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Measurement of local cerebral protein synthesis *in vivo*: Influence of recycling of amino acids derived from protein degradation

(aminoacyl-tRNA/amino acid recycling/brain/liver)

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ABSTRACT **A quantitative autoradiographic method for the determination of local rates of protein synthesis in brain *in vivo* is being developed. The method employs L-[1-^{14}C]leucine as the radiolabeled tracer. A comprehensive model has been designed that takes into account intracellular and extracellular spaces, intracellular compartmentation of leucine, and the possibility of recycling of unlabeled leucine derived from steady-state degradation of protein into the precursor pool for protein synthesis. We have evaluated the degree of recycling by measuring the ratio of the steady-state precursor pool distribution space for labeled leucine to that of unlabeled leucine. The values obtained were 0.58 in whole brain and 0.47 in liver. These results indicate that there is significant recycling of unlabeled amino acids derived from steady-state protein degradation in both tissues. Any method for the determination of rates of cerebral protein synthesis *in vivo* with labeled tracers that depends on estimation of precursor pool specific activity in tissue from measurements in plasma must take this recycling into account.**

The autoradiographic deoxyglucose method (1) for measurement of local cerebral glucose utilization provides a useful model for the design of methods to determine local rates of biochemical processes within tissues *in vivo*. We have used the same general approach to develop a method for the quantitative determination of local rates of protein synthesis, or, more precisely, amino acid incorporation into protein, in the nervous system (2, 3). The method is an application of the basic principles for the assay of rates of chemical reactions with isotopes adapted for the special conditions encountered *in vivo* (4). Briefly, the total amount of labeled product specific to the reaction of interest (in this case labeled protein) formed in the selected tissue over an interval of time is divided by the integrated specific activity (SA) of the precursor pool over the same interval and corrected for any isotope effect (4). This relationship can be expressed as follows:

$$R_i = \frac{P_i^*(T)}{\Phi \int_0^T (C_{pp}^*/C_{pp})(t)\,dt}, \qquad [1]$$

where R_i equals rate of the overall reaction in tissue i, P_i^* is amount of labeled product formed in tissue i between zero time and time T, $(C_{pp}^*/C_{pp})(t)$ is the ratio of the concentration of labeled tracer to that of the endogenous unlabeled substrate (i.e., SA) in the precursor pool at any time t, and Φ is a correction for any isotope effect. If there is no isotope effect, $\Phi = 1$.

The application of these principles to *in vivo* conditions is complicated, however, because the necessary variables readily measured directly *in vitro* must be determined indirectly *in vivo*. Quantitative autoradiography, for example, is used to achieve spatial localization of the label in the tissues, but it cannot distinguish between radiochemical species. It measures only total concentration of isotope and not the concentrations of the specific products of the reaction of interest. Unreacted labeled precursor molecules and labeled products of other biochemical reactions may also be present. In addition, some of the labeled products of the specific reaction under study may be removed from the tissue by the blood flow during the experimental period. Procedures must, therefore, be designed to avoid or correct for loss of specific product and presence of other labeled compounds. Another problem is the determination of the integrated SA of the precursor pool. Its direct determination would require measurement of the complete time courses of the concentrations of tracer and endogenous precursor compounds in each tissue of interest. This is obviously impossible in a conscious behaving animal. Instead, the time course of the SA in blood or plasma is measured, and correction is made for the effects of the lag of the tissue behind the blood or plasma and possible differences in the distribution spaces for the labeled and unlabeled precursors.

In our initial approach we attempted to deal with these problems in the design of the kinetic model and the experimental procedures (2, 3). Radioactive products of extraneous biochemical reactions were avoided by selection of L-[1-^{14}C]leucine as the labeled precursor. Carboxyl-labeled leucine, like other aliphatic, branched-chain amino acids, is either incorporated into protein or metabolized by only one pathway beginning with transamination rapidly followed by decarboxylation (5). Metabolized [1-^{14}C]leucine loses its label to $^{14}CO_2$, which is rapidly removed from brain by the circulation. Reincorporation of label from $^{14}CO_2$ is negligible because of its dilution with the CO_2 produced by glucose metabolism in brain. Therefore, ^{14}C remaining in the tissue exists only in labeled protein or free leucine. The administration of [1-^{14}C]leucine as an i.v. pulse followed by a long experimental period (e.g., 60 min) allows most of the free [^{14}C]leucine to be cleared from the tissue by its incorporation into protein, metabolism, or transport back to plasma. Residual free [^{14}C]leucine was experimentally removed by fixing the tissue sections in formalin before autoradiography (6). The long experimental period after the pulse served also to minimize the effects of the lag of tissue behind plasma on the estimation of the integrated SA of [^{14}C]leucine in the precursor pool.

Our initial model (2, 3) was based on these assumptions. (*i*) The tissue region i is homogeneous with respect to concentrations of amino acids, rates of blood flow, and rates of transport, metabolism, and incorporation of amino acids into protein. (*ii*) The concentration of unlabeled leucine in arterial plasma and rates of amino acid metabolism and protein synthesis are constant during the experimental period. (*iii*) There is no significant loss of label and no recycling of

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Abbreviations: SA, specific activity; Dns-, dansyl-, dimethylaminonaphthalene-1-sulfonyl-.

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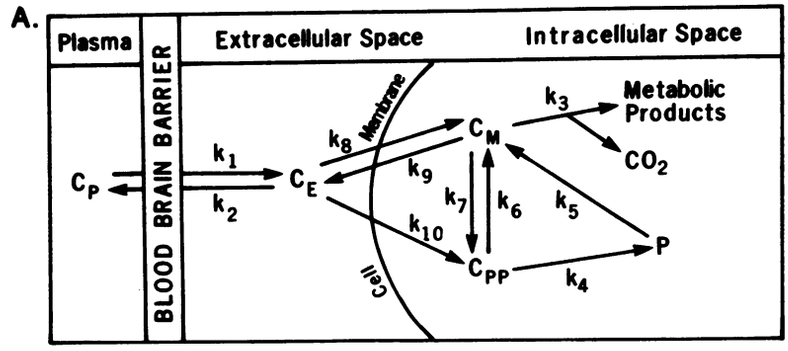
[^{14}C]leucine from the radioactive protein within an experimental period of 60 min because the average half-life of proteins in brain is several days (7). (*iv*) Concentrations of labeled leucine are sufficiently small that tracer theory holds, and there are no isotope effects associated with its use. (*v*) Labeled and unlabeled leucine enters the precursor pool for protein synthesis from the plasma (i.e., no separate extracellular and intracellular spaces). (*vi*) Unlabeled leucine derived from protein degradation does not recycle into the precursor pool for protein synthesis but is sequestered in a separate intracellular pool from which it is removed by either metabolism or transport back to plasma. This assumption was based on evidence from studies of isolated cells and tissues *in vitro* (8–10) and of intracellularly injected neurons in *Aplysia* (11), indicating that the source of essential amino acids for incorporation into protein is extracellular.

This early model obviously oversimplified events in tissues *in vivo*. Although *in vitro* studies suggested no recycling of unlabeled leucine derived from protein degradation, they did not rule out the possibility that recycling might occur *in vivo* by way of the extracellular space. The model was, therefore, expanded to include intracellular and extracellular spaces, intracellular compartmentation of free leucine, and admixture of unlabeled leucine released by protein breakdown with amino acids entering the tissue from plasma. A method was developed and applied in brain and liver to measure the degree of admixture. The results show that there is sufficient admixture to dilute the SA of the leucine entering the precursor pool from plasma by about 40% in brain and 50% in liver. Methods that depend on estimation of precursor pool SA from measurements in plasma to determine rates of cerebral amino acid incorporation into protein *in vivo* will underestimate the true rates unless this dilution is taken into account. Portions of this work have been presented in preliminary form (12).

THEORY

Although amino acids derived from protein breakdown might not mix with amino acids coming from extracellular sources within the cell *in vitro*, admixture could occur in the extracellular space *in vivo*. Our original model was, therefore, expanded; the first four assumptions were retained and the last two were discarded. Extracellular space, intracellular compartmentation of amino acids, and recycling of unlabeled but not labeled amino acids into the precursor pool were included (Fig. 1*A*). The equation for calculation of rates of protein synthesis (Fig. 1*B*) that was derived from this four-compartment model is more complex than the one from the earlier model (3), but its form is essentially the same. A constant, λ (Fig. 1*B*), is included to correct for possible differences in sources of input of labeled and unlabeled leucine to the precursor pool. λ equals the steady-state distribution ratio of the labeled leucine between precursor pool and plasma divided by the corresponding ratio for unlabeled leucine. This constant is necessary to estimate precursor pool SA in tissue from measured SA of leucine in plasma. If neither labeled nor unlabeled leucine derived from protein breakdown recycles and if there are no isotope effects, then $\lambda = 1.0$. Our comprehensive model includes the possibility of recycling of unlabeled but not labeled leucine from protein breakdown; in this case, λ would not equal 1.0 and must appear explicitly in the equation.

Experimental procedures can be designed to simplify the equation in Fig. 1*B*. The numerator of the equation is equivalent to P_i^*, the concentration of labeled leucine incorporated into protein in tissue i during the experimental period. Quantitative autoradiography measures C_i^*, the total concentration of label in the tissue, from which the concentrations of label remaining in the intracellular and extracellular leucine pools and in leucyl-tRNA (i.e., C_M^*, C_E^*, and C_{pp}^*, respectively, Fig. 1) must be subtracted to obtain P_i^*. There are no other labeled compounds because [1-^{14}C]leucine loses its label to $^{14}CO_2$ during its metabolism. Fixation of the brain sections in formalin prior to autoradiography washes out the free amino acid pools (i.e., C_M^* and C_E^*), and because the pool of tRNA-bound [^{14}C]leucine (C_{pp}^*) is so small compared to labeled protein (P_i^*) 60 min after a pulse of [1-^{14}C]leucine, its contribution to C_i^* is negligible. Therefore,



B.

$$R_i = \frac{C_i^*(T) - C_E^*(T) - C_M^*(T) - C_{pp}^*(T)}{\lambda\left[\int_0^T \frac{C_p^*(t)}{C_p}\,dt - \sum_{j=1}^{3} A_j \int_0^T \frac{C_p^*(t)}{C_p} e^{-\alpha_j(T-t)}\,dt\right]}$$

where λ is a constant, $0 < \lambda \leq 1$.

If $k_5P^* = 0$ and $k_5P = 0$, $\lambda = 1$.

If $k_5P^* = 0$ and $k_5P \neq 0$,

$$\lambda = \frac{(k_4 + k_6)[k_2k_9 + k_3(k_2 + k_8 + k_{10})]}{(k_4 + k_6)[k_2k_9 + k_3(k_2 + k_8 + k_{10})] + k_4[k_7(k_2 + k_8 + k_{10}) + k_9k_{10}]}$$

FIG. 1. (*A*) Diagrammatic representation of the comprehensive model of the behavior of leucine in brain. (*B*) Equation to calculate rates of protein synthesis derived from this model. In the model (*A*), C_p, C_E, C_M, and C_{pp} represent concentrations of free leucine (either labeled or unlabeled) in plasma, extracellular space, intracellular metabolic pool, and intracellular precursor pool for protein synthesis, respectively. *P* represents the total pool of leucine in cellular protein. The constants, k_1–k_{10}, inclusive, represent the rate constants for carrier-mediated transport of leucine from plasma to tissue, for transport back from tissue to plasma, for metabolic degradation of leucine, for leucine incorporation into protein, for release of free leucine by protein degradation, for deacylation of leucyl-tRNA, for transfer of leucine from the intracellular metabolic leucine pool to precursor pool for protein synthesis, for transport of leucine from extracellular space to intracellular metabolic leucine pool, for transport of leucine from intracellular metabolic pool to extracellular space, and for transport of leucine from extracellular space to intracellular precursor pool for protein synthesis, respectively. In the equation in *B*, C_p^* is the concentration of labeled leucine in the arterial plasma; R_i is the rate of leucine incorporation into protein in tissue i; C_E^*, C_M^*, C_{pp}^*, and P^* represent the concentrations in tissue i of the labeled species in the compartments defined in the model (*A*); C_i^* represents the total ^{14}C concentration in tissue i; A_j and α_j are constants composed of various combinations of the rate constants, and λ is the ratio of the precursor pool distribution space for labeled leucine in the tissue to that of unlabeled leucine.

under these conditions, $C_i^*(T) \approx P_i^*(T)$ and can serve as the numerator of the equation. The denominator of the equation represents the integrated SA of leucine in the precursor pool. It equals the product of λ and the difference between the integrated SA of leucine in arterial plasma and a series of convolution integrals that correct for the lag of the precursor pool in the tissue behind the plasma. After a pulse, the plasma [^{14}C]leucine concentration and SA fall with time while the integral of the plasma SA increases. The convolution integrals, however, decline with time, and the more rapid the turnover of free [^{14}C]leucine in the various tissue pools, the more rapid the decline. The half-life of the total free [^{14}C]leucine in rat gray matter has been experimentally determined to be about 3.5 min (C.B.S., unpublished results). With an experimental interval of 60 min (i.e., 17 half-lives, effectively infinite time) after the pulse the contributions of the convolution integrals become negligible. Thus,

$$\lim_{T\to\infty} \int_0^T [C_{pp}^*(t)/C_{pp}]dt = \lambda \int_0^T [C_p^*(t)/C_p]dt \qquad [2]$$

and the operational equation reduces to

$$R_i = \frac{C_i^*(T)}{\lambda \int_0^T [C_p^*(t)/C_p]dt}. \qquad [3]$$

The only variable in Eq. 3 not measured directly in each experiment is λ, which must be evaluated separately.

The values of the rate constants of which λ is composed (Fig. 1*B*) need not be individually determined to evaluate λ. λ can be determined experimentally on the basis of its functional definition as the ratio of the steady-state distribution ratios of labeled and unlabeled leucine between the precursor pool in the tissue and the arterial plasma (i.e., the ratio of the precursor pool distribution space for labeled leucine to that of unlabeled leucine). Therefore,

$$\lambda = \frac{(C_{pp}^*/C_p^*)}{(C_{pp}/C_p)}. \qquad [4]$$

By algebraic rearrangement λ equals the ratio of the SAs of leucine in the precursor pool and plasma. Thus,

$$\lambda = \frac{(C_{pp}^*/C_{pp})}{(C_p^*/C_p)}. \qquad [5]$$

Eq. 5 provides the theoretical basis for the experimental evaluation of λ. The immediate amino acid precursors for protein synthesis are tRNA-bound and can be isolated from other amino acid pools. To determine λ, a constant SA of leucine in arterial plasma is maintained until the brain reaches a steady state. The steady-state SAs of leucine in plasma and in brain aminoacyl-tRNA are then measured, and λ is calculated by Eq. 5.

MATERIALS AND METHODS

Chemicals. Chemicals and materials were obtained from the following sources: 5-[*methyl*-^{14}C]dimethylaminonaphthalene-1-sulfonyl chloride ([^{14}C]Dns-Cl) (SA, 70–110 mCi/mmol; 1 Ci = 37 GBq), New England Nuclear; L-[4,5-^{3}H]leucine (SA, 120–190 Ci/mmol) and L-[1-^{14}C]leucine (SA, 50–60 mCi/mmol), Amersham; unlabeled Dns-Cl and *Escherichia coli* tRNA, Sigma; Dowex 50-H$^+$ (AG 50W-X8, 200–400 mesh), Bio-Rad; polyamide TLC plates, BDH (Poole, U.K.); HPLC Supelcosil LC-18 columns, Supelco (Bellefonte, PA); vanadyl ribonucleoside complex and redistilled nucleic acid grade phenol, Bethesda Research Laboratories.

Animals. Normal male Sprague–Dawley rats (200–350 g) (Taconic Farms, Germantown, NY) were provided food and water *ad libitum*. Under light halothane anesthesia a femoral artery and vein were catheterized, the hindquarters were partially immobilized by a loose-fitting plaster cast, and at least 2 hr were allowed for recovery from anesthesia.

Experimental Procedure to Determine λ. Infusion schedules designed to achieve and maintain constant arterial plasma concentrations of labeled leucine for at least 60 min were determined by the method of Patlak and Pettigrew (13). A tracer dose of [^{3}H]leucine (7–10 mCi) was infused by way of the venous catheter according to the schedule by means of a computer-driven infusion pump. [^{3}H]Leucine was used to determine λ because higher SAs were needed to measure the very small amounts of tRNA-bound leucine in brain. The infusions were continued for ≈30 min (three rats) or ≈60 min (three rats). Timed arterial blood samples were drawn every 5 min during the infusion and centrifuged immediately to separate the plasma, which was deproteinized in 5% (wt/vol) trichloroacetic acid and stored at −20°C until assayed for leucine and [^{3}H]leucine concentrations. At the end of the infusion rats were decapitated, and brains and livers were quickly removed and chilled to 0°C in ice-cold 0.25 M sucrose.

Extraction and Purification of Aminoacyl-tRNA. Brain and liver from each animal were homogenized separately in 5 ml of 0.25 M sucrose (0°C) per g containing 10 mM vanadyl ribonucleoside complex to inhibit ribonuclease and 6 mg of uncharged tRNA added as a carrier. The homogenates were centrifuged at 100,000 × *g* for 1 hr. The pellets, which contained most of the RNA with high molecular weights, were discarded. The cytosolic protein and RNA were precipitated in 5% (wt/vol) trichloroacetic acid and removed by centrifugation (12,000 × *g*, 20 min). The supernatant solutions were stored at −70°C until assayed for [^{3}H]leucine SA. The precipitates containing the aminoacyl-tRNA were washed three times each in 3% (wt/vol) and 0.06% (wt/vol) $HClO_4$ to remove any remaining free amino acids, suspended in 0.3 M NaOAc (pH 5), and extracted with an equal volume of fresh water-saturated phenol containing 0.1% (wt/vol) 8-hydroxyquinoline as an antioxidant. The aqueous phase containing the aminoacyl-tRNA was separated, and residual phenol was removed by five extractions with diethylether. The aminoacyl-tRNA was precipitated overnight at −20°C following addition of 2.5 volumes of ethanol containing 0.12 M KOAc. The RNA precipitate was recovered by centrifugation at 0°C; washed three times in a 1:2.5 (vol/vol) mixture of 0.3 M NaOAc, pH 5/0.12 M KOAc in ethanol; dissolved in 0.3 ml of 50 mM Na_2CO_3 (pH 10); and incubated at 37°C for 90 min to deacylate aminoacyl-tRNA. Deacylated tRNA was precipitated overnight at −20°C by addition of 2.5 volumes of ethanol and removed by centrifugation (12,000 × *g*, 20 min). The supernatant solutions, which contained the previously tRNA-bound amino acids, were dried in a stream of N_2 and stored at −20°C until analyzed further.

Assay of SA of [^{3}H]Leucine by Derivatization with [^{14}C]-Dns-Cl. Amino acids in deproteinized plasma and acid-soluble fractions of cytosol from brain and liver were further purified by Dowex 50-H$^+$ column (2 × 0.5 cm) chromatography. The effluents from the columns were discarded, and the amino acids were eluted with 25–30% (wt/vol) NH_4OH. The eluates were evaporated to dryness and redissolved in 50 mM Na_2CO_3 (pH 10). The fractions containing the amino acids recovered from the deacylation of the tRNA extracted from brain and liver were redissolved in 50 μl of water. The volumes of all samples from plasma and tissues were adjusted to contain about 100 pmol of leucine in 50 μl. Reaction mixtures, containing 50 μl of sample and 25 μl of 15 mM [^{14}C]Dns-Cl (SA, 30 mCi/mmol) in acetonitrile, were incubated at room temperature for 30 min. Reactions were

stopped by addition of 2 μl of 88% (wt/vol) formic acid and 20 nmol of unlabeled Dns-leucine as carrier. Dns-leucine was purified from the reaction mixtures by the following procedure. The reaction mixtures were loaded onto Sep-Paks (Waters Associates). Dns-OH was first selectively eluted with 0.1% (wt/vol) acetic acid in 25% (vol/vol) methanol, and then the dansylated amino acids were eluted with 0.1% acetic acid in 100% methanol. The eluates were dried in a stream of N_2, dissolved in acetone, and subjected to two-dimensional TLC on polyamide plates (15 × 15 cm). The solvent systems were 1.8% (wt/vol) formic acid for the first dimension and benzene/acetic acid, 9:1 (vol/vol), for the second (14). The Dns-leucine spot was identified by UV fluorescence and cut out, and the Dns-leucine was eluted with acetone. The eluates were dried in a stream of N_2 and dissolved in 25 μl of 0.2 mM unlabeled carrier Dns-leucine in methanol. The Dns-leucine was isolated by reverse-phase HPLC on a 5-μm C_{18} column (150 × 4.6 mm). Samples were eluted at 1.2 ml/min over 30 min at room temperature with a linear gradient of 25–40% (vol/vol) acetonitrile in 15 mM orthophosphoric acid/8.6 mM triethylamine. Fractions were collected every 0.2 min, and those within the Dns-leucine peak, identified by fluorescence, were pooled, dried, dissolved in acetonitrile, and assayed for ^3H and ^{14}C by liquid scintillation spectroscopy (model LS 5801, Beckman). Counting efficiencies for ^3H and ^{14}C were 26% and 60%, respectively; spillover of ^{14}C into the ^3H channel was 6%. From the known SA of the [^{14}C]Dns-Cl and the $^3H/^{14}C$ ratio of the [^{14}C]Dns-[^3H]leucine, the SA of the [^3H]leucine in each sample was determined. The SA of the [^{14}C]Dns-Cl reagent was determined with each assay by dansylation of a [^3H]leucine standard.

Assay of SA of [^3H]Leucine by Direct Amino Acid Analysis. In one experiment (E, Table 1; Fig. 2) SA of [^3H]leucine was determined by liquid scintillation spectroscopy and assay of leucine by direct amino acid analysis. The samples were prepared as described above to the point of dansylation, then dried in a stream of N_2, dissolved in 0.5 ml of water, filtered through a 0.45-μm filter (Millipore), dried again, and redissolved in 100–200 μl of 0.2 M sodium citrate (pH 2.2). Amino acids in these samples were analyzed by post-column derivatization with *o*-phthalaldehyde and fluorimetric assay in a Beckman model 7300 amino acid analyzer. This system could measure 25–50 pmol of leucine (the range in the experimental samples) with a 3% error. Fractions, after passage through the detector, were collected every 0.25 min and assayed for ^3H by liquid scintillation spectroscopy. SA was calculated from total ^3H in all fractions in the leucine peak and the total leucine content was measured.

RESULTS

In six experiments the SA of [^3H]leucine in arterial plasma was maintained relatively constant long enough (30–60 min) for the leucine pools in brain and liver to reach steady states with respect to plasma. In all experiments the SAs of the tRNA-bound leucine in brain and liver were below those of the arterial plasma (Table 1; Fig. 2). According to Eq. **5**, the steady-state distribution spaces of the labeled leucine in the precursor pools for protein synthesis averaged 58% (λ = 0.58) and 47% (λ = 0.47) of the equivalent distribution spaces for the unlabeled leucine in brain and liver, respectively. The values in brain at 30 and 60 min were not significantly different, indicating that a steady state had indeed been achieved. Amounts of tRNA-bound [^3H]leucine and unlabeled leucine recovered from brain were small—i.e., 500–1500 dpm and 17–57 pmol, respectively. In all experiments except E (Table 1; Fig. 2*E*), the SA of [^3H]leucine was determined by the double-label [^{14}C]dansylation procedure; in experiment E, leucine was assayed by amino acid analysis with *o*-phthalaldehyde derivatization. The results were similar with both analytical techniques. The ratio of the distribution spaces for labeled and unlabeled leucine in the total acid-soluble pool of brain was 0.59, essentially the same as that for tRNA-bound leucine (Table 1).

Table 1. Ratios of SAs of tissue [^3H]leucine pools to plasma [^3H]leucine SA in steady state

Exp.	Plasma leucine level, μM	Pool SA/plasma SA: Brain, Acid-soluble leucine pool	Brain, tRNA-leucine pool	Liver, tRNA-leucine pool
		30-min experimental period		
A	186	0.62	0.59	0.41
B	203	0.52	0.59	0.48
C	241	0.56	0.54	0.46
		60-min experimental period		
D	270	0.65	0.62	0.59
E	190	ND	0.56	0.39
F	136	ND	0.57	0.46

ND, not determined.

DISCUSSION

The results of these studies show that in brain and liver of normal, conscious, adult rats there is significant recycling of leucine derived from protein degradation into the total acid-soluble amino acid pool and the precursor pool for protein synthesis. These findings confirm previous results in rat liver (15, 16), which showed that about 50% of the free amino acid pool is derived from protein catabolism. Our results also indicate that the degree of recycling is comparable in brain. Brain and liver appear then to be different from heart (17), where tRNA-bound labeled leucine has been reported to reach complete isotopic equilibrium with plasma leucine.

Our results in brain do not agree with a recent preliminary report (18), claiming that amino acids in the precursor pool for protein synthesis in brain are derived exclusively from plasma. Inasmuch as these experiments (18) were not carried out under steady-state conditions, it is difficult to interpret the results. Our results clearly show that under steady-state conditions, isotopic equilibrium between plasma [^3H]leucine and tRNA-bound [^3H]leucine is not achieved. There are at least two possible explanations for this finding: (*i*) an isotope effect associated with [^3H]leucine, an unlikely possibility, and (*ii*) another source of unlabeled leucine, in addition to plasma, contributing to the leucyl-tRNA pool—i.e., leucine derived from protein breakdown. This second possibility is more likely because *in vivo* there is an interstitial space between plasma and cells where admixture between amino acids from plasma and metabolic pools can occur. The absence or unrestricted communication of such a space with the external medium *in vitro* may explain the findings *in vitro* that the external medium is the exclusive source of essential amino acids for protein synthesis (8–10).

These results are pertinent to the design of methods for the determination of local rates of protein synthesis *in vivo* with radiolabeled tracers. They show that the integrated SA of the precursor amino acid pool in tissue cannot be predicted solely on the basis of the time course of the SA of the tracer in plasma. Dilution of the precursor pool by amino acid derived from protein degradation must be considered. Eq. **3** is valid for determining rates of protein synthesis when an i.v. pulse of [1-^{14}C]leucine is followed by a long experimental period and quantitative autoradiography is applied to formalin-fixed and washed tissue sections (3, 6), provided that λ is known. If λ is ignored or assumed to be 1, then only minimal possible rates of protein synthesis are determined. Actual rates may

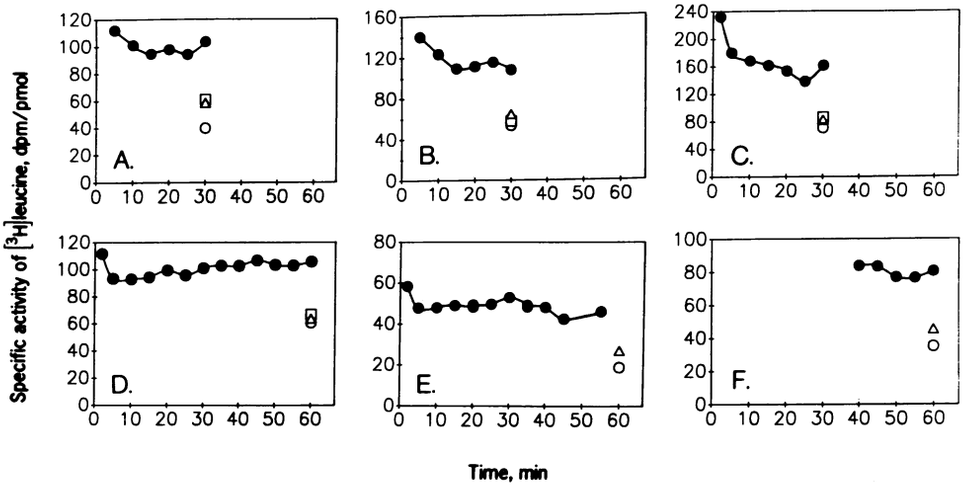


FIG. 2. Relationship between constant arterial plasma [^{3}H]leucine SA (●) and [^{3}H]leucine SAs in brain total acid-soluble (□), brain tRNA-bound (△), and liver tRNA-bound (○) pools. Constant arterial [^{3}H]leucine levels were maintained for 30 min (*A–C*) and 60 min (*D–F*) before killing the rats. [^{3}H]Leucine SA was assayed by the *o*-phthalaldehyde fluorescence procedure in the experiment shown in *E* and by double-label dansylation in all other experiments. In one experiment (*F*), plasma samples were taken only between 40 and 60 min for analysis of [^{3}H]leucine SA.

be greater depending on the rate of protein breakdown and the true value of λ. It may then be impossible to distinguish increased rates of protein synthesis from decreased rates of protein degradation and vice versa.

The four-compartment model in Fig. 1*A* takes into account all possible routes by which recycling of leucine might occur. Although λ (Fig. 1*B*) is expressed in terms of the rate constants that characterize these routes, in the present studies the values for λ were determined experimentally on the basis of a direct, operational definition (Eq. 5) and are valid regardless of the relationships among the tissue compartments. The value in brain, however, applies only to the brain as a whole in normal, awake rats. Its variation in brain regions and its stability in different biological states are unknown. We must now attempt to determine λ locally in different brain regions. A regional method would also facilitate examination of its stability in different experimental states. With a regional method for determining λ it would become possible to distinguish between changes in rates of protein synthesis and rates of protein degradation. Dunlop *et al.* (19) have proposed "flooding" the amino acid pools with massive loading doses of radioactive valine to dilute the effects of recycling of unlabeled valine. Examination of the effects of "flooding" doses of leucine on λ is necessary to determine whether they raise its value toward unity.

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