Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport.

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Research Article

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Evidence for Entry of Plasma Insulin into Cerebrospinal Fluid through an Intermediate Compartment in Dogs

Quantitative Aspects and Implications for Transport

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Abstract

To study the route by which plasma insulin enters cerebrospinal fluid (CSF), the kinetics of uptake from plasma into cisternal CSF of both insulin and [^{14}C]inulin were analyzed during intravenous infusion in anesthetized dogs. Four different mathematical models were used: three based on a two-compartment system (transport directly across the blood–CSF barrier by nonsaturable, saturable, or a combination of both mechanisms) and a fourth based on three compartments (uptake via an intermediate compartment). The kinetics of CSF uptake of [^{14}C]inulin infused according to an "impulse" protocol were accurately accounted for only by the nonsaturable two-compartment model (determination coefficient [R^2] = 0.879±0.044; $\bar{x}$±SEM; n = 5), consistent with uptake via diffusion across the blood–CSF barrier. When the same infusion protocol and model were used to analyze the kinetics of insulin uptake, the data fit (R^2 = 0.671±0.037; n = 10) was significantly worse than that obtained with [^{14}C]inulin (P = 0.02). Addition of a saturable component of uptake to the two-compartment model improved this fit, but was clearly inadequate for a subset of insulin infusion studies. In contrast, the three-compartment model accurately accounted for CSF insulin uptake in each study, regardless of infusion protocol (impulse infusion R^2 = 0.947±0.026; n = 10; P < 0.0001 vs. each two-compartment model; sustained infusion R^2 = 0.981±0.003; n = 5). Thus, a model in which insulin passes through an intermediate compartment en route from plasma to CSF, as a part of a specialized transport system for the delivery of insulin to the brain, best accounts for the dynamics of this uptake process. This intermediate compartment could reside within the blood–CSF barrier or it may represent brain interstitial fluid, if CNS insulin uptake occurs preferentially across the blood–brain barrier. (*J. Clin. Invest.* 1991. **88**:1272–1281.) Key words: central nervous system • compartmental model • insulin action • mathematical model • MLAB modeling program • simulation

Introduction

Brain insulin has been implicated in a number of physiological processes including the regulation of food intake and body weight (1). The uptake of insulin from plasma into the central nervous system (CNS) and subsequent binding to neuronal insulin receptors have been proposed as the primary means by which insulin acts within the brain (2). Although insulin uptake has been demonstrated into both cerebrospinal fluid (CSF)[1] (3) and brain parenchyma (4), the primary route by which insulin enters the CNS and the mechanisms underlying this uptake process remain to be identified. Attempts to quantify the uptake of peptides such as insulin across the blood–brain barrier with conventional methods (see reference 5 for review) have been hindered by several factors, including degradation of labeled peptide and binding of the peptide to brain vasculature. These difficulties have led to studies of insulin uptake into CSF, an alternative approach that avoids these problems. Such studies have demonstrated that CSF insulin levels slowly parallel plasma levels in a variety of species (6–9), including human (10), and suggest that plasma is an important source of insulin in the CNS, and further, that the CSF may represent a means for delivering insulin to target sites in the brain. The interpretation of the relationship between plasma and CSF insulin levels, however, has been limited by a lack of information regarding the route by which insulin enters the CSF from plasma. Such information is critical for the quantitative assessment of insulin uptake into the CNS using this approach.

Recent observations from our laboratory have suggested that the mechanism(s) responsible for insulin uptake into CSF differ(s) from that of most other solutes (8). Specifically, we observed a delay of over 30 min which preceded any rise in CSF insulin levels during clamped, euglycemic hyperinsulinemia with the physiological range. This delay is uncharacteristic for solutes that enter CSF by diffusion across the choroid plexus, the primary site of CSF formation (11) and raises the possibility that diffusion of insulin across the blood–CSF barrier is restricted, and that its uptake into CSF requires an intermediate step involving specialized cellular processing or internalization. Receptor-mediated insulin transport (which has been demonstrated in peripheral tissues both in vitro [12] and in vivo [13]) at the level of either the blood–brain or blood–CSF barrier provides a rational basis for this hypothesis, and is further supported by the observation that CSF insulin uptake exhibits specificity (8).

In the current studies we report the analysis of the kinetics of uptake from plasma into CSF of both insulin and inulin (a biologically inert marker of diffusion similar in size to insulin)

1. *Abbreviations used in this paper:* CSF, cerebrospinal fluid; IRI, immunoreactive insulin; RMS, residual mean squared deviation(s).

during intravenous infusions, using both two- and three-compartment mathematical models. We hypothesized that if the uptake occurs primarily by diffusion across the blood–CSF barrier, a simple linear, two-compartment model would account for the kinetics of this uptake. This model was predicted to apply to inulin, in that it enters CSF via this mechanism (14). If the uptake of insulin occurs directly from plasma into CSF, but is saturable, then a modification of this two-compartment model should account for the uptake process. Finally, if the CSF uptake of either insulin or inulin involves passage through an intermediate compartment, a three-compartment representation should more accurately model this uptake process.

Methods

Study animals and insulin infusion protocols

Study animals and conditions. Normal adult male mongrel dogs weighing 20–40 kg were studied. A licensed veterinarian supervised care of the animals. The dogs were housed in individual cages that included a 5 × 20 ft area for exercise and were fed 0.5 kg/d of dry standard dog laboratory diet (Wayne Pro-Mix, Allied Mills, Inc., Memphis, TN) with unlimited access to water. Body weight was measured immediately before each study. After an overnight fast, animals were anesthetized with thiamylol (Surital, Parke-Davis, Morris Plains, NJ), 20 mg/kg i.v., intubated, and placed on a ventilator delivering 1–2% halothane or 2–3% isoflurane with 40% O_2. Intravenous catheters were placed in superficial veins of fore- and hindlimbs (one for infusion and one for sampling), and the cisternum magnum was cannulated by the placement of a 22-gauge, 1.5-in spinal needle utilizing sterile technique. The hub of the spinal needle was connected via low-capacity sterile tubing (0.6 ml) to a sterile three-way stopcock and 1-ml syringe for CSF sampling. The CSF in the dead space was cleared before taking each sample.

Insulin infusion

Intravenous insulin administration was carried out according to one of two protocols: (a) a brief, high-dose insulin infusion (80–400 mU/kg · min^{-1} × 5–10 min; studies 1–10) followed by sampling of plasma and CSF for up to 7 h ("impulse protocol"), or (b) a "sustained infusion" of insulin (10–15 mU/kg · min^{-1} × 4–12 h; studies 11–15). During the impulse protocol, blood samples (2.5 ml) were obtained at t = −15, −5, 2, 4, 6, 8, 10, 12, 15, 20, and 30 min and thereafter at 30-min intervals for 400–500 min. CSF samples (0.4 ml) were obtained at t = −15, −5, 6, 15, 30, 60, 90, 120, 150, and 180 min and thereafter at 60-min intervals until the end of the study. For the sustained infusion protocol, plasma and CSF samples were obtained at t = −15, −5, 5, 15, and 30 min and at 30–60-min intervals thereafter for up to 12 h. Euglycemia was maintained in all studies by variable-rate i.v. infusion of 50% dextrose with on-line monitoring of blood glucose levels achieved via a hand-held, computerized glucose meter (Glucoscan, Lifescan, American Medical Systems, Cincinnati, OH). Blood and CSF samples were placed on ice before processing. After blood separation, both plasma and CSF were frozen at −70°C until assay. Plasma levels of immunoreactive insulin (IRI) were measured by radioimmunoassay using a modification of the double-antibody method (15). CSF IRI was measured using a further modification of this method which enhances its sensitivity (8, 16). Plasma glucose was determined by the glucose oxidase method with a glucose autoanalyzer (Beckman Instruments, Inc., Brea, CA).

Inulin infusion

Studies utilizing an i.v. infusion of [^{14}C]inulin (sp act = 2.5 mCi/g; 97% radiochemical purity; mol wt 5,000–5,500; New England Nuclear, Boston, MA) were performed according to the impulse protocol described above for insulin. 125 μCi of [^{14}C]inulin dissolved in 15 ml of

normal saline were infused i.v. over 5 min beginning at t = 0 min. Blood (2.5 ml) and CSF (0.5 ml) samples were collected as described for studies 1–10 above. Samples were immediately placed on ice, and blood samples subsequently separated into plasma. Plasma and CSF radioactivity was determined by liquid scintillation counting, and expressed as disintegration per minute per milliliter.

Mathematical models

The schematic basis for the two- and three-compartment mathematical models is diagrammed in Fig. 1. Both models utilize differential equations to represent the rate of change of either insulin (*INS*) or inulin (*INU*) within the CSF as a function of the plasma level. In order to clarify the terms used in the equations, the two-compartment models are described for inulin, and the three-compartment model described for insulin. However, the kinetics of uptake of each solute was analyzed with all four models. These models were designed to study the dynamics of the CSF uptake of exogenously infused insulin. Therefore, basal plasma and CSF insulin levels were subtracted from all values in each data set before modeling, such that for modeling purposes the concentration in each compartment was set equal to zero at t = 0 min, and it was not necessary to assume that at the basal state all CSF IRI must be derived from plasma.

The two-compartment model (Fig. 1 *A*) uses a single differential equation to express the rate of change of inulin in CSF (INU_{CSF}, in concentration units) as a function of its rate of entry into CSF (the plasma level $[INU_P]$ · a rate constant, k_5) minus its rate of removal from CSF (the CSF level · a rate constant, k_4):

$$\frac{d}{dt}\text{INU}_{CSF}(t) = k_5 \cdot \text{INU}_P(t) - k_4 \cdot \text{INU}_{CSF}(t),$$

where INU_{CSF} = CSF inulin concentration, INU_P = plasma inulin concentration, k_5 = fractional rate constant for inulin uptake into CSF (min^{-1}), and k_4 = fractional rate constant for CSF inulin removal (min^{-1}).

Two modifications of this model were also analyzed in order to test the hypothesis that uptake occurs as a saturable process within a two-compartment system. Specifically, we modified k_5 such that it varied as a saturable function of the plasma level, based on the Michaelis-Menten equation:

$$k_5 = \frac{V_{max}}{K_m + \text{INS}_P(t)},$$

where K_m = the plasma level at which half-maximal transport occurs (Michaelis-Menten constant; μU/ml) and V_{max} = the maximal transport rate via a saturable process (μU · min^{-1}). In order to minimize the number of parameters to be identified, K_m was defined as 300 μU/ml, the value of the dissociation constant of the insulin receptor (17).[2]

Thus, the equation

$$\frac{d}{dt}\text{INS}_{CSF}(t) = \frac{k_{5'}}{300 + \text{INS}_P(t)} \cdot \text{INS}_P(t) - k_4 \cdot \text{INS}_{CSF}(t)$$

was utilized to analyze all studies, such that $k_{5'}$ represented V_{max}. The model based on this equation was designated the 2.1-compartment model. A further modification of this model was also developed in

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2. The 2.1-compartment model was also used to analyze insulin infusion studies with the substitution of the following values for K_m: 100, 600, and 1,000 μU/ml. These did not result in any further improvement in the data fit obtained in studies 6–9. In addition, a version of this model was tested in which K_m was not specified, but was identified as a third parameter. This resulted in a failure of this model to converge on an optimal curve fit, despite providing appropriate initial parameter estimates.
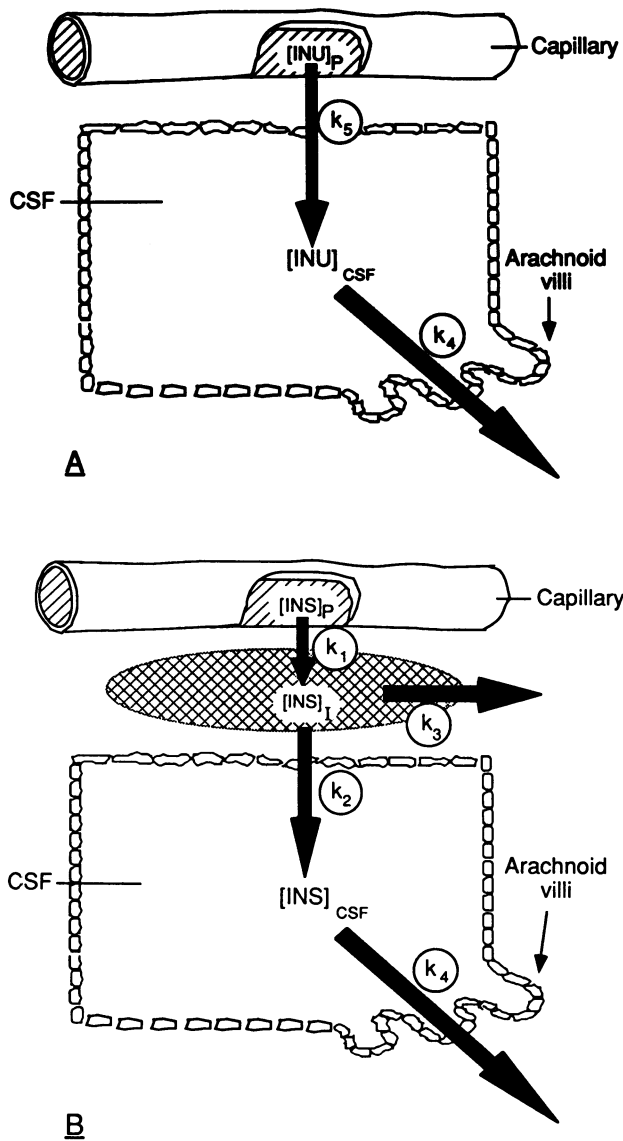
Figure 1. Schematic basis for the four mathematical models used to assess uptake into CSF from plasma. Studies conducted with both insulin and inulin were analyzed with each of the four models. (*A*) In the two-compartment system, uptake occurs primarily across the blood–CSF barrier. This pathway is indicated for inulin, where plasma inulin (INU_P) enters CSF at a rate characterized by a transfer rate constant, k_5 (min^{-1}). In the event that this uptake is saturable, two modifications of this model were also analyzed in which k_5 varies as a saturable function of the plasma level either with (2.2-compartment) or without (2.1-compartment) a nonsaturable component of uptake. CSF inulin (INU_{CSF}) is removed (arrow across arachnoid villi) at a rate characterized by the constant k_4 (min^{-1}). (*B*) In the three-compartment system, depicted for insulin, uptake occurs by penetration of plasma insulin (INS_P, $\mu U/ml$) into an intermediate compartment which has a volume V_1 (ml), at a rate characterized by k_1 ($ml \cdot min^{-1}$). The precise nature of this compartment is not specified by the model, but is presumed either to reside within the blood–CSF barrier or to represent brain interstitial fluid, if uptake occurs across the blood–brain barrier. The turnover of insulin in this compartment (INS_I, μU) is largely independent of CSF formation, occurring at a rate characterized primarily by k_3 ($ml \cdot min^{-1}$). A small fraction of INS_I enters CSF, which has volume V_{CSF} (ml), at a rate characterized by k_2 (min^{-1}). CSF insulin (INS_{CSF}) removal occurs at a rate characterized by k_4 (min^{-1}), a process largely dependent on bulk flow across arachroid villi or other structures.

order to account for the possibility that both saturable and nonsaturable components contribute to insulin uptake:

$$\frac{d}{dt} INS_{CSF}(t) = \frac{k_5}{300 + INS_P(t)} \cdot INS_P(t)$$

$$+ k_6 \cdot INS_P(t) - k_4 \cdot INS_{CSF}(t),$$

where k_6 = the rate constant for nonsaturable insulin uptake into CSF (min^{-1}). The model based on this equation was designated the 2.2-compartment model.

The optimal curve fit of the rate of change of INU_{CSF} as a function of INU_P was performed with each of these equations using the NIH mathematical modeling program, M LAB (18), operating via a mainframe computer (DEC 10, Digital Equipment Corp., Marlboro, MA). In deriving the optimal data fit, values were identified for both parameters k_4 and k_5 (as well as k_6, for the 2.2-compartment model). The identification of each parameter was accompanied by a fractional standard deviation, a measure of the variability in the combination of parameter values which could have been used to produce the optimal curve fit.

The detailed derivation of the equations used in the three-compartment model is provided in Appendix I. The three-compartment model (Fig. 1 *B*) assumes that insulin entering the CSF is first taken up from plasma into an intermediate compartment. Some fraction of the insulin in the intermediate compartment gains access to the CSF, at a rate which is small compared to the overall turnover of insulin in the intermediate compartment (i.e., $k_3/V_1 \gg k_2$). The rationale for this latter assumption is that preliminary models which assumed that insulin in the intermediate compartment was largely transferred into the CSF were unable to model the insulin infusion studies. The rate of insulin entry into CSF as a function of the plasma level, therefore, is dependent on two factors: (*a*) the rate of change of insulin levels in the intermediate compartment in response to changes in the plasma level, and (*b*) the rate at which insulin in the intermediate compartment enters the CSF. Once in CSF, insulin is removed at rate characterized by a fractional rate constant identified by the model, as in the two-compartment model. Two differential equations characterize the rate of change of insulin both in the intermediate compartment and in the CSF compartment:

$$\frac{d}{dt} INS_I(t) = k_1 \cdot INS_P(t) - \frac{k_3}{V_1} \cdot INS_I(t) - k_2 \cdot INS_I(t)$$

$$\frac{d}{dt} INS_{CSF}(t) = k_2 \cdot INS_I(t) - k_4 \cdot INS_{CSF}(t),$$

where INS_I = insulin content (μU) in the intermediate compartment; INS_P = plasma insulin level ($\mu U/ml$); INS_{CSF} = insulin content (μU) in CSF; V_1 = volume of the intermediate compartment (ml); k_1 = rate constant for insulin uptake from plasma into brain ISF ($ml \cdot min^{-1}$); k_3 = turnover rate constant for insulin in the intermediate compartment ($ml \cdot min^{-1}$); k_2 = fractional rate constant for insulin entry into CSF from the intermediate compartment (min^{-1}); k_4 = fractional rate constant for removal of insulin from CSF (min^{-1}), as in the two-compartment model; (see Fig. 1 *B*). In order to minimize the number of parameters, the equations were reduced to (see Appendix I):

$$\frac{d}{dt} X = INS_P(t) - \left(\frac{k_3}{V_1} + k_2\right) \cdot X$$

$$\frac{d}{dt} \frac{INS_{CSF}(t)}{V_{CSF}} = \left[\left(\frac{k_2 \cdot k_1}{V_{CSF}}\right) \cdot X\right] - k_4 \cdot \frac{INS_{CSF}(t)}{V_{CSF}},$$

where $X = [INS_I(t)]/k_1$ ($\mu U \cdot min$)/ml and V_{CSF} = CSF volume (ml). Curve fitting based on this model was again performed with the M LAB program. This resulted in the identification of three parameters: $k_3/V_1 + k_2$, $(k_2 \cdot k_1)/V_{CSF}$, and k_4. Since $k_3/V_1 \gg k_2$, the parameter $k_3/V_1 + k_2$ can be reduced to k_3/V_1.

Comparison of model fits and statistical methods

Unless otherwise indicated, data are expressed as mean±SEM. The level of significance for all statistical comparisons was considered to be $P < 0.05$.

In order to compare the accuracy of the curve fit generated by the two models for a given set of plasma and CSF data, the two-compartment models were each analyzed as a limiting special case of the three-compartment model and should in general have had a larger residual sum of squared deviations of the data from the curve fit. The need for a three-compartment model was assessed by using an F-test based on the residual sums of squares from the two fits obtained for each data set (see Appendix II). A second approach was to examine the observed values minus the fitted values, called the residual values. A "run" is a sequence of residuals that are all positive or all negative. When the residual values are plotted versus time, models that do not fit tend to have too few runs (because over regions the fitted curve is consistently either too low or too high for the data). We examined whether or not the two-compartment models had statistically too few runs compared to the three-compartment model using the one-sided sign test.

The accuracy of the two-compartment model fits of data obtained from insulin infusion studies was compared to that obtained using [^{14}C]inulin with a Wilcoxon two-sample test applied to the R^2 values for the fit obtained in each study. These comparisons were performed only on studies in which the same infusion and sampling protocol was employed (impulse protocol).

Results

Analysis of impulse infusion studies with insulin. The high-dose impulse infusion protocol resulted in an increase of both plasma and CSF insulin levels, illustrated for studies 1–5 in Fig. 2 *A*. The plasma insulin time course (*upper panel*) was characterized by peak mean insulin levels of $\sim 8,000$ μU/ml achieved at $t = 6$ min, followed by a decline toward basal values over 3 h. In the CSF (*lower panel*), mean insulin levels gradually rose from basal values of ~ 0.5 μU/ml to peak values of 4.5 μU/ml by $t = 90$ min. Over the subsequent 5.5 h, there was a continuous decline in CSF insulin to 2 μU/ml. The mean values (±SEM) for the plasma and CSF insulin levels are provided in Table I. Plasma glucose levels were maintained between 65 and 105 mg/dl by a variable rate glucose infusion in all studies (data not shown). On visual inspection of the bottom panel of Fig. 2 *A*, it is clear that the three-compartment model can account for the kinetics of CSF insulin uptake, whereas the nonsaturable two-compartment model cannot ($P < 0.0001$). A saturable two-compartment model (2.1-compartment) provided a significant improvement in the fit of these data vs. the nonsaturable version ($P < 0.0001$), but no further improvement was provided by a model which combines both saturable and nonsaturable uptake mechanisms (2.2-compartment).

Fig. 2 *B* illustrates plasma and CSF insulin levels for studies 7–10, in which insulin was infused i.v. at rates $< 10\%$ of those used in studies 1–5 (16.5 vs. 176 mU/kg·min^{-1}), thus resulting in lower peak plasma and CSF insulin levels (Table I). Visual inspection of the curve fits shows the superior accuracy of the three-compartment model compared with any of the two-compartment models ($P < 0.0001$ vs. each of the 2-, 2.1-, and 2.2-compartment models). This was confirmed by comparing the mean accuracy of the curve fits generated by each model in each of the studies employing the impulse protocol (individual data and curve fits not shown), as measured by both the determination coefficient (R^2) and the residual mean squared deviations (RMS) of the data from the curve fit. Table II indicates that for the studies in Fig. 2, *A* and *B*, as well as for

the impulse studies as a group (studies 1–10) the R^2 value was higher, and RMS value lower for the fit generated by the three-compartment compared to any of the two-compartment models. This difference in accuracy of the curve fit between the two types of models was highly significant, particularly for the low dose impulse studies (studies 7–10; $P < 0.0001$), indicating that the addition of the third compartment improved the fit of the data, whether or not a saturable two-compartment model was employed. Furthermore, the impulse studies generally had fewer positive and negative runs under each of the two-compartment models than with the three-compartment model, again demonstrating the better fit of the three-compartment model ($P < 0.05$ for three-compartment vs. each of the three two-compartment models).

The middle panels of Fig. 2, *A* and *B*, indicate the predicted relative time course of insulin levels in the intermediate compartment, generated by the three-compartment model. The concentration of insulin in this compartment is predicted to be intermediate between the plasma and CSF levels, but absolute values could not be determined since they are dependent on variables which could not be explicitly identified (k_2, V_{CSF}, and V_I). The relative time course, however, was predicted by the three-compartment model in the process of generating the model fit of the data. The turnover rate constant for insulin in this compartment ($k_3/V_I = 0.037\pm0.006$ min^{-1}; Table III) corresponds to a half-life of insulin of $\sim$ 15 min. Thus, the rate of insulin turnover is predicted to be much greater in the intermediate compartment than in the CSF ($k_4 = 0.0089\pm0.0018$ min^{-1}). The values for each of the three parameters identified by the three-compartment model tended to be similar in magnitude between studies. Thus, the standard error of the mean of each of the three parameters values was $\le 20\%$ for studies 1–10 (Table III).

Analysis of sustained insulin infusion studies. The plasma insulin values for study 11, representative of the sustained infusion protocol (studies 11–15), is indicated in the top panel of Fig. 3.[3] After the institution of euglycemic hyperinsulinemia at supraphysiologic levels, there was an interval of at least 15 min with no change in CSF insulin levels, followed by a gradual rise in CSF insulin concentrations to nearly 25 μU/ml at 12 h (Fig. 3, *bottom panel*). Upon visual inspection, there is little difference in the accuracy of the fit obtained with the four different models. However, the RMS values for the sustained infusion studies as a group (Table II) were significantly lower in the three-compartment (RMS = 0.838±0.080) than the two-compartment model (RMS = 0.973±0.129; $P < 0.0001$). The addition of a saturable component to the two-compartment model (both with and without a nonsaturable component) increased the accuracy of fit over the nonsaturable two-compartment model ($P < 0.0001$) to values not significantly different from the three-compartment model. However, in contrast to the three-compartment model, both saturable two-compartment models identified at least one parameter with a value < 0 in the majority of studies (three of five studies for the 2.1-, and four of five for the 2.2-compartment model), values which cannot apply physiologically.

From the predicted time course of the relative insulin concentration in the intermediate compartment (INS$_I$), derived

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3. Several different infusion protocols were employed for the sustained infusion studies. Therefore, it was not possible to present the mean of all five studies in one figure.

A

B
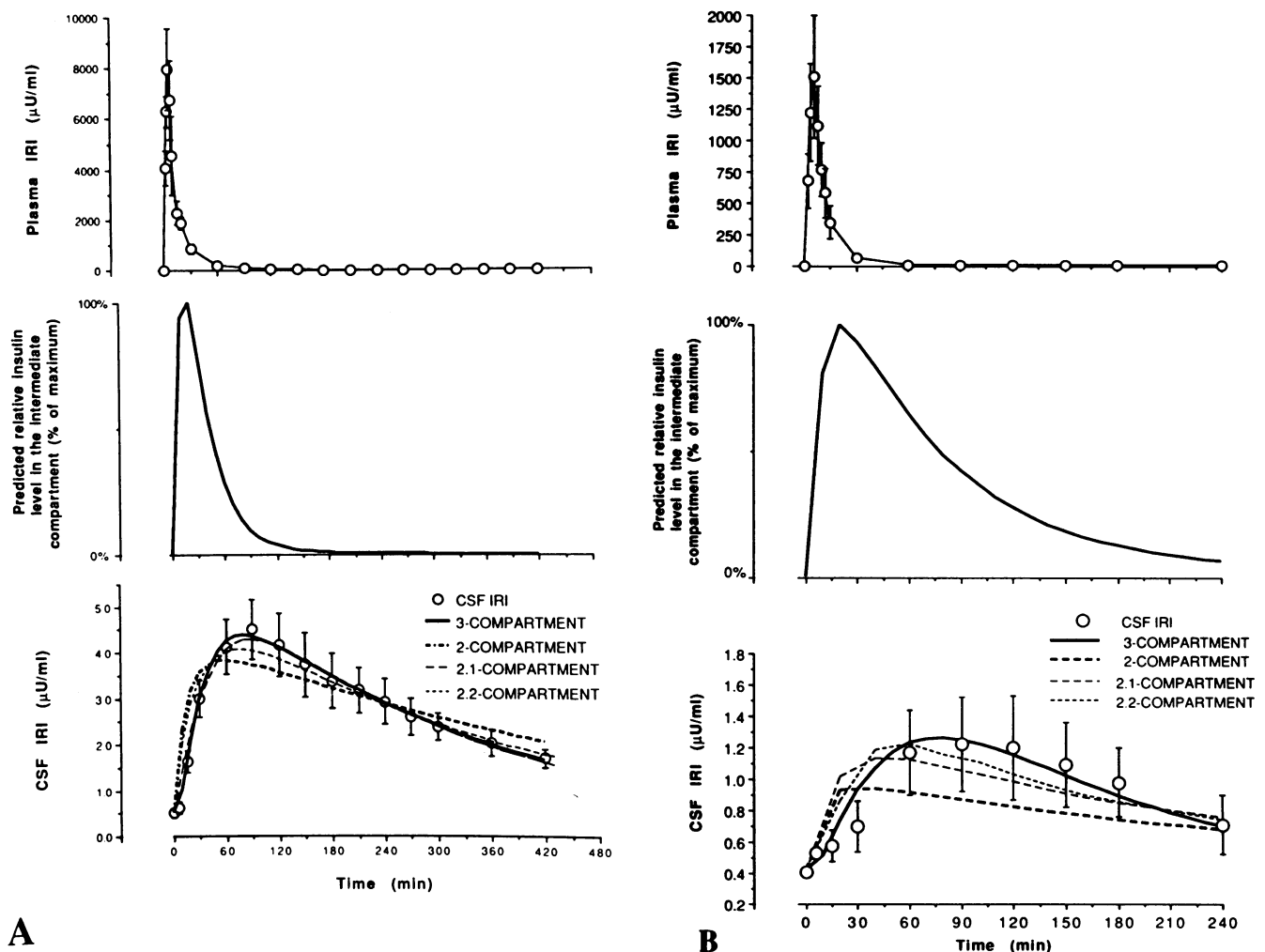
Figure 2. (A) Upper panel: time course of mean (±SEM) plasma insulin levels during insulin infusion according to the impulse protocol (mean infusion rate = 176 ± 23 mU/kg·min^{-1} over 5 min; see methods) for studies 1–5. Lower panel: time course of mean CSF insulin levels (O) for the same studies and mean curve fit generated by the three-compartment model (solid line) and the three different two-compartment models (broken lines). The R^2 for the mean three-compartment and nonsaturable two-compartment fits were 0.976 and 0.772, respectively (P < 0.0001). The 2.1- and 2.2-compartment model fits (R^2 = 0.956 and 0.961, respectively) were significantly improved over that obtained with the nonsaturable two-compartment model ($P \le 0.0001$). The mean parameter values identified by the three-compartment model were k_3/V_1 = 0.054 min^{-1}; $(k_2 \cdot k_1)/V_{CSF}$ = 23.4·10^{-7} min^{-2}; k_4 = 0.0042 min^{-1}. Middle panel: time course of relative insulin levels in the intermediate compartment, as predicted by the three-compartment model, as a percent of maximal levels. (Study 6 was excluded from this figure as it was conducted at a much higher insulin infusion rate [326 mU/kg·min^{-1}].) (B) Upper panel: mean (±SEM) plasma insulin levels for studies 7–10 (mean insulin infusion rate = 16.5 ± 4.5 mU/kg·min^{-1} over 5 min). Lower panel: time course of CSF insulin levels (O) and mean curve fit generated by the four models. The mean R^2 value for the three-compartment model (0.912) was significantly greater than for any of the two-compartment models (0.549, 0.645, and 0.746 for 2-, 2.1-, and 2.2-compartment model fits, respectively; P < 0.0001 for each two-compartment model vs. three-compartment model). Mean parameter values identified by the three-compartment model were: k_3/V_1 = 0.017 min^{-1} ($k_1 \cdot k_2/V_{CSF}$ = 25.6·10^{-7} min^{-2}; k_4 = 0.0154 min^{-1}. Middle panel: time course of relative insulin levels in the intermediate compartment, as predicted by the three-compartment model.

from the three-compartment model (Fig. 3, middle panel), it is evident that the temporal dynamics parallel that of plasma more so than do CSF levels. The parameters identified by the 3-compartment model (Table III) for studies 11–15 exhibited a greater degree of variability than was observed in studies using the impulse protocol, both within and between studies. This indicates that less information necessary for modeling purposes was provided by the sustained infusion than by the impulse infusion protocol.

Analysis of uptake of [^{14}C]inulin into CSF. After i.v. infusion over 5 min (impulse protocol), plasma levels of [^{14}C]inulin peaked at t = 6 min and subsequently fell over the

8 hr study period (Table I, Fig. 4). CSF [^{14}C]inulin levels rose to peak values by t = 90 min and then gradually declined over the subsequent 6 h. On visual inspection, the CSF [^{14}C]inulin curve fit derived from the nonsaturable two-compartment model (lower panel) was far more accurate than was generated using this model on data obtained during insulin infusions with the same protocol (Fig. 2). Confirming this impression, the mean R^2 value for the [^{14}C]inulin studies was significantly greater than was observed during comparable insulin studies conducted with the impulse protocol when analyzed by the nonsaturable two-compartment model (R^2 = 0.879±0.044 vs. 0.723±0.036; P = 0.02). Thus, the nonsaturable two-compart-

Table I. Mean (±SEM) Plasma and CSF Insulin (μU/ml) and [^{14}C]Inulin (dpm/ml) Levels for High-Dose (Studies 1-5, Insulin Infusion Rate = 176±23 mU/kg·min^{-1} over 5 min*) and low-dose (Studies 7-10, 16.5±4.5 mU/kg·min^{-1} over 5 min) "Impulse" Infusion Studies, and for [^{14}C]Inulin Infusion Studies

Time (min)	Insulin studies 1-5		Insulin studies 7-10		Inulin studies 1-5	
	Mean plasma IRI (±SEM)	Mean CSF IRI (±SEM)	Mean plasma IRI (±SEM)	Mean CSF IRI (±SEM)	Mean plasma inulin (±SEM)	Mean CSF inulin (±SEM)
	μU/ml	μU/ml	μU/ml	μU/ml	dpm/ml	dpm/ml
0	3 (1.2)	0.51 (0.20)	3 (0.6)	0.41 (0.04)	0 (0)	0.00 (0.00)
2	4,090 (1,340)		678 (440)		38,280 (7,100)	
4	6,300 (1,220)		1,225 (780)		69,690 (9,072)	6.80 (4.00)
6	7,950 (3,200)	0.61 (0.26)	1,513 (980)	0.53 (0.08)	69,606 (11,556)	
8	6,760 (3,100)		1,120 (620)		56,410 (10,144)	
10	4,570 (3,140)		765 (424)		48,806 (5,248)	23.80 (13.20)
12			583 (400)		41,562 (6,724)	
15	2,300 (900)	1.63 (0.48)	348 (258)	0.58 (0.20)	37,578 (3,636)	
20	1,900 (480)				29,552 (2,764)	35.40 (12.00)
30	874 (420)	3.00 (0.80)	63 (42)	0.7 (0.32)	23,662 (2,032)	68.20 (13.20)
60	175 (140)	4.12 (1.20)	13 (6)	1.17 (0.54)	14,428 (1,404)	97.20 (20.00)
90	77 (20)	4.50 (1.30)	12 (6)	1.22 (0.60)	9,206 (1,020)	118.20 (32.00)
120	37 (10)	4.17 (1.40)	7 (4)	1.20 (0.66)	6,080 (856)	102.60 (32.40)
150	26 (6)	3.73 (1.40)	8 (2)	1.09 (0.54)	4,144 (712)	111.80 (28.00)
180	17 (4)	3.37 (1.20)	8 (4)	0.98 (0.44)	2,926 (560)	92.00 (22.80)
210	13 (4)	3.17 (1.00)			2,310 (392)	
240	11 (6)	2.92 (1.00)	7 (4)	0.71 (0.38)	1,756 (468)	86.80 (20.40)
270	10 (4)	2.58 (0.80)			1,436 (436)	
300	8 (4)	2.38 (0.60)	7 (2)	0.45 (0.04)	1,134 (428)	85.60 (24.00)
330	8 (4)				884 (320)	
360	8 (4)	2.03 (0.60)	8 (2)	0.44 (0.02)	624 (228)	81.60 (14.80)
390	8 (4)				484 (196)	
420	8 (4)	1.70 (0.40)			378 (164)	73.80 (21.60)
450					344 (136)	
480					282 (124)	60.00 (17.60)

* Study 6 was excluded from the presentation of the mean data due to the high insulin infusion rate (325 mU/kg·min^{-1} over 5 min) used compared to the other studies.

ment model accounts for the kinetics of uptake of [^{14}C]inulin, but not insulin, with a high degree of accuracy.

In contrast, the 2.2-compartment model fit the inulin data in only three of five studies, the 2.1-compartment model fit zero of five and the three-compartment model fit only two of five studies (data not shown). Thus, the kinetics of inulin uptake are most consistent with a nonsaturable, two-compartment model. Furthermore, whereas the three-compartment model accurately accounts for insulin uptake, it is inappropriate for the evaluation of the uptake of inulin into CSF.

The parameters identified by the nonsaturable two-compartment model for [^{14}C]inulin included the rate constants for penetration of the blood–CSF barrier (k_5 = 55.3±4.7 min^{-1} × 10^{-6}; $\bar{x}$±SEM) and for turnover in CSF (k_4 = 0.0025±0.0003 min^{-1}) (Fig. 1 A). Both were identified with a high degree of confidence (standard deviations < 25% of the parameter value in each study. Individual parameter values were also comparable between inulin studies (standard errors of 9% and 14% for parameters for k_5 and k_4, respectively). This reproducibility of parameter identification further supports the validity of the two-compartment model for inulin uptake, and suggests that the dynamics of this process are similar from one animal to the next. Thus, the uptake of inulin into CSF appears to occur primarily across the blood-CSF barrier as a two-compartment, nonsaturable system.

Discussion

The hypothesis that plasma insulin enters the brain to act as a satiety signal reflecting body adiposity in the long-term regulation of food intake and body weight has received considerable experimental support (1). Several additional roles for insulin in the CNS have also been proposed and are currently under investigation (19–21). Although it is likely that brain insulin is derived primarily from plasma, the means by which this occurs has not been established. Since insulin transport mediated by insulin receptors has been demonstrated across the endothelium of peripheral tissues (13), and since insulin receptors are expressed by both the blood–brain (2, 9) and blood–CSF barriers (22), it is reasonable to propose that such specialized transport contributes to the uptake of insulin into the CNS (2, 19). In this case, one might anticipate that the kinetics of CSF insulin uptake would differ from those characterizing solute uptake via diffusion alone.

In the current studies, we analyzed the kinetics of CSF insulin uptake from plasma using mathematical modeling techniques based on both two- and three-compartment uptake systems. Our results indicate that the three-compartment model accounts for the kinetic relationship between plasma and CSF insulin levels with a high degree of accuracy in each of 15 studies, using several different insulin infusion protocols, whereas

Table II. Mean Accuracy of (±SEM) of the Model Fits Obtained with the Three-Compartment Model Compared to That Obtained with Three Different Versions of the Two-Compartment Model

	Model							
	Three-compartment		Two-compartment					
			2-C		2.1-C[||]		2.2-C[||]	
Insulin study	R^2	RMS	R^2	RMS	R^2	RMS	R^2	RMS
High-dose impulse (studies 1–5)	0.976±0.005*	0.213±0.052*	0.772±0.018	0.647±0.128	0.956±0.007‡	0.264±0.038‡	0.961±0.008‡	0.255±0.037‡
Low-dose impulse (studies 7–10)	0.903±0.026*	0.088±0.033*	0.549±0.027	0.223±0.079	0.645±0.052§	0.187±0.062§	0.746±0.068§	0.162±0.051§
All impulse studies (studies 1–10)	0.947±0.015*	0.242±0.090*	0.671±0.037	0.809±0.364	0.831±0.054‡	0.357±0.131‡	0.876±0.043‡	0.329±0.118‡
Sustained infusion (studies 11–15)	0.981±0.003‡	0.838±0.080‡	0.975±0.004	0.973±0.129	0.973±0.005	0.940±0.101	0.983±0.004	0.824±0.143

Accuracy was assessed by both the determination coefficient (R^2) and by the residual mean squared deviations of the data from the curve fit (RMS) (see Methods and Appendix II). * $P < 0.0001$ vs. each of the three types of two-compartment model. ‡ $P < 0.0001$ vs. two-compartment model. § $P \leq 0.02$ vs. two-compartment model. [||] Model fits were obtained with identification of negative parameter values in the majority of sustained infusion studies.

the two-compartment model based on nonsaturable uptake, in general, does not. The difference in the accuracy of the data fit provided by these two models was greatest with the impulse infusion protocol, where is it evident that a nonsaturable two-component system is inappropriate for modeling CSF insulin uptake in all 10 studies. Interestingly, a modification of the two-compartment model to allow for the possibility that CSF insulin uptake occurs via a saturable process (2.1-compartment model) improved the data fit in the sustained infusion studies and in those impulse studies employing high rates of insulin infusion. However, when impulse insulin infusion studies were conducted at lower infusion rates (studies 7–10), it was clear that such modifications fail to provide an appropriate representation of the uptake process. Nonetheless, these findings are provocative in that they raise the possibility that saturable transport may play a role in CSF insulin uptake, and further studies are warranted to address this issue. An additional modification of this model which permits both saturable and nonsaturable mechanisms to contribute to uptake (2.2-compartment model) did not significantly improve the accuracy of fit over the 2.1-compartment model. Since this model involves the identification of three parameters instead of two (as in the three-compartment model), it suggests that the superior fit obtained with the three-compartment model is not simply the consequence of increasing the number of parameters used. When the kinetics of CSF insulin uptake during the impulse infusion protocol were analyzed using any of the two-compartment models as a "limited special case" of a three-compartment model (i.e., a three-compartment model with one compartment reduced to an infinitely small size), the need for the addition of the third compartment in order to account for the kinetics of insulin uptake was highly significant ($P < 0.0001$). Thus, while these findings do not establish our three-compartment model as being physiologically correct, they indicate that the simpler, two-compartment system of uptake is unlikely to apply to the uptake of insulin into CSF.

In contrast, the nonsaturable two-compartment model accurately accounted for the kinetics of [^{14}C]inulin uptake into CSF, a process likely to be mediated by diffusion across the blood–CSF barrier (14). As expected, modifications to allow for saturable uptake could not account for this uptake process. Although the possibility cannot be excluded that inulin degra-

dation products make a minor contribution to the observed dynamics of CSF inulin uptake, this possibility seems unlikely, since the efficiency of uptake is similar to that reported previously (14). Thus, CSF insulin uptake from plasma appears to involve mechanisms fundamentally different from those which apply to the uptake of inulin (a molecule of similar size to insulin), and passage through an intermediate compartment appears to be a key difference. These findings are in keeping with our previous observation of a delay in the uptake of insulin into CSF which occurs during physiologic elevations of insulin in plasma (8).

The kinetics of insulin uptake from plasma into CSF in the basal state (after an overnight fast) may differ from those which apply during exogenous insulin infusion. In the basal state, levels of immunoreactive insulin in CSF are quite low ($\sim 0.5 \ \mu U/ml$), but as a proportion of plasma levels (~ 10–15%), are in excess of the steady-state value predicted by the three-compartment model ($\sim 2\%$, based on mean parameter values obtained during insulin infusion according to the impulse protocol). Although this discrepancy results from only a small increase in basal CSF insulin levels over the predicted value (0.3–$0.4 \ \mu U/ml$), the implication that the three-compartment model may not apply to the basal state warrants comment. First, it is possible that the kinetic relationship between plasma and CSF insulin levels identified by the three-compartment model does apply in the basal state, but that proteins or other solutes in CSF cause a small degree of interference with our radioimmunoassay for insulin (which is capable of discriminating concentrations of $0.20 \ \mu U/ml$ from zero with 95% confidence when applied to solutions prepared in an assay buffer system [8]), thus modifying the observed basal relationship. This is the interpretation which we believe best accounts for the available data. However, it is also possible that a small amount of insulin in CSF is not derived from plasma, but originates from de novo insulin synthesis within the CNS itself (23). Finally, saturable transport of insulin has been suggested to occur across the blood–brain barrier via a process involving endothelial insulin receptors (2). In this instance, the efficiency of entry into CSF would be greater at low than at high plasma insulin levels, and therefore the parameters characterizing insulin uptake in the basal state would differ from those which apply during exogenous insulin infusion. Further studies will
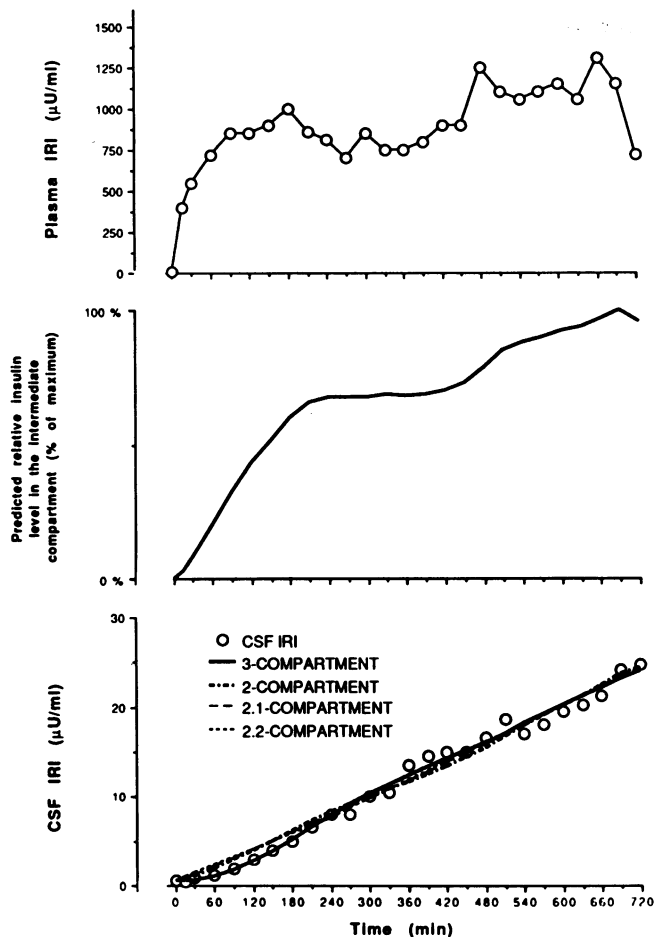
Figure 3. *Upper panel*: time course of plasma insulin levels during insulin infusion according to one of the sustained infusion protocols (insulin infusion rate = 10 mU/kg·min^{-1}; study 11). *Lower panel*: time course of insulin levels in CSF for the same study, with curve fits generated by the three two-compartment models (R^2 = 0.982, 0.985, and 0.985 for 2-, 2.1-, and 2.2-compartment, respectively) and the three-compartment model (R^2 = 0.991). *Middle panel*: time course of relative insulin levels in the intermediate compartment as predicted by the three-compartment model.

be necessary to determine which of these possibilities account for our observations. It should be stressed, however, that our modeling procedures were designed to analyze the kinetics of the CSF uptake of insulin during intravenous infusion, and are independent of the basal relationship between insulin levels in plasma and CSF.

Our results indicate that insulin traverses an intermediate compartment en route from CSF to plasma. The identity of this compartment remains open to further study. One possibility is that it resides within the blood–CSF barrier, for example, as the epithelium of the choroid plexus. This concept is attractive, since specialized transport of key nutrients (see reference 24 for review) as well as peptide hormones such as prolactin (25) are thought to occur at this site. Furthermore, the choroid plexus is richly populated with insulin receptors (22). However, this hypothesis has several shortcomings. Chiefly, it is evident that although the CSF uptake of insulin is kinetically distinct from that of inulin, it is no more efficient, at least with the infusion protocols employed for these studies. This fact, combined with the observation that concentrations of both in-

sulin and insulin receptors in the brain are unrelated to the proximity to the cerebral ventricles or subarachnoid space (16) have raised questions regarding the role of CSF in the delivery of insulin to target sites in the brain.

This raises the alternative interpretation that insulin entering the choroid plexus is largely metabolized via a receptor-mediated process, but that some fraction escapes degradation and "spills over" into CSF after binding to insulin receptors on choroidal epithelium and subsequent cellular internalization. In this instance, insulin uptake into CSF would be unrelated to the means by which it enters the brain. This would account for the delay in appearance of insulin in CSF, for the three-compartmental nature and for the relative inefficiency of the uptake process. However, it would not account for the observation that CSF insulin uptake occurs at a rate fivefold greater than that of proinsulin (8), a peptide with reduced affinity for the insulin receptor which would therefore not be expected to be degraded more rapidly than insulin. When viewed collectively, the data are more consistent with an insulin transport process specific for insulin, but with uptake occurring primarily into a compartment which is not CSF, but which is in communication with it.

Thus, it is possible that insulin uptake into both brain and CSF occurs primarily across the blood–brain barrier endothelium (2), rather than the blood–CSF barrier. In this case, the intermediate compartment would represent brain interstitial fluid, the compartment within which brain insulin action occurs. Since bulk flow of brain interstitial fluid across ventricular ependyma may account for as much as 30% of CSF formation (26), it is likely that a fraction of the insulin in this compartment enters CSF via this route. However, most of the insulin in brain interstitial fluid would be expected to be removed relatively rapidly by binding to neuronal and glial insulin receptors, or to be lost via cervical lymph rather than by entry into CSF (27). This notion is supported indirectly by the dynamics of insulin in the intermediate compartment predicted by the three-compartment model. Thus, the half-life of insulin in this compartment is predicted to be ≈ 15 min (similar to that observed in interstitial fluids elsewhere in the body [28]), far less than that in CSF. Moreover, insulin in the intermediate compartment appears to have limited access to CSF, since, when the model was constructed such that entry into

Table III. *Mean Rate Constants Identified by the Three-Compartment Model for the Two Types of Insulin Infusion Protocols*

Insulin study protocol	Parameter (±SE)		
	k_3/V_1	$k_1 \cdot k_2/V_{CSF}$	k_4
	min^{-1}	$min^{-2} \times 10^{-7}$	min^{-1}
Impulse protocol (studies 1–10)	0.037±0.006	23.4±3.1	0.0089±0.0018
Sustained infusion protocol (studies 11–15)	0.012±0.004	12.3±1.8	0.0099±0.0037

k_3/V_1 is the rate constant for insulin turnover in the intermediate compartment (based on the assumption that $k_3/V_1 \gg k_2$, ∴ $k_3/V_1 + k_2 \approx k_3/V_1$ [see Methods and Appendix I]); $k_1 \cdot k_2/V_{CSF}$ is the composite rate constant for insulin uptake from plasma through the intermediate compartment and into CSF; k_4 is the CSF insulin turnover rate constant.
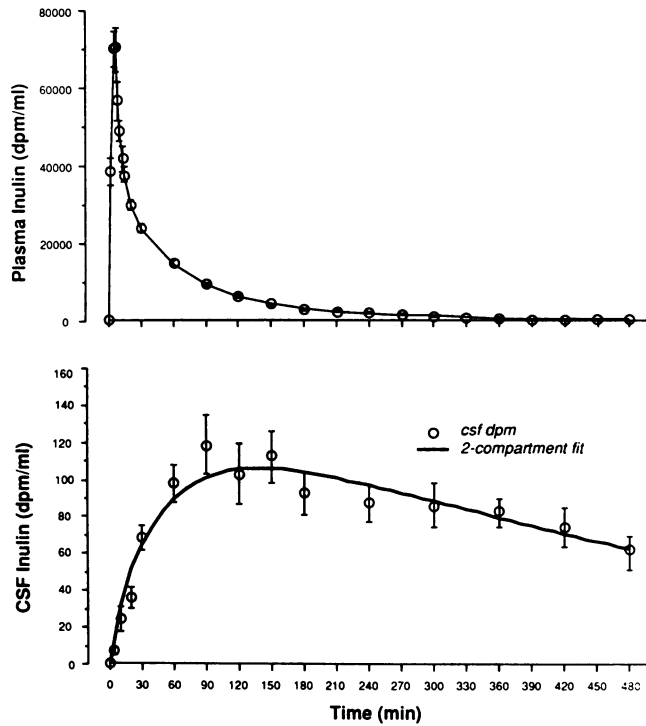
Figure 4. Upper panel: time course of mean (±SEM) plasma
[^{14}C]inulin levels during [^{14}C]inulin infusion according to the im-
pulse protocol for inulin studies 1–5. *Lower panel*: time course of
corresponding [^{14}C]inulin levels in CSF with mean curve fit gener-
ated by the two-compartment model ($R^2 = 0.879\pm0.044$; RMS
$= 7.07\pm1.20$). The data could not be fit by the three-compartment
model in three of five studies, by the 2.1-compartment model in five
of five studies, nor by the 2.2-compartment model in two of five
studies (data not shown).

CSF was the primary means by which insulin is removed from
the intermediate compartment, modeling efforts were unsuc-
cessful (data not shown). Insulin uptake occurring primarily
into brain interstitial fluid is therefore consistent both with a
three-compartmental system of uptake and with certain physio-
logical aspects of the relationship between brain interstitial
fluid and CSF. However, the concept that CSF insulin is de-
rived primarily from brain interstitial fluid hinges on the condi-
tion that the blood–CSF barrier represents an impediment to
CSF insulin uptake. While it is possible that insulin receptors in
the choroid plexus bind insulin in the process of CSF forma-
tion, such that choroid plexus-derived CSF is essentially free of
insulin, this possibility is as yet untested.

Perhaps the most provocative information provided by the
three-compartment model is the predicted time course for the
kinetics of insulin uptake from plasma into the intermediate
compartment (Figs. 2 and 3, *middle panels*). Although it was
not possible to determine absolute values for insulin levels in
this compartment, the dynamics of the relative changes of insu-
lin within it are predicted to parallel those in plasma much
more so than do CSF levels. Thus, not only are the predicted
levels in the intermediate compartment intermediate in magni-
tude between plasma and CSF levels, but the fluctuations in the
plasma levels should be reflected much more dynamically in
this compartment than is seen in the CSF. In the event that this
compartment represents brain interstitial field, it is likely that
brain interstitial fluid insulin levels (in contrast to those in
CSF) are quite sensitive to short-term fluctuations in plasma

levels, as is the case with other insulin-sensitive tissues. Since
insulin is postulated to represent satiety signal within the CNS
which varies in amplitude as a function of the plasma level (1,
19), the concept that insulin levels in brain interstitial fluid are
higher than in CSF and dynamically related to plasma levels
(in comparison to those in CSF) has important implications.

In summary, we find that the uptake of insulin from plasma
into CSF can be distinguished from that of inulin, which enters
the CSF via diffusion across the blood–CSF barrier, in that it
appears to require passage through an intermediate compart-
ment. Such specialized handling of insulin may reflect a trans-
port system designed to regulate the rate of delivery of insulin
from plasma into the CNS.

Appendix I

*Method for mathematical modeling with a
three-compartment system*

Fig. 1 *B* depicts the basis for the three-compartment model used for the
mathematical analysis of the kinetics of insulin uptake from plasma
into CSF. Plasma insulin (INS_P, $\mu U/ml$) enters an intermediate com-
partment (INS_I, μU) with volume V_I(ml), at a rate characterized by
the rate constant k_1($ml \cdot min^{-1}$). Insulin is cleared from the interme-
diate compartment at a rate characterized by a second constant,
k_3($ml \cdot min^{-1}$). The turnover of insulin in this compartment was as-
sumed to be independent of CSF formation (i.e., the quantity of insulin
cleared from this compartment via entry into CSF was assumed to be
small compared to that which is cleared by other mechanisms), for the
purpose of simplifying the model. CSF insulin (INS_{CSF}, μU) was as-
sumed to be derived from the intermediate compartments at a rate
characterized by $INS_I(t) \cdot k_2$($\mu U \cdot min^{-1}$), where k_2 represents a third
rate constant (min^{-1}). The rate of change of insulin concentration
within the intermediate compartment (in units of $\mu U \cdot min^{-1}$) was ex-
pressed by the differential equation:

$$\frac{d}{dt} INS_I(t) = k_1 \cdot INS_P(t) - \frac{k_3}{V_I} \cdot INS_I(t) - k_2 \cdot INS_I(t), \quad (A1)$$

Within the CSF compartment, insulin is removed at a rate character-
ized by a rate constant k_4 (min^{-1}). The rate of change of CSF insulin
content ($\mu U \cdot min^{-1}$) was therefore described by:

$$\frac{d}{dt} INS_{CSF}(t) = k_2 \cdot INS_I(t) - k_4 \cdot INS_{CSF}(t). \quad (A2)$$

Because CSF insulin was measured in concentration units ($\mu U/ml$),
Eq. A2 was divided by the CSF volume, V_{CSF} (ml):

$$\frac{d}{dt} \frac{INS_{CSF}(t)}{V_{CSF}} = \frac{k_2}{V_{CSF}} \cdot INS_I(t) - k_4 \cdot \frac{INS_{CSF}(t)}{V_{CSF}}. \quad (A3)$$

The rate of change of CSF insulin therefore has units of $\mu U/ml \cdot min^{-1}$. In order to minimize the number of parameters in the two
equations, Eq A1 was divided by k_1: let $X = [INS_I](t)/k_1$ ($\mu U/ml \cdot min$), then

$$\frac{d}{dt} X = INS_P(t) - \left(\frac{k_3}{V_I} + k_2\right) \cdot X. \quad (A4)$$

If we assume that $k_3/V_I \gg k_2$, then $k_3/V_I + k_2 \approx k_3/V_I$. Thus, Eq. A4
can be reduced to:

$$\frac{d}{dt} X = INS_P(t) - \frac{k_3}{V_I} \cdot X. \quad (A5)$$

Equation (A3) was therefore revised to include X:

$$\frac{d}{dt} \frac{INS_{CSF}(t)}{V_{CSF}} = \left[\left(\frac{k_2 \cdot k_1}{V_{CSF}}\right) \cdot X\right] - k_4 \cdot \frac{INS_{CSF}(t)}{V_{CSF}}. \quad (A6)$$

The intent of these models was to identify the kinetics of uptake of
the insulin which was infused intravenously. Therefore, the basal val-
ues for insulin in the plasma and CSF were subtracted from all plasma
and CSF insulin levels in each data set, such that for modeling pur-

poses, insulin levels in plasma, CSF and the intermediate compartment were equal to zero at $t = 0$ min. The initial conditions were therefore defined for the CSF curve fit by setting both the value for X and the model-derived CSF value at $t = 0$ min equal to zero for each study. Estimates for the three parameters $(k_3/V_1, [k_2 \cdot k_1]/V_{CSF}, k_4)$ at $t = 0$ min were provided to the M LAB program before modeling the data sets. Given the input of plasma insulin values $[INS_P(t), \mu U/ml]$ and paired CSF insulin values (INS_{CSF}/V_{CSF}) derived from each study, this program generated fitted values of both the CSF insulin concentration $(\mu U/ml)$ and X as a function of time. This was accomplished using an iterative process which simultaneously adjusted the three parameters $[k_3/V_1(min^{-1}), (k_2 \cdot k_1)/V_{CSF}(min^{-2}), and k_4(min^{-1})]$ for the best fit using Eqs. A5 and A6. Since the time course for both the input into the intermediate compartment from the plasma (or first compartment) and output from the intermediate compartment (into the CSF or third compartment) are known, there is a unique solution which includes the identification of the three parameters as well as the internal variable, X; i.e., the model is identifiable (29). The time course of change of X (generated by the modeling program) is directly related to the predicted time course of insulin levels in the intermediate compartment. The calculation of absolute values of insulin levels in this compartment was not performed, since it requires definition of k_1, as well as V_1 and V_{CSF}, none of which were directly determined in these studies.

Appendix II

Statistical method for comparing accuracy of data fits

The statistical method employed for comparing the residual sums of squares from the data fits obtained using the two models was assessed using an F-test. Because the fits were nonlinear and absolute optimums may not have been reached, there was one insulin infusion study where the two-compartment model had a slightly smaller residual sums of squares than the three compartment model. In this case the P value was set to 0.975. Under the null hypothesis that all F-statistics do indeed have the correct distribution, the negative of twice the sum of the natural logarithms of the P values has a χ^2 distribution with degrees of freedom equal to twice the number of experiments summed (30). The P value for this χ^2 statistic was used to assess the need for a three compartment model. This approach assumes both that the asymptotic estimation theory holds and that the "errors" or "deviation from the true curve" are statistically independent. These assumptions will hold only approximately.

Acknowledgments

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