_f[1]	D-[^{14}C]Glucose I_b[2]	3-*O*-methyl-D-[^{14}C]glucose I_b[2]	^{3}HOH, brain/mix[3]	D-[^{14}C]Glucose, brain/mix[3]	3-*O*-methyl-D-[^{14}C]glucose, brain/mix[3]
Olfactory bulb	0.74	40.0±3.3	30.0±3.0	1.03±0.22	0.41	0.31
Caudate-putamen nucleus	1.02	32.6±3.7	25.6±2.8	1.30±0.15	0.42	0.33
Thalamus-hypothalamus	1.06	35.4±5.4	27.6±3.2	1.26±0.17	0.45	0.35
Inferior-superior colliculi	1.42[4]	36.8±6.6	28.1±2.8	1.45±0.23	0.53	0.41

[1] Cerebral blood flow v_f, ml/g per min [24].
[2] Mean brain uptake index (I_b±S.D.) based on data from five rats.
[3] Volumes of distribution (ml/g×100) 15 s after common carotid injection. Values for ^{3}HOH were determined directly (Methods). Values for glucose and 3-*O*-methyl-D-glucose were determined from respective brain uptake index and ^{3}HOH volume of distribution for each region.
[4] Average cerebral blood flow of inferior colliculus (1.74 ml/g per min) and superior colliculus (1.10 ml/g per min).

such an extent that virtually no glucose that is taken up by brain from blood is lost due to efflux back to blood. This condition would infer that the intracerebral glucose level approaches zero; related studies indicate the intracerebral glucose level falls below 0.5 μmol/g wet brain after only 24 h of starvation in the rat [3, 6].

Regional uptake studies

The brain uptake index of D-[^{14}C]glucose and 3-*O*-methyl-D-[^{14}C]glucose in each of the four brain regions analyzed is presented in Table VI. Since the brain uptake index is a ratio of brain uptake of the test substance versus the tritiated reference tracer, the regional brain uptake index cannot be directly related to regional cerebral blood flow because the brain uptake of the ^{3}HOH reference is largely flow-limited [20] and will vary with the regional cerebral blood flow. Therefore, the regional volumes of distribution of ^{3}HOH were determined at 15 s following carotid injection. The regional volumes of distribution of glucose and 3-*O*-methyl-D-glucose were determined from the respective brain uptake indices (Table VI) and the ^{3}HOH distribution volumes for each region. The regional volumes of distribution ($\times$ 100) for ^{3}HOH, D-[^{14}C]glucose, and 3-*O*-methyl-D-[^{14}C]glucose are presented in Table VI in conjunction with the reported cerebral blood flow for each region in the cat [24]. The brain uptake for water, glucose or 3-*O*-methyl-D-glucose increased in a roughly linear relationship with regional cerebral blood flow ranging from lowest in the olfactory bulb to highest in the colliculi. As the regional cerebral blood flow is increased 48 % (from the olfactory bulb to the colliculi), the uptake of ^{3}HOH, D-[^{14}C]glucose and 3-*O*-methyl-D-[^{14}C]glucose increases 29 %, 23 % and 24 %, respectively. These values, particularly for ^{3}HOH, slightly underestimate the actual increase in uptake with blood flow as no correction for back-diffusion is made. The data in Table VI are a form of clearance data and are not to be confused with extraction data. Clearance is a rate (ml/g per min) which increases with blood flow; extraction is expressed as a dimensionless fraction and decreases with increasing blood flow [36]. The demonstration of a relationship between brain uptake and blood flow by regional analyses provides an approximation of data that is more elegantly obtained by perfused brain preparations [12, 35]. These regional uptake studies of hexoses and water (Table VI) emphasize the need for caution in interpreting regional differences in brain uptake index. Factors such as differences in blood-brain barrier permeability and cerebral blood flow may influence variations in regional brain uptake index values.

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