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Research Article

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Saturable Transport of Insulin from Plasma into the Central Nervous System of Dogs In Vivo

A Mechanism for Regulated Insulin Delivery to the Brain

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Abstract

By acting in the central nervous system, circulating insulin may regulate food intake and body weight. We have previously shown that the kinetics of insulin uptake from plasma into cerebrospinal fluid (CSF) can best be explained by passage through an intermediate compartment. To determine if transport kinetics into this compartment were consistent with an insulin receptor-mediated transport process, we subjected overnight fasted, anesthetized dogs to euglycemic intravenous insulin infusions for 90 min over a wide range of plasma insulin levels (69–5,064 μU/ml) (n = 10). Plasma and CSF samples were collected over 8 h for determination of immunoreactive insulin levels, and the kinetics of insulin uptake from plasma into CSF were analyzed using a compartmental model with three components (plasma → intermediate compartment → CSF). By sampling frequently during rapid changes of plasma and CSF insulin levels, we were able to precisely estimate three parameters (average standard deviation 14%) characterizing the uptake of insulin from plasma, through the intermediate compartment and into CSF (k_1k_2); insulin entry into CSF and insulin clearance from the intermediate compartment ($k_2 + k_3$); and insulin clearance from CSF (k_4). At physiologic plasma insulin levels (80±7.4 μU/ml), k_1k_2 was determined to be 10.7 $\times 10^{-6} \pm 1.3 \times 10^{-6}$ min^{-2}. With increasing plasma levels, however, k_1k_2 decreased progressively, being reduced sevenfold at supraphysiologic levels (5,064 μU/ml). The apparent K_M of this saturation curve was 742 μU/ml (~ 5 nM). In contrast, the rate constants for insulin removal from the intermediate compartment and from CSF did not vary with plasma insulin ($k_2 + k_3$ = 0.011±0.0019 min^{-1} and k_4 = 0.046±0.021 min^{-1}). We conclude that delivery of plasma insulin into the central nervous system is saturable, and is likely facilitated by an insulin-receptor mediated transport process. (*J. Clin. Invest.* 1993. 92:1824–1830.) Key words: central nervous system • compartmental model • insulin action • SAAM modeling program

Introduction

Several lines of investigation suggest that circulating insulin levels within the physiologic range exert biologic effects on functioning of the central nervous system (CNS). For example, experimental elevations of plasma insulin to postprandial values suppress feeding behavior (1, 2), an effect also observed during direct insulin infusion into the brain (3). That this occurs despite the extensive network of endothelial tight junctions in cerebral vasculature comprising the blood-brain barrier, which limits diffusion of peptides of this size, suggests that circulating insulin may enter the brain via a facilitated transport process (4). In vitro studies (5, 6) demonstrating that insulin transport across vascular endothelium is dependent on its binding to endothelial insulin receptors have led to the hypothesis that receptor-mediated insulin transport may facilitate the delivery of circulating insulin to target tissues in vivo. Moreover, brain microvascular endothelium expresses insulin receptors (7), and the uptake of plasma insulin into cerebrospinal fluid (CSF)[1] demonstrates specificity when compared to analogues with reduced affinity for the insulin receptor, such as proinsulin (8). These and other studies (9) provide support for the hypothesis that the entry of circulating insulin into the brain is facilitated by an insulin receptor-mediated transport process. However, quantitative measurements of insulin transport kinetics into the CNS have yet to address the possibility of saturable insulin transport.

In our previous work (10), we provided evidence that insulin uptake from plasma into CSF requires passage through an intermediate compartment, and reported on the use of a compartmental model to facilitate the analysis of this uptake process. We now report the quantitation of the dose-response relationship between the plasma insulin level and kinetics of insulin uptake from plasma, through an intermediate compartment, and into CSF. We hypothesized that if insulin receptors, localized to either the blood-brain or blood-CSF barrier, facilitate this uptake process, then the rate constant characterizing CSF insulin uptake should be saturable, decreasing in magnitude with increasing plasma insulin levels. Moreover, the plasma insulin concentration at which half-maximal transport occurs (K_M) should be comparable to the dissociation constant (K_D) of the insulin receptor.

Methods

Study animals and conditions. Six normal adult male mongrel dogs weighing 20–30 kg were studied after an overnight fast. Their care was supervised by a licensed veterinarian. The dogs were housed in individual cages that included a 5 × 20 ft area for exercise and were fed 0.3 kg/d of dry standard dog laboratory diet (Wayne Pro-Mix, Allied

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1. *Abbreviations used in this paper:* CSF, cerebrospinal fluid; IRI, immunoreactive insulin.

Mills, Inc., Memphis, TN) with unlimited access to water. For each study, each dog was anesthetized with thiamylol (Surital, Parke-Davis, Morris Plains, NJ), 20 mg/kg i.v., and was placed on mechanical ventilation, with 1–2% halothane and 40% O_2. Intravenous catheters were placed for both sampling and infusion, and the cisternum magnum was cannulated with a no. 22 gauge spinal needle for sampling of CSF, as reported previously (10). Body weight was measured immediately before each study. All studies were approved by the Animal Care Committee of the Seattle VA Medical Center.

Insulin infusion and sampling. The study protocol consisted of a 90-min i.v. insulin infusion period with sampling of plasma and CSF for a total of 490 min. Intravenous insulin was administered as a 3-min primed infusion (8.5–50 mU/kg-min) commencing at $t = 0$ min, followed by a continuous infusion for 87 min at 20% of the primed infusion rate. Blood samples (1.8 ml) were obtained at $t = -10, -5, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, 25, 30, 35, 40, 65$, and 90 min, at 5-min intervals for $90 \leq t \leq 150$ min, and at 20-min intervals for $150 \leq t \leq 490$ min. CSF samples (0.4 ml) were obtained at $t = -10, -5, 20, 40, 65, 90, 95, 100, 110, 120, 135$, and 150 min and thereafter at 20-min intervals until 490 min. In three studies (P81, P520, and P1195), additional blood and CSF samples were taken at 20-min intervals for $510 \leq t \leq 590$ min. Euglycemia was maintained in all studies by a variable-rate i.v. infusion of 50% dextrose with on-line monitoring of blood glucose levels using 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 −20°C until assay. Plasma levels of immunoreactive insulin (IRI) were measured in duplicate by radioimmunoassay using a modification of the double-antibody method (11). CSF IRI was measured in triplicate using a further modification of this method which enhances its sensitivity (8, 12). Plasma glucose was determined by the glucose oxidase method with a glucose autoanalyzer (Beckman Instruments, Inc., Brea, CA). The average plasma insulin concentration during infusion, P_{ave}, was determined by taking the average of plasma insulin values during the interval $1 \leq t \leq 90$ min. Two dogs were studied once at either a low or high insulin dose, and four were studied twice, at both a low and high insulin dose. Thus, 10 independent data sets were obtained.

Multicompartmental model. A schematic illustration of the multicompartment model used to interpret the data is shown in Fig. 1; this model was previously described in Schwartz et al. (10). Because of the delay in the appearance of insulin in the CSF after intravenous insulin infusion, insulin entry from plasma into CSF can best be explained by passage through an intermediate compartment, $INS_I(t)$. Insulin in plasma, $INS_P(t)$, enters CSF, $INS_C(t)$, after passing through $INS_I(t)$, and irreversible loss can occur from both $INS_C(t)$ and $INS_I(t)$. $INS_P(t)$ provides a functional description of the plasma data providing input to the model. By assuming that flux of insulin from the CNS back into plasma has a negligible influence on the plasma concentration, the need to postulate a structure for the kinetics of insulin outside of the brain is eliminated.

In this model, k_1 represents the uptake of plasma insulin into the intermediate compartment, and k_2 represents the movement of insulin from the intermediate compartment into CSF. k_3 represents insulin clearance from the intermediate compartment, and k_4 represents insulin clearance from CSF. All rate constants are expressed in min^{-1}.

Using this model, the rates of change of exogenously administered insulin in the intermediate compartment, $INS_I(t)$, and of CSF insulin, $INS_C(t)$, are described by the following differential equations:

$$\frac{d}{dt} INS_I(t) = k_1 INS_P(t) - (k_2 + k_3) INS_I(t) \quad (1)$$

$$\frac{d}{dt} INS_C(t) = k_2 INS_I(t) - k_4 INS_C(t) \quad (2)$$

$$INS_P(0) = INS_I(0) = INS_C(0) = 0 \quad (3)$$

All insulin variables, $INS_x(t)$, are expressed in concentration units of microunits per milliliter. To obtain the initial conditions of Eq. 3, basal plasma and CSF levels were subtracted from all values of each data set before modeling, such that for modeling purposes the insulin concentration in each compartment was set equal to zero at $t = 0$ min. Therefore, only the kinetics of infused insulin were analyzed, and it was not necessary to assume that at the basal state all CSF IRI must be derived from plasma. Because the intermediate compartment was not directly sampled, only the rate constant characterizing insulin clearance from CSF, k_4, can be identified independently, whereas k_1, k_2, and k_3 are not independently identifiable. Instead, the product k_1k_2, which represents the throughput of insulin from plasma, through the intermediate compartment, and subsequently into CSF, and sum $(k_2 + k_3)$, which represents insulin flux from the intermediate compartment into CSF as well as insulin clearance from the intermediate compartment, were estimated. A detailed derivation of unique parameter identification is provided in the Appendix.

Parameter identification. By using the mathematical modeling program SAAM (13) on a model 486 computer (Gateway 2000, Gateway, Sioux City, SD), the rate constants k_1k_2 (min^{-2}), $(k_2 + k_3)$ (min^{-1}), and k_4 (min^{-1}) were estimated from the plasma and CSF insulin data. To reduce the number of samples and therefore total fluid volumes required per study, only CSF insulin data points taken during $t \geq 90$ min were used for modeling. It can be shown that as long as enough plasma and CSF samples are taken and steady state is reached, the same parameters will be obtained from CSF samples taken at $0 \leq t \leq 3$, $3 \leq t \leq 90$, or $t \geq 90$ min (14). The intraassay errors for the plasma and CSF insulin radioimmunoassays were assessed by measuring eight samples of various insulin concentrations (data not shown). These error estimates (plasma insulin assay 4% for concentrations < 80 μU/ml, 12% otherwise; CSF insulin assay 11%) were used by SAAM to determine the optimal curve fit and to identify the rate constant parameters. For each parameter, the corresponding coefficient of variation (CV), also known as the fractional standard deviation, was determined from the covariance matrix generated by SAAM.

Independent estimation of the CSF insulin clearance parameter, k_4. To obtain an independent estimate of k_4, the CSF insulin clearance parameter, insulin (750 μU in 750 μl of saline) was infused over 1 min into the cisternum magnum of three dogs which had been fasted overnight, anesthetized, and maintained on mechanical ventilation. Immediately before the infusion was administered, 750 μl of CSF was removed from the cisternum magnum, in order to preserve the original volume of CSF during the infusion. CSF samples (0.4 ml) were obtained at $t = -10, -5, 2, 5, 10, 15, 25$, and 40 min, and thereafter at 20-min intervals till 240 min. The resulting CSF levels decayed biexponentially, and were fitted to a two-compartment model. This two-compartment analysis has previously been used to model transport kinetics of substances administered intracerebroventricularly, with one compartment representing CSF and the other representing brain interstitium (15, 16). The CSF clearance rate was calculated by dividing the estimated bolus concentration administered by the area under the curve of the two exponentials (17).

Estimation of K_M, the plasma insulin concentration at which half-maximal transport occurs. To estimate K_M, the plasma insulin concentration at which half-maximal transport occurs, the transport velocity for various plasma insulin concentrations was required. Velocity is determined by multiplying k_1, the rate constant representing uptake of plasma insulin into the intermediate compartment, by the corresponding plasma insulin concentration during infusion, P_{ave}. As k_1 could not be uniquely identified, the product k_1k_2, which estimated k_1, was used for this calculation. Therefore, velocity$*k_2$, which is directly proportional to the rate of insulin uptake from plasma into CSF, was instead calculated. Velocity$*k_2$ due to nonspecific uptake is linear, such that at high plasma insulin levels, velocity$*k_2$ does not plateau. As this linear component is readily apparent at high plasma insulin levels, velocity$*k_2$ due to nonspecific uptake was estimated through graphical analysis as $2 \times 10^{-7} * P_{ave}$. This estimate was subtracted from velocity$*k_2$ to isolate velocity$*k_2$ due to saturable uptake (18). Velocity$*k_2$ was then plotted as a function of P_{ave}. K_M was calculated by using a variation of the Michaelis-Menten equation,

$$\text{Velocity} * k_2 = \frac{V_{MAX} k_2 P_{ave}}{P_{ave} + K_M}, \quad (4)$$

to fit the resulting curve. In this equation, V_{MAX} = maximum velocity.

Results

The 90-min intravenous insulin infusion protocol resulted in a characteristic time course of change in CSF IRI levels, illustrated in Fig. 2. During i.v. insulin infusion, CSF insulin levels rose gradually, reaching peak values at 105 min (15 min after discontinuation of infusion), and fell exponentially thereafter. The estimated parameters and corresponding coefficients of variation for each study are given in Table I. The mean CV for all estimated parameters was 14%.

The data in Table I demonstrate that studies conducted at low plasma insulin levels are associated with relatively high values of k_1k_2. This was evident when examining all dogs as a group, as well as when comparing results obtained in separate studies at two different plasma levels. Examining data obtained from two studies in individual dogs, the value of k_1k_2 was higher in studies conducted at lower plasma insulin levels in all cases, whereas no consistent relationship between either ($k_2 + k_3$) or k_4 and the plasma level was evident.

To further characterize these relationships, k_1k_2, ($k_2 + k_3$), and k_4 were plotted as a function of the average plasma insulin concentration during infusion, P_{ave}, to determine dose-response relationships. As shown in Fig. 3, the parameter characterizing insulin uptake from plasma into CSF varied inversely with plasma insulin levels, being reduced sevenfold from physiologic to supraphysiologic levels. The apparent K_M for this transport process was determined from a Michaelis-Menten dose-response curve based on the data in Table I. As shown in Fig. 4, the apparent K_M was approximated to be 742 μU/ml ($\sim$ 5 nM). In contrast, the mean rate constant values for insulin removal from the intermediate compartment ($k_2 + k_3$) and insulin clearance from CSF (k_4) did not appear to vary with plasma insulin ($k_2 + k_3$ = 0.011±0.0019 min^{-1} and k_4 = 0.046±0.021 min^{-1}) (Fig. 5). Therefore, delivery of circulating insulin into the intermediate compartment and subsequently into CSF demonstrates saturable kinetics, whereas insulin clearance from the intermediate compartment does not. Data are insufficient to assess the possibility that a saturable process also contributes to CSF insulin clearance.

To validate the model-derived parameter representing CSF insulin clearance, the mean CSF clearance rate constant was also determined independently by modeling data from intracisternal insulin injection (n = 3). The intracisternal insulin injection protocol resulted in a characteristic time course of change in CSF IRI levels, illustrated in Fig. 6. The mean CSF insulin clearance rate determined from these studies was 0.028 ± 0.0080 min^{-1}, which is within the error of the mean value of k_4 determined during i.v. insulin infusion (Fig. 7). These results validate the CSF insulin clearance parameter identified during intravenous insulin infusion.

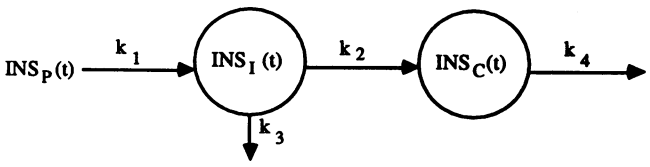

Figure 1. Schematic illustration of the pharmacokinetic model used to analyze the kinetics of insulin uptake from plasma into CSF in the dog. In this model, k_1 represents the uptake of plasma insulin into the intermediate compartment, hypothesized to occur across the blood-brain barrier, and k_2 represents the transport of insulin from the intermediate compartment into CSF. k_3 represents insulin clearance from the intermediate compartment, and k_4 represents insulin clearance from CSF. All rate constants are expressed in min^{-1}. Modified from Schwartz et al. (10).

Table I. Identified Parameters from Optimal Curve Fits of Data from Nine studies in Dogs of Various Insulin Doses

Study	P_{ave}	k_1k_2	$k_2 + k_3$	k_4
	μU/ml	min^{-2}	min^{-1}	
P69	69	10.2e-6	0.0086	0.060
		(14%)	(11%)	(16%)
P81	81	9.1e-6	0.014	0.043
		(14%)	(28%)	(16%)
P83	83	11.5e-6	0.011	0.10
		(16%)	(8%)	(19%)
P86	86	12.0e-6	0.012	0.047
		(10%)	(19%)	(23%)
P520	520	6.7e-6	0.011	0.026
		(4%)	(5%)	(8%)
P989	989	7.6e-6	0.0095	0.035
		(12%)	(18%)	(22%)
P1195	1195	3.8e-6	0.011	0.034
		(16%)	(24%)	(22%)
P1203	1203	3.1e-6	0.012	0.032
		(10%)	(11%)	(19%)
P2076	2076	3.4e-6	0.011	0.042
		(13%)	(12%)	(18%)
P5064	5064	1.6e-6	0.0073	0.042
		(8%)	(6%)	(12%)

In studies P81 and P1194, plasma insulin samples were not taken at t = 1, 2, 3, 4, 6, 8, 10, 13, and 16 min, which resulted in higher coefficients of variation than in studies in which these samples were obtained. Studies conducted in the same dog were P69 and P989, P81 and P1203, P520 and P1194, and P86 and P5064.

Discussion

In the current studies, we analyzed the kinetics of CSF insulin uptake from plasma using a previously presented multicompartmental model (10). Our results indicate that the efficiency with which circulating insulin enters CSF via passage through an intermediate compartment varies inversely with the plasma insulin concentration. These data support the hypothesis that insulin entry into the CNS from plasma is facilitated by a saturable transport process. As the efficiency of uptake into the CNS decreases sevenfold with increasing plasma insulin concentration, we estimate that 85% of this uptake is mediated by a saturable process at physiologic plasma insulin levels (< 100 μU/ml). The apparent K_M of this transport process was 742 μU/ml ($\sim$ 5 nM), which is similar to the K_D of the insulin receptor identified in brain microvasculature (2 nM) (19). Therefore, data are consistent with the hypothesis that insulin receptors facilitate transport of plasma insulin into the central nervous system.

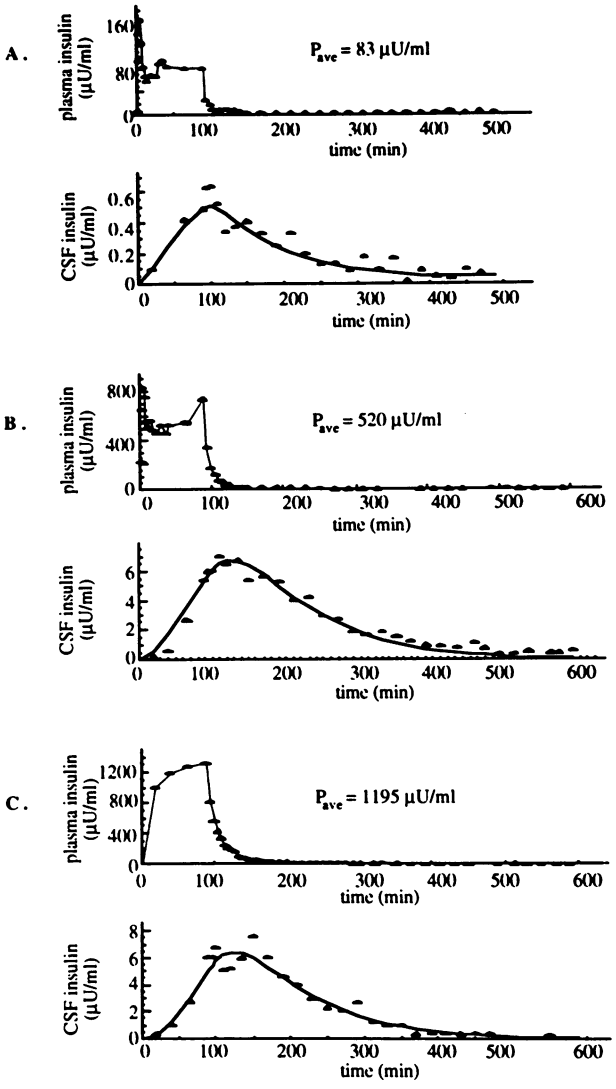

Figure 2. Representative studies in which the kinetics of CSF uptake of intravenously infused insulin were determined. Insulin was infused during $0 \leq t \leq 90$ min. Studies shown are representative of low (*A*), medium (*B*), and high (*C*) insulin doses. *Upper panels:* time course of plasma insulin. *Lower panels:* time course of CSF insulin and optimal curve fit, from which rate constant parameters were derived.

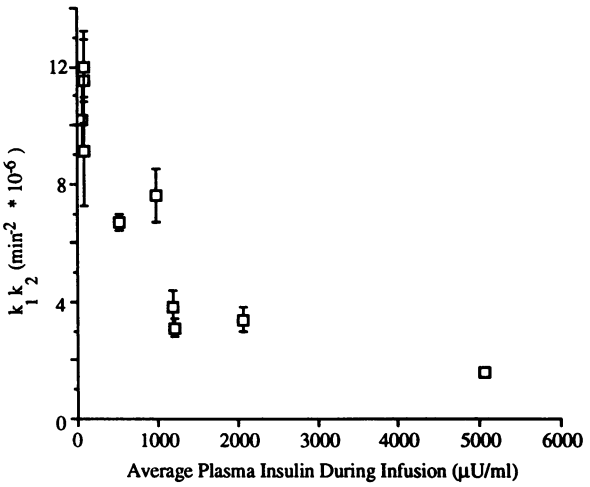

Figure 3. Dose-response curve illustrating the relationship between the rate of uptake of plasma insulin into the intermediate compartment and subsequently into CSF. $k_1 k_2$ decreases sevenfold with increasing plasma insulin levels from the physiologic to supraphysiologic range during intravenous insulin infusion. Each error bar represents the fractional standard deviation associated with the identified parameter value.

Two barrier systems exist in the central nervous system which regulate solute movement between the blood and CNS. The first of these is the blood-brain barrier, which arises from epithelial-like tight junctions that virtually cement adjoining capillary endothelium together in the brain microvasculature. Similarly, the blood-CSF barrier arises from tight junctions between adjacent epithelial cells investing the choroid plexus, the primary site of CSF formation. In general, most solutes gain access from blood to CSF via the blood-CSF barrier at the choroid plexus. Transport of solutes such as Na^+ occurs directly from blood to CSF, and does not necessitate postulating transport through an intermediate compartment (16). Thus, while insulin may gain access to CSF via either of the CNS barrier systems, the observation that plasma insulin passes through an intermediate compartment en route to CSF attests not only to the uniqueness with which insulin enters the CSF, but suggests that a barrier to diffusion of insulin exists across the choroid plexus. One hypothesis to account for this observation is that insulin entering the choroid plexus is sequestered by choroidal insulin receptors, which are expressed at high concentrations, preventing its diffusion into CSF, and are possibly responsible for degrading insulin following binding, as occurs in the placenta (20, 21).

The intermediate compartment through which insulin passes en route to CSF likely represents either brain ISF or choroid plexus epithelium. Although the identity of this compartment can not be established without undertaking the difficult task of sampling the compartment itself, data are available which indirectly address these two possibilities. The observation that insulin concentrations in rat brain extracts are ~ 30–

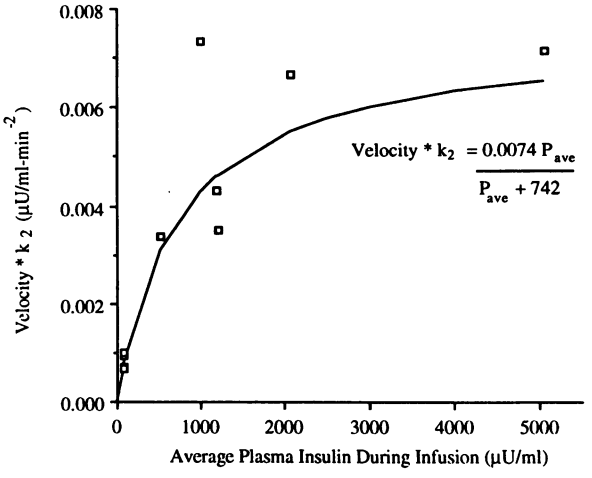

Figure 4. Michaelis-Menten curve, based on calculated $k_1 k_2$, the rate of insulin uptake from plasma into intermediate compartment and subsequently into CSF, for various insulin doses. The apparent K_M, the concentration during which half maximal transport occurs, is 742 μU/ml (~ 5 nM).

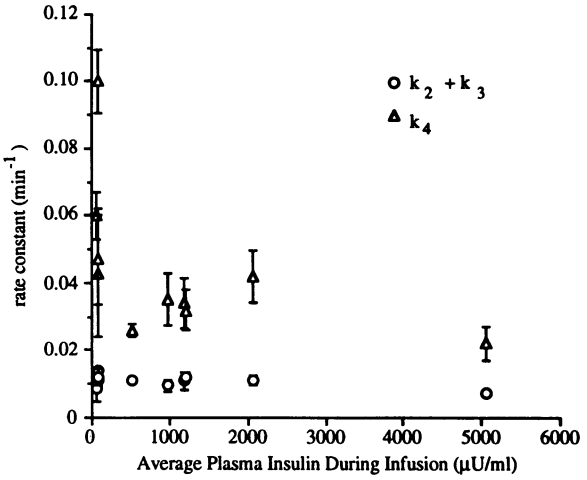

Figure 5. Dose-response curves illustrating how rates of insulin transport into CSF and insulin clearance from intermediate compartment ($k_2 + k_3$) and rate of CSF insulin clearance (k_4) vary with average plasma insulin level during infusion. Each error bar represents the fractional standard deviation associated with the identified parameter value.

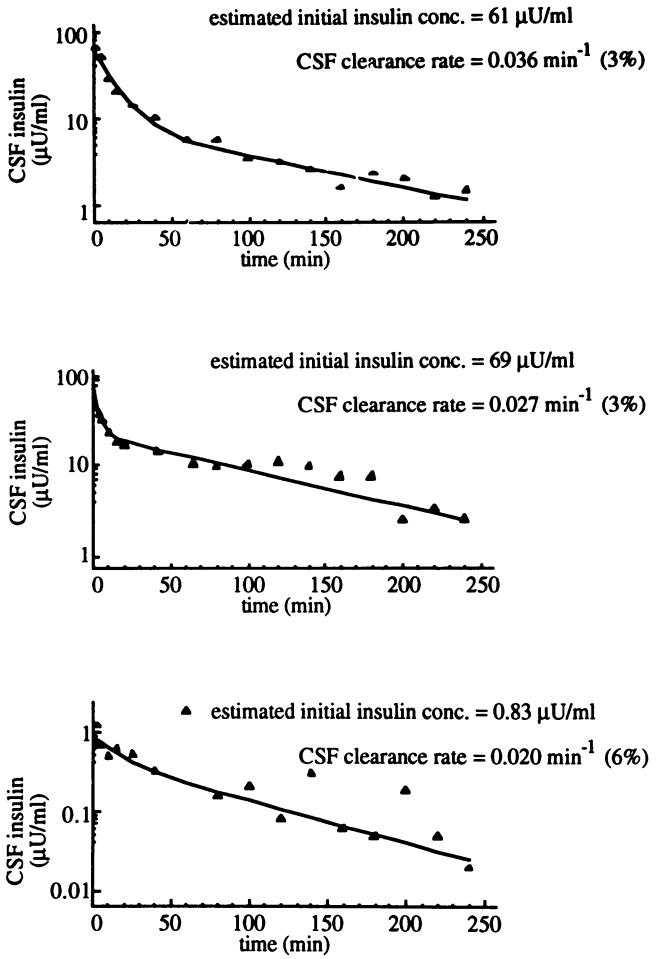

Figure 6. Studies in which the CSF clearance of intracerebroventricularly injected insulin was determined. Insulin was administered as a 1-min infusion at $t = 0$ min. Each percentage in parentheses is the fractional standard deviation associated with the identified parameter value. The average CSF insulin clearance rate for these studies was 0.028±0.0080 min^{-1}.

40% of plasma levels (22), whereas CSF levels are < 5% of plasma levels, for example, suggests that insulin uptake occurs preferentially across the blood-brain barrier, rather than the blood-CSF barrier. Moreover, blood-brain barrier endothelium expresses insulin receptors (4, 23), and after intracarotid injection of radiolabeled insulin, penetration of insulin tracer into brain parenchyma was inhibited by coadministration of excess insulin in newborn rabbits (9), suggesting a saturable transport process within the blood-brain barrier. Taken together, these data support the hypothesis that a saturable transport system operates at the level of the blood-brain barrier to promote the delivery of circulating insulin to target sites in the brain. Analogous blood-brain barrier transport systems have been described for glucose (24), amino acids (25), plasma proteins such as transferrin (26), and peptides such as enkephalins (27). If transport across the blood-brain barrier represents the major route by which circulating insulin enters the CNS, as suggested by these observations, then the intermediate compartment identified in our studies may represent brain ISF.

Alternatively, the intermediate compartment could represent the intracellular space of the choroid plexus epithelium. As noted above, the choroid plexus expresses insulin receptors at high levels, and likely concentrates circulating insulin within the blood-CSF barrier. Having entered the intracellular space of the choroidal epithelium, insulin in this compartment could subsequently be released into CSF. However, the dynamics of CSF insulin uptake are qualitatively similar whether sampled from lumbar sac in man (28) or cisterna magna in dogs (8, 10), suggesting that the choroid is unlikely to represent the major source of insulin in CSF. Further, the teleologic rationale for a specialized transport system for insulin across the choroid into CSF is unclear, since this uptake results in relatively low CSF insulin levels, and recent studies have established that infusion of insulin into CSF is an inefficient means for its delivery into the brain. For example, 100–1,000-fold greater CSF infusion rates are required to achieve effects on food intake comparable to those resulting from direct intrahypothalamic insulin delivery (29). For these reasons, we favor the hypothesis that brain ISF represents the primary compartment into which circulating insulin enters within the central nervous system, with subsequent movement into CSF. Further studies are required to confirm this possibility.

Insulin exerts a variety of actions within the CNS (see reference 23 for review), including behavioral responses (i.e., sup-

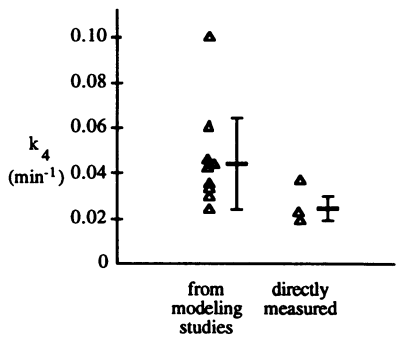

Figure 7. Range of estimated k_4, the CSF insulin clearance rate constant, by two methods: derived from modeling CSF insulin changes in response to an intravenous insulin infusion (mean value 0.046±0.021 min^{-1}), and directly estimated from an intracerebroventricular insulin bolus injection (mean value 0.028±0.0080 min^{-1}). Each error bar represents the fractional standard deviation associated with the mean parameter value.

pression of food intake), regulation of neuropeptide biosynthesis and catecholamine metabolism, and activation of the sympathetic nervous system. Collectively, these central actions promote a state of negative energy balance, favoring weight loss. These observations have led to the hypothesis that circulating insulin acts in the brain as a negative feedback signal proportional to body adipose mass in the process of long-term body weight regulation (30, 31). From the available data, we believe that plasma insulin is delivered to brain interstitial fluid at least in part via an insulin receptor-mediated transcytotic process. As most insulin-receptor mediated actions are subject to a variety of regulatory influences, it is possible that this insulin transport process may also be regulated, which would be unlikely were transport dependent solely on nonsaturable mechanisms such as diffusion. Theoretically, regulation of CNS insulin transport could alter the efficiency with which circulating insulin gains access to target sites in the brain, and could thus represent an important determinant of the level about which body weight is maintained.

Receptor-mediated insulin transport from vasculature to tissue interstitium may regulate processes other than body weight. The movement of insulin from blood into the interstitium of tissues is an important determinant of the rate of insulin action on carbohydrate utilization in vivo. Yang et al. (32) have shown a strong correlation between the dynamics of thoracic duct lymph insulin, which is derived from interstitial fluid, and insulin mediated glucose utilization. Further, lymph insulin is proportional to glucose utilization within the physiologic but not pharmacologic range of insulin, indicating that transcapillary insulin transport is rate-limiting for insulin action in this range (33). Thus, the movement of insulin from vasculature to interstitium is a critical step in determining tissue sensitivity to insulin, and it is possible that defects in transport may contribute to the pathogenesis of insulin resistance in conditions such as non-insulin-dependent diabetes mellitus and obesity. It should be stressed, however, that while we have demonstrated saturable uptake of insulin into CSF, studies have yet to establish a saturable mechanism of insulin transport into peripheral interstitial fluid. It is possible that mechanisms operating within brain vasculature differ from those of other tissues where insulin uptake is concerned.

It should be noted that the estimated k_1k_2 values in the supraphysiologic range of plasma insulin levels in the current studies are about half those calculated previously (10). These differences are probably attributable to modified plasma and CSF sampling schedules, which have improved parameter estimates. Specifically, the number of samples taken during rapid changes of plasma and CSF insulin levels was increased. This change of sampling times reduced the variability of estimated parameters, compared to those generated using conventional sampling procedures (34). In our previous work, estimated parameters often had associated coefficients of variation > 100%. In contrast, the mean coefficient of variation for 30 identified parameters in the current studies is 14%. In both cases, the true parameter value was located within the coefficient of variation of each estimate. However, because the coefficients of variation have been reduced in the current studies, the mean estimates are now much closer to the true parameters than in the previous work.

In the current studies, we measured k_4, the rate of insulin clearance from CSF, to be 0.046 ± 0.021 min^{-1}. This clearance rate was independently verified by intracerebroventricular administration of insulin, and is much higher than the rate of bulk flow CSF clearance in the dog (0.0025 ± 0.0003 min^{-1}), as determined from previous inulin studies (10, 15, 16). This large disparity between CSF clearance rates suggests that mechanisms other than bulk flow contribute to insulin removal from CSF. One possibility is that insulin receptors on the choroid plexus bind and remove/degrade insulin in CSF, as suggested by Manin et al. (35). While this would imply a saturable mechanism for CSF insulin removal, saturability would not be demonstrable at such low CSF insulin levels as occurred in our studies (< 10 μU/ml). Thus, while CSF insulin removal may involve a saturable mechanism, further study is required to address this possibility.

In summary, we have demonstrated that uptake of plasma insulin into the central nervous system is saturable. This mechanism is consistent with insulin binding to blood-brain barrier and/or blood-CSF insulin receptors and subsequent transcytosis into the central nervous system.

Appendix

To determine if model parameters are uniquely identifiable, the system impulse response, that is, the cause-effect relationship between input and output, is analyzed. For this system, the CSF impulse response is:

$$\mathrm{INS_C}(t) = \frac{k_1k_2}{k_4 - k_2 - k_3}\left[e^{-k_4t} - e^{-(k_2+k_3)t}\right] \quad (5)$$

For theoretical, or a priori, identifiability, all the rate constants must be uniquely identified from Eq. 4. For the function $D(e^{-\alpha t} - e^{-\beta t})$, D, α, and β can be determined from a semilog plot of the natural log of this function versus time. Therefore, k_4 can be identified, as can the quantity $(k_2 + k_3)$. These three constants make up the denominator of the terms multiplying the exponentials, so the numerator (k_1k_2) can also be identified. In order to uniquely identify k_1, k_2, and k_3, more information is needed (36). Therefore, this model is not theoretically identifiable.

Eqs. 1 and 2 can be rearranged to simplify parameter identification by dividing both sides of Eq. 1 by k_1, subtracting and adding the same term to Eq. 1, and rewriting Eq. 2 in terms of $\mathrm{INS_I}(t)/k_1$:

$$\frac{\mathrm{d}\,\mathrm{INS_I}(t)}{\mathrm{d}t\quad k_1} = \mathrm{INS_P}(t) - \frac{\mathrm{INS_I}(t)}{k_1}(k_2 + k_3 - k_1k_2 + k_1k_2) \quad (6)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{INS_C}(t) = k_2 \cdot k_1\frac{\mathrm{INS_I}(t)}{k_1} - k_4\mathrm{INS_C}(t) \quad (7).$$

These equations are realized in the rearranged model of Fig. 8.

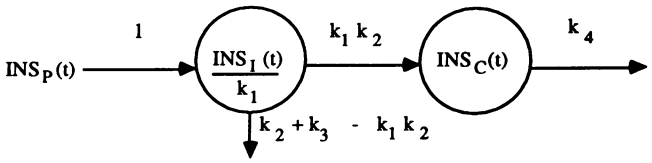

Figure 8. Schematic illustration of the rearranged pharmacokinetic model used to analyze the kinetics of insulin uptake from plasma into CSF.

The parameters identified with this model are k_1k_2, $(k_2 + k_3 - k_1k_2)$, and k_4. Once k_1k_2 and $(k_2 + k_3 - k_1k_2)$ are determined, $(k_2 + k_3)$ is also known.

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