Pergamon

0197-0186(95)00022-4

Neurochem. Int. Vol. 27, No. 4/5, pp. 407–416, 1995
Elsevier Science Ltd. Printed in Great Britain

EFFECTS OF AGING ON REGIONAL RATES OF CEREBRAL PROTEIN SYNTHESIS IN THE SPRAGUE-DAWLEY RAT: EXAMINATION OF THE INFLUENCE OF RECYCLING OF AMINO ACIDS DERIVED FROM PROTEIN DEGRADATION INTO THE PRECURSOR POOL

C. BEEBE SMITH, Y. SUN and L. SOKOLOFF

Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20892, U.S.A.

(Received 9 November 1994; accepted 14 January 1995)

Abstract—The quantitative autoradiographic L-[1-^{14}C]leucine method for determination of regional rates of cerebral protein synthesis ($lCPS_{leu}$) requires knowledge of the degree of recycling of leucine derived from protein degradation into the precursor pool for protein synthesis. The influence of recycling can be evaluated by measuring λ, the steady state ratio of the leucine specific activity in the precursor amino acid pool (tRNA-bound leucine) to that in the arterial plasma. To define the changes in $lCPS_{leu}$ during the process of normal aging in the rat we have evaluated λ in middle-aged (14 months) and aged (24 months) rats and compared its values with those obtained previously in young adult rats (two months of age). The results show that the value of λ is the same in all three age groups, and that there is no change with aging in the fraction of leucine in the precursor pool derived from protein degradation.

Our previously reported regional rates of protein synthesis in young adult and aged rats were based on the assumption that there was no recycling of leucine derived from protein degradation into the precursor pool for protein synthesis [Ingvar M. C., Maeder P., Sokoloff L. and Smith C. B. (1985) *Brain* **108,** 155–170]. These values have been recalculated in the present study in order to take into account the appropriate correction for recycling. The recalculated rates are higher than those reported previously, but the effects of aging in the brain as a whole and in some specific brain regions are confirmed. Decreased $lCPS_{leu}$ was observed by middle-age, and in this cross-sectional study did not appear to decrease further. Of the 39 brain regions examined decreases were found throughout the brain with some proclivity for the brain stem. In comparison with young adults the weighted average rate of protein synthesis in the brain as a whole was found to be decreased by 16 and 11% in the middle-aged and aged groups, respectively.

We previously reported that normal aging in the Sprague-Dawley rat is accompanied by decreased rates of leucine incorporation into protein in the brain as a whole and in some specific brain regions (Ingvar *et al.*, 1985). Brain regions involved in visual and auditory function appeared to be particularly affected, but rates of protein synthesis were also statistically significantly decreased in several regions involved in motor function (substantia nigra, inferior olive, vestibular nucleus, and red nucleus) and in two limbic areas (dentate gyrus of the hippocampus and nucleus accumbens) in the older rats. Notably, there was a significant age-related decrease in $lCPS_{leu}$ in the locus coeruleus which contains the cell bodies of origin of the major ascending noradrenergic innervation of the cortex. Two of the four white matter regions examined were statistically significantly decreased in the older rats as was the olfactory cortex. The method used to determine the rates of protein synthesis in that study was an earlier version of the quantitative autoradiographic L-[1-^{14}C]leucine method (Smith *et al.*, 1980) that was based on a simple two-compartment model for the behavior of leucine in brain and assumed no recycling of leucine derived from protein degradation into the precursor pool for protein synthesis. This assumption was based on the results of *in vitro* studies which showed that extracellular amino acids are preferentially incorporated into protein (Gainer *et al.*, 1975; Robertson and Wheatley, 1979).

Abbreviations: λ, the fraction of an amino acid in the tRNA-bound pool that is derived from the plasma; ψ, the fraction of an amino acid in the tissue free amino acid pool that is derived from the plasma.

Since then, however, we have experimentally determined *in vivo* in young, adult, male, Sprague-Dawley rats that about 58% of the leucine in the precursor pool comes from the arterial plasma and the remainder from protein degradation (Smith *et al.*, 1988). Rates of protein synthesis reported previously (Ingvar *et al.*, 1985) were, therefore, underestimates of the true rates in the young adult rats and possibly also in the aged rats. The apparent decrease in protein synthesis with aging may then have reflected a decrease in the relative contribution of leucine in the precursor pool that is derived from the plasma due to either an increase in protein degradation or a decrease in blood–brain barrier transport of leucine or both.

We have extended our kinetic model for the behavior of leucine in brain (Smith *et al.*, 1988) to include the extracellular space and three intracellular pools of leucine: a free pool, a tRNA-bound pool, and a protein-bound pool. Recycling of leucine from protein degradation into the precursor pool, reflected in the tRNA-bound amino acid pool, can take place either directly within the cell or via the extracellular space. An equation for the determination of rates of protein synthesis was derived from this model and simplified by appropriate experimental design (Smith, 1991):

$$R_i = \frac{P_i^*(T)}{\lambda_i \int_0^T \frac{C_p^*(t)}{C_p} dt} \quad (1)$$

where R_i is the regional rate of protein synthesis, $P_i^*(T)$ is the total concentration of ^{14}C in protein in the tissue i at time T,

$$\int_0^T \frac{C_p^*(t)}{C_p} dt$$

is the integrated specific activity of leucine in arterial plasma, and λ_i is the factor that corrects the integrated specific activity of leucine measured in the arterial plasma for its dilution in the precursor pool by unlabelled leucine derived from protein breakdown. λ_i is a constant between 0 and 1.0 which is equal to the fraction of the leucine in the precursor pool for protein synthesis in tissue i that is derived from the plasma. A value for λ_i of 1.0 indicates that there is no recycling of leucine derived from protein breakdown, and a value of 0 indicates that the entire precursor pool is composed of recycled leucine. λ_i can be determined as the ratio of the apparent steady state leucine specific activity in the precursor pool in the tissue to that of the arterial plasma (Smith *et al.*, 1988). In the whole brain of the conscious, young adult, male rat this ratio (λ_{WB}) equals 0.58 (Smith *et al.*, 1988). In the present study we have examined the effects of aging on the value of λ_{WB}, and we have used the age-appropriate values of λ_{WB} to recalculate local rates of cerebral protein synthesis ($lCPS_{leu}$) in young adult, middle-aged, and aged rats.

EXPERIMENTAL PROCEDURES

Materials

Chemicals and materials were obtained from the following sources: L-[3,4,5-^{3}H]leucine (spec.act., 155 Ci/mmol), Du Pont-NEN, Wilmington, De. U.S.A.; *Escherichia coli* tRNA, Sigma Chemical Co., St. Louis, Mo., U.S.A.; vanadyl ribonucleoside complex and redistilled nucleic acid-grade phenol, Bethesda Research Laboratories, Gaithersburg, Mo, U.S.A.; L-norleucine, Cyclochemicals, Division of Travenol Laboratories, Los Angeles, Ca., U.S.A.; 5-sulfosalicylic acid, Fluka Chemie AG, Buchs, Switzerland.

Animals

All procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the NIMH Animal Care and Use Committee. Male, Sprague-Dawley rats (14 and 24 months of age) were obtained from Zivic-Miller Labs, Inc. (Zelienople, Pa, U.S.A.) and maintained for two weeks in the central animal facility under controlled conditions of normal humidity and temperature with standard alternating 12 h periods of light and darkness before initiating any experimental procedure. Food and water were provided *ad libitum*. Under light halothane anesthesia (1.5% halothane, 70% nitrous oxide/30% oxygen) a femoral artery and vein were catheterized. The catheters were tunnelled under the skin and exited at the back of the neck, and at least 4 h were allowed for recovery from anesthesia. Throughout the experimental procedure the rats were allowed to move freely in their cages.

Physiological variables

Physiological variables were measured to evaluate each animal's physiological state. Arterial blood pressure, pH, pCO_2, pO_2, and hematocrit, arterial plasma glucose concentrations, and rectal temperature were measured as previously described (Smith *et al.*, 1991).

Procedure to determine λ_{WB} and ψ_{WB}

λ_{WB} and ψ_{WB} were determined by the method described by Smith *et al.* (1988). λ_{WB} is the apparent steady state ratio of the specific activity of leucine in the tissue precursor pool of the whole brain to that of the arterial plasma when the arterial plasma specific activity is maintained constant for a prolonged period (Smith *et al.*, 1988). The time necessary to achieve this apparent steady state between brain and plasma for leucine is 60 min in young adult rats (Smith *et al.*, 1988), but we have extended the period in the present study to 90 min to be sure that an apparent steady state was achieved in the older animals. The steady state is designated as apparent because it pertains to the free and tRNA-bound leucine pools only; a steady state for [^{3}H]leucine in brain protein is not even approached during the 90 min experiments (Lajtha *et*

al., 1976). Analagously, ψ_{WB} is the steady state ratio of the specific activity of leucine in the tissue free amino acid pools to that in the arterial plasma.

In order to determine λ_{WB} and ψ_{WB} a constant arterial plasma specific activity for [^{3}H]leucine was maintained for 90 min by means of a programmed infusion of [^{3}H]leucine (40 mCi) as previously described (Smith *et al.*, 1988). [^{3}H] Leucine was used to determine λ_{WB} because its high specific activity was needed to measure the specific activity of the leucine in the very small pool of leucyl-tRNA in tissues. The specific activities of [^{3}H]leucine in arterial plasma and in the acid-soluble and tRNA-bound pools in tissues were determined as described below. During the infusion timed arterial blood samples, approximately 40 μl, were drawn every 5 or 10 min. Because the total volume (i.e., 550 μl) of blood sampled over the entire experimental interval was less than 5% of the blood volume, it was not necessary to replace the blood during the experiment. Blood samples were centrifuged immediately to separate the plasma, which was deproteinized in 4% (W/V) sulfosalicylic acid containing norleucine (0.01 mM) as an internal standard for amino acid analysis of the free amino acid pool. The deproteinized plasma samples were stored at -70°C until assayed for leucine and [^{3}H]leucine concentrations. At the end of the infusion the rats were decapitated, and the brain was quickly removed and chilled to 0°C in ice-cold 0.25 M sucrose.

Extraction and purification of aminoacyl-tRNA

Brains were homogenized by means of a motor-driven, loose fitting, all glass homogenizer in 10 ml of 0.25 M sucrose (0°C) containing 10 mM vanadyl ribonucleoside complex to inhibit ribonuclease and 0.02 mM L-norleucine added as an internal standard for analysis of the tissue free amino acids. Uncharged *Escherichia coli* tRNA (6 mg) was added as carrier. The homogenates were centrifuged at 100,000 ***g*** for 1 h, and a pure aminoacyl-tRNA fraction was isolated from the supernatant fraction as previously described (Smith *et al.*, 1991). Briefly, the supernatant fraction was treated with trichloroacetic acid, the precipitated protein and nucleic acids were washed repeatedly to remove free amino acids, and the aminoacyl-tRNA was separated from protein by phenol extraction. The aminoacyl-tRNA was hydrolyzed at pH 10, and the specific activity of the previously tRNA-bound, but now free amino acids, was determined as described below.

Extraction of tissue free amino acid pools

A 100 μl volume of cytosol fraction, i.e., the supernatant solution derived from the 100,000***g*** centrifugation of each tissue homogenate, was deproteinized by the addition of an equal volume of a solution of 8% (W/V) sulfosalicylic acid and stored at -70°C until assayed for leucine and [^{3}H]leucine concentrations. At the time of these assays the samples were thawed, mixed and centrifuged for 30 min at 5000***g*** at 4°C to remove precipitated protein.

Assay of specific activity of [^{3}H]leucine

Specific activities of [^{3}H]leucine in deproteinized plasma, tissue acid-soluble fractions, and in the fractions derived from the deacylation of the aminoacyl-tRNA were assayed by post-column derivatization with *o*-phthaldehyde and fluorometric assay as described previously (Smith *et al.*, 1988). The leucine concentration and the specific activity of [^{3}H]leucine in the acid-soluble pool in the brain were corrected for contamination by leucine in the residual blood contained in the brain (Smith *et al.*, 1991). Leucine recovered from the tRNA-bound amino acid fraction in the tissues was uncontaminated by leucine derived from any blood in the brain because all free amino acids in the tissue were separated from this fraction during the extraction procedure.

Calculation of λ_{WB} and ψ_{WB}

λ_{WB} was calculated from the ratio of the measured steady state specific activity of the tRNA-bound leucine in the brain to that of the acid-soluble leucine in the arterial plasma, and ψ_{WB} was calculated from the ratio of the measured steady state leucine specific activity in the total tissue free amino acid pool to that of the arterial plasma. The time course of the specific activity in arterial plasma and the specific activity of tRNA-bound leucine and the free leucine pool in tissue at the end of the experimental interval were determined as described above. The apparent steady state free leucine activity in the arterial plasma was calculated as the mean of the specific activities determined from 50 min to the end of the experimental period. In some of the experiments the specific activity of leucine in the arterial plasma was not constant during the first 30 min but from 50 min on remained within $\pm$ 10% of the mean and showed no overall trend to increase or decrease over the entire interval.

Recalculation of $lCPS_{leu}$ in aged rats

Regional rates of protein synthesis were recalculated by means of equation 1 with determined age-specific values of λ_{WB}. Values for measured concentrations of total ^{14}C in tissue protein and the integrated specific activity of leucine in arterial plasma were those used in our previously reported study (Ingvar *et al.*, 1985). In our previous study we had combined the middle-aged and aged rats into one group because there were only four rats in the aged group and because values of $lCPS_{leu}$ appeared to be similar in the middle-aged and aged animals. In this reexamination of the data we have treated the middle-aged and aged animals separately. Two animals were eliminated from the original study; one young adult rat because the surgical preparation of this rat under halothane anesthesia was inordinately long and one aged rat because of a rectal temperature well below the range of all of the rats studied.

Statistical analyses

Values of λ_{WB} and ψ_{WB}, physiological variables, and $lCPS_{leu}$ determined in the aging rats were compared with those of young adults by means of Dunnett's *t*-tests. Because our study is a survey of the effects of aging on $lCPS_{leu}$ no corrections were made for the multiple brain regions (39 brain regions and the brain as a whole) compared in the analysis. By the very conservative Bonferroni criteria for multiple comparisons of non-independent data only a *P*-value ≤ 0.0013 would achieve the 95% confidence level in the statistical analysis of the effects of aging on $lCPS_{leu}$.

RESULTS

Physiological status

The physiological status (Table 1) of the middle-aged and aged groups of rats was compared with that of the young adult control rats in which the value of

Table 1. Physiological variables in aging rats†

	Young adult‡ (9)	Middle-aged (3)	Aged (3)
Age (months)	2	14	24
Body weight (g)	243±5	828±75***	785±67***
Brain weight (g)	1.90±0.02	2.19±0.01***	2.36±0.06***
Body temperature (°C)	37.7±0.2	38.1±0.1	37.6±0.4
Hematocrit (%)	44.0±0.4	47.0±1.7	48.7±2.7
Arterial blood			
Mean pressure (mm Hg)	106±2	114±2*	101±11
pH	7.45±0.01	7.47±0.01	7.44±0.01
pCO_2(mm Hg)	40.3±0.9	37.5±1.8	39.8±1.5
pO_2 (mm Hg)	78.7±1.4	79.4±3.6	80.8±4.3
Arterial plasma			
Glucose concentration (mM)	9.2±0.4	8.2±0.6	7.1±0.6*

† Values are the means±SEM for the number of animals indicated in parentheses.
‡ From Sun *et al.* (1992).
* Statistically significantly different from young adult rats, $P < 0.05$, Dunnett's *t*-test.
** Statistically significantly different from young adult rats, $P < 0.01$, Dunnett's *t*-test.
*** Statistically significantly different from young adult rats, $P \ll 0.01$, Dunnett's *t*-test.

λ_{WB} was previously determined (Sun *et al.*, 1992). The body and brain weights of the middle-aged and aged rats were statistically significantly higher than those of the young adult rats. Arterial blood pressure was slightly but statistically significantly higher in the middle-aged rats. In the aged rats plasma glucose concentration was statistically significantly lower by 23% than that of the young adults, but none of the aged rats was in the hypoglycemic range. All other physiological variables measured were similar in the three groups of rats.

Concentrations of leucine in brain amino acid pools

Arterial plasma and brain free leucine concentrations were similar in both groups of older rats compared with young adults (Table 2), but tRNA-bound leucine was statistically significantly lower by 36% in the aged rats. In the middle-aged rats the 26% reduction in the concentration of tRNA-bound leucine was very close to statistical significance. We also measured the concentrations of valine, isoleucine, and phenylalanine in the tRNA-bound amino acid pool in brain. Concentrations of tRNA-bound valine were reduced by 32 and 38% in the middle-aged rats, respectively, and concentrations of tRNA-bound phenylalanine were reduced by 60 and 71% in the middle-aged and aged rats, respectively. Isoleucine concentrations in the tRNA-bound pool in both the middle-aged and aged rats were similar to those measured in young adults.

Values of λ_{WB} and ψ_{WB} during aging

Values of both λ_{WB} and ψ_{WB} were considerably less than 1.0 in all three age groups (Table 3), indicating that neither the free nor the tRNA-bound leucine pools in the tissue came close to achieving isotopic equilibrium with the arterial plasma. Therefore, in all three age groups significant portions of both the free and the tRNA-bound leucine pools were derived from protein degradation. Values of both λ_{WB} and ψ_{WB} in the middle-aged and aged rats were not statistically significantly different from the values in young adult rats. The means±SEM's for λ_{WB} and ψ_{WB} in all three groups ($n = 15$) were 0.58±0.01 and 0.49±0.01, respectively.

Rates of protein synthesis in whole brain during normal aging

Compared with young adult rats mean rates of protein synthesis in the brain as a whole were statistically

Table 2. Effects of aging on leucine concentrations in plasma and brain amino acid pools†

Age group	*n*	Plasma (nmol/ml)	Brain free leucine (nmol/g)	Brain tRNA-bound leucine (nmol/g)
Young adults‡	9	154±9	55±4	0.096±0.006
Middle-aged	3	181±15	59±1	0.071±0.007
Aged	3	151±6	51±3	0.062±0.004*

† Values are means±SEM.
‡ Values from Sun *et al.* (1992).
* Statistically significantly different from young adult controls, $P < 0.05$, Dunnett's *t*-test.

Table 3. Effects of aging on steady state ratios of [^{3}H]leucine specific activities in tissue pools to arterial plasma [^{3}H]leucine specific activity*

Age	Steady state ratio of [^{3}H]leucine specific activities	
	ψ_{WB}† Free amino acid pool/plasma	λ_{WB}‡ tRNA-bound pool/plasma
Young adults§	0.49±0.02 (9)	0.58±0.01 (9)
Middle-aged	0.49±0.01 (3)	0.59±0.01 (3)
Aged	0.49±0.03 (3)	0.56±0.01 (3)

* Values are means±SEM of the individual ratios determined in the number of animals indicated in parentheses.
† ψ_{WB}, the fraction of leucine in the acid-soluble pool in whole brain derived from the arterial plasma.
‡ λ_{WB}, the fraction of leucine in the precursor pool for protein synthesis in whole brain derived from the arterial plasma.
§ Values from Sun *et al.* (1992).

significantly decreased in both the middle-aged and aged rats by 16 and 11%, respectively (Table 4).

lCPS$_{leu}$ during normal aging

In the telenecephalon (Table 5) there were statistically significant decreases in the middle-aged rats in the olfactory and visual cortex, CA1 pyramidal cells of the hippocampus, the supragranular zone of the dentate gyrus, the genu of the corpus callosum, and the internal capsule. Decreases from values in the young rats ranged from 17–24%. In the diencephalon (Table 6) statistically significant decreases were found in the lateral geniculate and paraventricular nuclei in the middle-aged rats. There were no statistically significant effects in the aged rats in any of the regions examined in the telencephalon or the diencephalon. Of the six mesencephalic regions examined (Table 6) four were statistically significantly decreased in the middle-aged rats, i.e. the red nucleus, the zona compacta of the substantia nigra, the inferior colliculus, and the locus coeruleus. The inferior colliculus was also statistically significantly decreased in the aged rats. In the pons and medulla (Table 7) five of the six regions examined were statistically significantly decreased in the middle-aged rats, and two of these regions, the lateral lemniscus and the inferior olive, were also significantly decreased in the aged animals. Rates of protein synthesis in cerebellar white matter were significantly decreased by 21% in the middle-aged rats compared to young adults (Table 7).

DISCUSSION

The results of the present studies demonstrate that normal aging in the rat is accompanied by widespread decreases in local rates of cerebral synthesis. The present study is, to our knowledge, the first study of the effects of aging on cerebral protein synthesis in rats in which the quantitative autoradiographic method for measurement of regional rates of cerebral protein synthesis with correction for recycling of amino acids derived from protein degradation into the precursor pool for protein synthesis has been used. Without correction for recycling, rates of protein synthesis determined with a radiolabeled tracer amino acid may

Table 4. Whole brain: effects of aging on rates of cerebral protein synthesis†

Age group	*n*	Cerebral protein synthesis (nmol leucine incorporated into protein/g/min)‡	Percent change from young adult
Young adult (6 months)	5	7.4±0.1	
Middle-age (15 months)	8	6.2±0.3**	−16
Aged (23 months)	3	6.6±0.2*	−11

† Recalculated with correction for recycling from the data of Ingvar *et al.* (1985).
‡ Values are means±SEM.
* Statistically significantly different from young adult, $P < 0.05$, Dunnett's *t*-test.
** Statistically significantly different from young adult, $P < 0.01$, Dunnett's *t*-test.

Table 5. Telencephalon: effects of aging on local rates of cerebral protein synthesis (nmol leucine incorporated into protein/g/min)†

Region	Young adult (6 months)	Middle-aged (15 months)	Aged (23 months)
Cortex			
Parietal	9.1±0.3	7.9±0.5	8.6±0.5
Olfactory	13.6±0.4	10.6±0.7**	12.9±0.6
Frontal	9.2±0.1	8.6±0.5	8.9±0.2
Sensorimotor	9.3±0.3	8.4±0.5	8.5±0.1
Visual	10.0±0.4	8.3±0.4*	8.9±0.5
Auditory	9.2±0.3	8.1±0.4	9.2±0.5
Hippocampal formation and septal area			
Pyramidal cells, CA1	11.7±0.7	8.9±0.5*	11.7±1.5
Dentate gyrus, supragranular zone	14.8±1.2	11.8±0.7*	13.2±0.4
Septal nucleus, lateral	7.1±0.3	6.1±0.4	6.6±0.4
Basal ganglia			
Nucleus accumbens, medial	6.9±0.6	5.5±0.5	5.7±0.4
Caudate-putamen	6.6±0.2	5.7±0.4	5.8±0.3
Globus pallidus	4.1±0.2	3.8±0.2	3.9±0.2
Amygdala, lateral	10.3±0.7	9.2±0.5	8.3±1.1
White matter			
Corpus callosum, splenium	3.6±0.2	3.1±0.2	3.1±0.2
Corpus callosum, genu	4.0±0.14	3.2±0.2**	3.6±0.2
Internal capsule	3.7±0.1	2.9±0.2**	3.3±0.2

† Recalculated with correction for recycling from data of Ingvar *et al.* (1985). Values are means±SEM for the number of animals indicated in the parentheses.
* Statistically significantly different from young adults rats, $P < 0.05$, Dunnett's *t*-test.
** Statistically significantly different from young adult rats, $P < 0.01$, Dunnett's *t*-test.

be underestimated, and it cannot be assumed that the correction for recycling is constant across all ages.

In the equation for computation of $lCPS_{leu}$ (equation 1) the constant λ_{WB} corrects for the dilution of the integrated specific activity of leucine measured in arterial plasma by the contribution of unlabeled leucine derived from protein breakdown to the precursor pool for protein synthesis. The constant λ_{WB} was evaluated in middle-aged and aged rats and found to be identical to the value previously reported for young adult rats (Sun *et al.*, 1992). The mean value of λ_{WB} (0.58) determined in all three age groups of rats was used to recalculate $lCPS_{leu}$ in brain as a whole and in all 39 brain regions analyzed in our previous study (Ingvar *et al.*, 1985) in which no correction for recycling was applied. It was assumed that the correction for recycling of leucine is constant from region to region at all ages examined. This is a reasonable assumption inasmuch as previous studies in normal, conscious, adult rats have shown that the values of λ_i in most grey matter regions fall within ±5% of the value of 0.58, the average measured for the brain as a whole (Sun *et al.*, 1992) and remain so even in conditions in which $lCPS_{leu}$ has been shown to be changed, including the regenerating hypoglossal nucleus of the adult rat in which $lCPS_{leu}$ is increased by 20–30% (Sun *et al.*, 1993). Rates of protein synthesis reported (Tables 4–7) are the *actual* rates because effects of recycling are taken into account.

Survival effect

In our previous study (Ingvar *et al.*, 1985) we had combined the middle-aged and aged groups into one group for the purpose of statistical comparisons with the young adults because we had observed that the values in the two older groups were overlapping and not statistically significantly different from each other. In the present study $lCPS_{leu}$ in the middle-aged and aged rats are reported separately in order to chart more clearly the course of normal aging. The data show that in most structures mean values for $lCPS_{leu}$ are similar in the two older groups probably due to a survival effect (Schaie, 1977), a common artifact of cross-sectional aging studies. The aged group in our study represents about 8% of the original rat population (Ingvar *et al.*, 1985), a selected population of survivors that possibly aged at a slower rate. This point is illustrated in Fig. 1 in which individual values for $lCPS_{leu}$ in the olfactory cortex (A), dentate gyrus of the hippocampus (B), visual cortex (C), inferior colliculus (D), vestibular nucleus (E), lateral lemniscus (F), cochlear nucleus (G), and inferior olivary nucleus (H) are plotted vs age. In comparison with controls the mean rates of $lCPS_{leu}$ in these regions in the middle-aged rats were statistically significantly decreased by 17–27%, but in the aged rats they were closer to the rates in controls (Table 5–7). Figure 1 shows that in each region the values measured in all three of the aged rats were similar to the high values

Table 6. Diencephalon and mesencephalon: effects of aging on local rates of cerebral protein synthesis (nmol leucine incorporated into protein/g/min)†

Region	Young adult (6 months)	Middle-aged (15 months)	Aged (23 months)
Diencephalon			
Habenula	19.8±1.4 (4)	18.0±1.7 (4)	14.6±3.4 (3)
Thalamus, ventral nucleus	9.4±0.2 (5)	8.5±0.6 (8)	9.2±0.3 (3)
Thalamus, posterior lateral nucleus	7.0±0.3 (4)	6.3±0.4 (4)	6.8±0.2 (3)
Medial geniculate nucleus	9.6±0.4 (5)	8.2±0.4 (8)	8.3±1.1 (3)
Lateral geniculate nucleus	9.1±0.3 (5)	7.1±0.4* (8)	7.6±0.4 (3)
Hypothalmus, ventral medial	9.6±0.7 (5)	8.4±0.8 (3)	9.4±0.7 (2)
Hypothalamus, paraventricular	20.5±0.8 (5)	17.3±0.5* (4)	19.6±0.6 (3)
Hypothalamus, supraoptic	21.9±1.7 (3)	20.4±2.9 (4)	23.7±0.1 (2)
Hypothalamus, mamillary body	8.6±0.4 (5)	8.4±0.7 (8)	8.8±0.4 (3)
Mesencephalon			
Red nucleus	9.3±0.4 (5)	6.9±0.4** (8)	7.3±1.4 (2)
Substantia nigra, compacta	3.6±0.2 (5)	3.0±0.2* (8)	3.0±0.1 (3)
Interpeduncular nucleus	9.7±0.4 (5)	9.1±0.4 (8)	8.6±0.5 (3)
Superior colliculus	8.6±0.4 (5)	7.3±0.4 (8)	7.9±0.6 (3)
Inferior colliculus	11.1±0.3 (5)	8.4±0.2*** (8)	9.4±0.6* (3)
Locus ceruleus	13.7±0.4 (5)	10.0±0.6** (7)	11.6±2.0 (2)

† Recalculated with correction for recycling from data of Ingvar *et al.* (1985). Values are means±SEM for the number of animals indicated in the parentheses.
* Statistically significantly different from young adult rats, $P < 0.05$, Dunnett's *t*-test.
** Statistically significantly different from young adult rats, $P < 0.01$, Dunnett's *t*-test.
*** Statistically significantly different from young adult rats, $P \ll 0.01$, Dunnett's *t*-test.

measured in the middle-aged rats. With respect to $lCPS_{leu}$ in these brain regions, the aged survivors resemble the fittest of the middle-aged animals.

Aging and rates of protein synthesis in the brain as a whole

In the present study the rates of protein synthesis in the brain as a whole are statistically significantly lower in both groups of older rats compared with young adult controls (Table 4). Other *in vivo* studies of the effects of aging on rates of cerebral protein synthesis have used the "flooding" technique (Dunlop *et al.*, 1975) in an attempt to circumvent any possible effects of recycling of amino acids derived from protein degradation on the precursor pool specific activity. In the "flooding" technique it is assumed that the administration of large doses of the tracer amino acid at low specific activity might so enhance the contribution of the plasma amino acid to the tissue amino acid pools that the relative contribution of the amino acid derived from protein degradation would become negligible. With this technique and [^{3}H]leucine as the tracer amino acid it was shown that in grossly dissected brain regions of young adult (three months of age), female, Long-Evans hooded rats (Dwyer *et al.*, 1980) and in the brain as a whole of young (one month of age), male, Fisher 344 rats (Fando *et al.*, 1980) rates of protein synthesis were statistically significantly higher than rates measured in older animals. In both of these studies lower rates were seen by middle-age. In another study in which the "flooding" technique was used with [^{14}C]valine as the tracer amino acid (Avola *et al.*, 1988) rates of protein synthesis in grossly dissected brain regions from young adult (four months old), middle-aged (12 months old), and aged (24 months old) male, albino, Wistar rats were compared and found to be the same. Our studies in young adult, Sprague-Dawley rats on the effects of

Table 7. Pons, medulla oblongata, and cerebellum: effects of aging on local rates of cerebral protein synthesis (nmol leucine incorporated into protein/g/min)†

Region	Young adult (6 months)	Middle-aged (15 months)	Aged (23 months)
Pons			
Superior olivary nucleus	8.1 ± 0.4 (4)	7.0 ± 0.4 (5)	7.7 ± 0.3 (2)
Cochlear nucleus, ventral	11.4 ± 0.6 (5)	9.4 ± 0.6* (7)	9.5 ± 0.1 (2)
Lateral lemniscus, ventral	10.3 ± 0.5 (5)	8.3 ± 0.4* (7)	7.8 ± 0.4* (3)
Pontine grey matter	7.0 ± 0.4 (5)	5.2 ± 0.3** (7)	6.1 ± 0.3 (3)
Vestibular nucleus, superior	13.1 ± 0.5 (5)	10.7 ± 0.4** (8)	11.9 ± 0.2 (3)
Medulla oblongata			
Inferior olivary nucleus	10.6 ± 0.3 (5)	7.8 ± 0.4*** (6)	8.3 ± 0.5** (3)
Cerebellum			
Cortex	10.4 ± 0.5 (5)	9.0 ± 0.4 (8)	10.7 ± 0.3 (3)
White matter	3.0 ± 0.1 (5)	2.4 ± 0.1* (8)	2.8 ± 0.3 (3)

† Recalculated with correction for recycling from data of Ingvar *et al.* (1985). Values are means ± SEM for the number of animals indicated in the parentheses.
* Statistically significantly different from young adult rats, $P < 0.05$, Dunnett's *t*-test.
** Statistically significantly different from young adult rats, $P < 0.01$, Dunnett's *t*-test.
*** Statistically significantly different from young adult rats, $P \ll .01$, Dunnett's *t*-test

"flooding" with L-valine on the contribution of valine derived from protein degradation to the precursor pool indicate that under "flooding" conditions the precursor pool in brain is not totally overwhelmed by valine coming from the plasma and that recycling is still significant and cannot be ignored (Smith *et al.*, 1991).

Regional specificity of effects of aging on $lCPS_{leu}$

The effects of aging on $lCPS_{leu}$ in the recalculated data (Tables 5–7) are very similar to what we had found previously (Ingvar *et al.*, 1985). In the present analysis in which we separated the middle-aged and aged groups and compared them with the young adults, most of the statistically significant effects were found in the middle-aged group, probably because there were only three rats in the aged group. Two regions (nucleus accumbens and superior colliculus) that had been statistically significantly decreased in the combined middle-aged/aged group were no longer statistically significantly decreased in either the middle-aged or aged groups. Interestingly, several other brain regions (CA1 pyramidal cells of the hippocampus, genu of the corpus callosum, and the paraventricular nuclei of the hypothalamus), which previously had not been statistically significantly affected in the combined older animals, were statistically significantly decreased in the middle-aged rats.

Effects of aging on $lCPS_{leu}$ were more prevalent in the more caudal parts of the brain and in white matter. Statistically significant effects were found in 31 and 22% of the regions examined in the telencephalon and diencephalon, respectively, whereas 67 and 83% of the regions examined in both the mesecephalon and the pons, respectively, were statistically significantly affected. This predilection for changes in the brain stem contrasts with results of studies of neuronal loss in both humans (Konigsmark and Murphy, 1972; Monagle and Brody, 1974) and rats (Goldman and Coleman, 1981) which show that brain stem nuclei are relatively spared. It is possible that the age-related decreases in $lCPS_{leu}$ are more akin to the dendtritic atrophy seen in the senescent brain (Geinisman *et al.*, 1978).

Of the four areas of white matter examined we found statistically significant decreases in $lCPS_{leu}$ in three of them: genu of corpus callosum, internal capsule and cerebellar white matter. Axonal degeneration has been reported to accompany aging in the rat in most white matter regions (Naranjo and Greene, 1977), but since protein is synthesized in cell bodies it is difficult to see how even the loss of axons would result in decreased rates of protein synthesis in white matter. Studies of neuroglia in the aging brain have shown that in general the numbers of oligodendroglia and astroglia remain constant while microglia tend to proliferate (Vaughan and Peters, 1974). It is possible that in the face of axonal degeneration oligo-

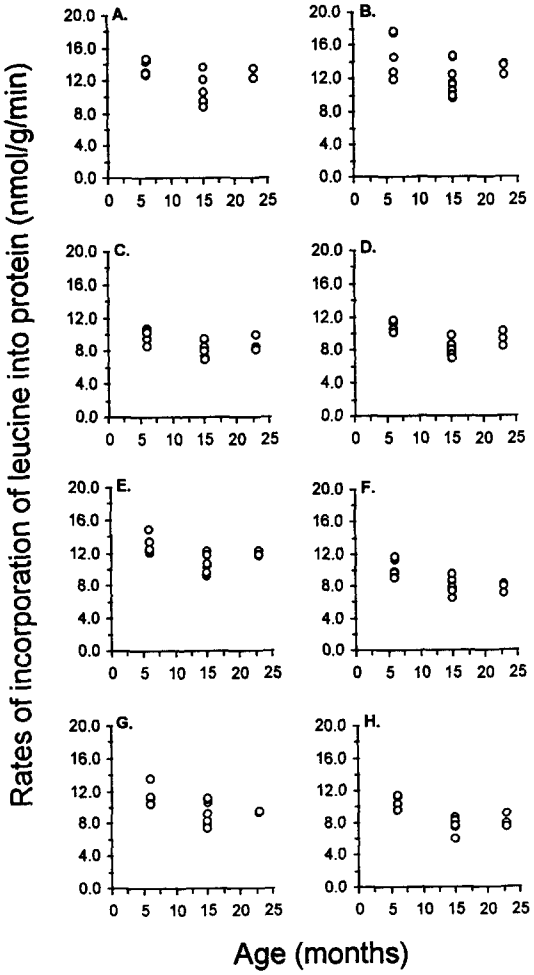

Fig. 1. Effects of aging on $lCPS_{leu}$ in olfactory cortex (A), dentate gyrus of the hippocampus (B), visual cortex (C), inferior colliculus (D), vestibular nucleus (E), Lateral lemniscus (F), cochlear nucleus (G), and inferior olivary nucleus (H). Each point represents the value of $lCPS_{leu}$ in a single rat.

dendroglia may survive but their synthesis of myelin proteins is reduced.

Effects of aging on leucine concentrations in brain amino acid pools

The decreased rates of protein synthesis found in the brain as a whole in the older groups of rats are consistent with the lower concentrations of brain tRNA-bound leucine in the older rats compared with young adults. Similarly, in our study of protein synthesis and brain development (Sun *et al.*, 1995) higher rates of protein synthesis in the young animals were accompanied by higher concentrations of tRNA-bound leucine. Free leucine concentrations in brain were also higher in the developing rats compared with young adults whereas in the aged rats free leucine concentrations were unaffected.

Lack of an effect of aging on the value of ψ_{WB} and λ_{WB}

Values of ψ_{WB} remain constant from 7 days of age to 24 months of age (Sun *et al.*, 1995; Table 3), indicating that there are no age-related changes in the relative contribution of leucine derived from protein degradation into the total acid-soluble amino acid pool in brain. The common use, therefore, of the acid-soluble pool specific activity as a measure of the precursor pool activity in studies of age-related changes in brain protein synthesis is problematic. Values for λ_{WB} in developing rats are lower than those of young adults, indicating that at these young ages higher rates of protein degradation accompany the higher rates of protein synthesis (Sun *et al.*, 1995). This relationship can be clarified by expressing λ_{WB} in terms of the relative fluxes of leucine into the precursor pool from plasma and protein degradation:

$$\lambda_{WB} = \frac{\text{flux}_{plasma}}{\text{flux}_{plasma} + \text{flux}_{proteolysis}} \quad (2)$$

We had expected that in normal aging, in which rates of protein synthesis are decreased, that the value of λ_{WB} would also be increased due to a decrease in protein degradation. The lack of an effect of aging on the value of λ_{WB} (Table 3) suggests that the rate of protein degradation in brain does not change with senescence or that there is a decrease in flux of leucine from plasma into the brain that is about equal in magnitude to the decrease in amino acid derived from protein degradation.

Acknowledgements—We are grateful to Ms G. E. Deibler for carrying out the amino acid analyses and to Ms Jane Macedonia for technical assistance.

REFERENCES

Avola R., Condorelli D. F., Ragusa N., Renis M., Alberghina M., Giuffrida Stella A. M. and Lajtha A. (1988) Protein synthesis rates in rat brain regions and subcellular fractions during aging. *Neurochem. Res.* **13,** 337–342.

Dunlop D. S., van Elden W. and Lajtha A. (1975) A method for measuring brain protein synthesis rates in young and adult rats. *J. Neurochem.* **24,** 337–344.

Dwyer B. E., Fando J. L. and Wasterlain C. G. (1980) Rat brain protein synthesis declines during postdevelopmental aging. *J. Neurochem.* **35,** 746–749.

Fando J. L., Salinas M. and Wasterlain C. G. (1980) Age-dependent changes in brain protein synthesis in the rat. *Neurochem. Res.* **5,** 373–383.

Gainer H., Barker J. L. and Wollberg Z. (1975) Preferential incorporation of extracellular amino acids into neuronal proteins. *J. Neurochem.* **25,** 177–179.

Geinisman Y., Bondareff W. and Dodge J. T. (1978) Dendritic atrophy in the dentate gyrus of the senescent rat. *Am. J. Anatomy* **152,** 321–330.

Goldman G. and Coleman P. D. (1981) Neuron numbers in locus coeruleus do not change with age in Fisher 344 rat. *Neurobiol. Aging* **2,** 33–36.

Ingvar M., Maeder P., Sokoloff L. and Smith C. B. (1985) Effects of aging on local rates of cerebral protein synthesis. *Brain* **108,** 155–170.

Konigsmark B. W. and Murphy E. A. (1972) Volume of the ventral cochlear nucleus in man: its relationship to neuronal population and age. *J. Neuropath. exp. Neurol.* **31,** 304–316.

Lajtha A., Latzkovits L. and Toth J. (1976) Comparison of turnover rates of proteins of the brain, liver and kidney in mouse *in vivo* following long term labeling. *Biochim. Biophys. Acta* **425,** 511–520.

Monagle R. D. and Brody H. (1974) The effects of age upon the main nucleus of the inferior olive in the human. *J. comp. Neurol.* **155,** 61–66.

Naranjo N. and Greene E. (1977) Use of reduced silver staining to show loss of connections in aged rat brain. *Brain Res. Bull.* **2,** 71–74.

Robertson J. H. and Wheatley D. N. (1979) Pools and protein synthesis in mammalian cells. *Biochem. J.* **178,** 699–709.

Schaie K. W. (1977) Quasi-experimental research designs in the psychology of aging. In: *Handbook of the Psychology of Aging.* (Birren J. E. and Schaie K. W. (Eds.), pp. 39–58. van Nostrand Reinhold, New York.

Smith C. B. (1991) The measurement of regional rates of cerebral protein synthesis *in vivo*. *Neurochem. Res.* **16,** 1037–1045.

Smith C. B., Davidsen L., Deibler G., Patlak C., Pettigrew K and Sokoloff L. (1980) A method for the determination of local rates of protein synthesis in brain. *Trans. Am. Soc. Neurochem.* **11,** 94.

Smith C. B., Deibler G. E., Eng N., Schmidt K. and Sokoloff L. (1988) Measurement of local cerebral protein synthesis *in vivo*: influence of recycling of amino acids derived from protein degradation. *Proc. Natn. Acad. Sci. U.S.A.* **85,** 9341–9345.

Smith C. B., Sun Y., Deibler G. E. and Sokoloff L. (1991) Effect of loading doses of L-valine on relative contributions of valine derived from protein degradation and plasma to the precursor pool for protein synthesis in rat brain. *J. Neurochem.* **57,** 1540–1547.

Sun Y., Deibler G. E., Jehle J., Macedonia J., Dumont I., Dang T. and Smith C. B. (1995). Rates of local cerebral protein synthesis in the rat during normal postnatal development. *Am. J. Physiol.* **268,** R549–561.

Sun Y., Deibler G. E. and Smith C. B. (1993) Effects of axotomy on protein synthesis in the rat hypoglossal nucleus: examination of the influence of local recycling of leucine derived from protein degradation into the precursor pool. *J. Cerebr. Blood Flow Metab.* **13,** 1006–1012.

Sun Y., Deibler G. E., Sokoloff L. and Smith C. B. (1992) Determination of regional rates of cerebral protein synthesis adjusted for regional differences in recycling of leucine derived from protein degradation into the precursor pool in conscious adult rats. *J. Neurochem.* **69,** 863–873.

Vaughan D. W. and Peters A. (1974) Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: an electron microscopie study. *J. Neurocyt.* **3,** 405–429.