Age-dependent decrease in the amount of eukaryotic initiation factor 2 in various rat tissues

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Recent studies have suggested that the decline in protein synthesis that occurs in rat liver and brain during development and aging is associated with a decrease in the activity of eukaryotic initiation factor 2 (eIF-2). One way in which eIF-2 activity could be decreased in tissue extracts would be through a decrease in the activity of the GDP exchange factor, eIF-2B. In the present study, the activity of eIF-2B was measured in tissue extracts and was found to be less in older than in younger rats. Thus a decrease in eIF-2B activity could account for part of the decrease in protein synthesis that occurs during aging. Another way in which eIF-2 activity could be decreased would be through a decrease in amount of the protein. Therefore the amount of eIF-2 in various tissues was quantified by protein immunoblot analysis. We found that the amount of eIF-2 relative to total protein tended to fall with increasing age. Furthermore, eIF-2 content was directly proportional to the rate of protein synthesis in the tissues examined. Finally, slot-blot analysis of polyadenylated RNA revealed no significant change in the relative abundance of eIF-2 α mRNA with age. The last-mentioned experiments suggest that the synthesis of eIF-2 may be regulated through changes in the efficiency of translation of eIF-2 α mRNA rather than through changes in gene transcription.

INTRODUCTION

The rate of protein synthesis undergoes decremental changes in many organs and tissues during aging. In particular, protein synthesis in rat brain decreases with age, as measured *in vivo* [1–3], in brain slices *in vitro* [1,3] and in cell-free systems from brain [1]. The mechanism involved in the age-dependent decrease in protein synthesis in brain is unknown. However, recent studies have shown that the activity of eukaryotic initiation factor 2 (eIF-2) decreases in both rat and mouse brain in parallel with the decrease in the rate of protein synthesis [4–6].

eIF-2 plays a crucial role in the first step in peptide-chain initiation, the binding of initiator methionyl-tRNA_i^{Met} (Met-tRNA_i^{Met}) to the 40 S ribosomal subunit to form a 43 S preinitiation complex (reviewed in [7,8]). A decrease in the eIF-2-mediated binding of Met-tRNA_i^{Met} to 40 S ribosomal subunits results in an inhibition of protein synthesis in such diverse systems as virally infected cells (reviewed in [9]) and amino-acid-deprived rat liver [10]. Indeed, changes in eIF-2 activity in rat liver have been proposed to play an essential role in the age-related decrease in protein synthesis observed in that tissue [11].

One mechanism by which eIF-2 activity could be altered in tissues from different-aged rats would be through a change in the activity of eukaryotic initiation factor 2B (eIF-2B), which catalyses the exchange of GDP bound to eIF-2 for free GTP. Phosphorylation of the α -subunit of eIF-2 converts it into a form that has much higher affinity for eIF-2B than unphosphorylated eIF-2, which results in a sequestration of eIF-2B into an inactive complex with eIF-2·GDP (reviewed in [7,8]). Therefore, phosphorylation of the α -subunit of eIF-2 results in an apparent inhibition of eIF-2 activity, since eIF-2·GDP does not bind Met-tRNA_i^{Met}. A second mechanism by which eIF-2 activity could be altered during aging would be through a change in the amount of the protein.

In the present study, we have measured eIF-2B activity in extracts from brain and liver from 1-, 4- and 10-month-old rats.

In addition, we have examined the amount of eIF-2 present in brain, liver, lung, spleen and kidney from these rats, using a polyclonal antibody specific for the β -subunit of rat eIF-2. The relative abundance of mRNA coding for the α -subunit of eIF-2 present in brain, lung and liver was also determined. We show that eIF-2B activity is decreased in tissue from 10-month-old as compared with 1-month-old rats. In addition, we show that the amount of eIF-2 relative to total protein decreases with increasing age in all tissues examined and that eIF-2 content is directly proportional to the rate of protein synthesis. However, the relative abundance of eIF-2 α mRNA shows no significant change in any of the conditions examined. The latter result suggests that the synthesis of eIF-2 relative to other proteins is regulated during aging through alterations in the efficiency of translation of eIF-2 mRNA.

MATERIALS AND METHODS

Animals and experimental design

Male Sprague–Dawley rats were provided food and water *ad libitum* and maintained on a 12 h-light/12 h-dark cycle. The ages of the animals used for this study were 1 month $\pm$ 3 days, 4 months $\pm$ 2 weeks and 10 months $\pm$ 1 month. Rats were killed by anoxia with CO₂. Tissues were removed in the following order: liver, kidneys, spleen, lungs and brain; they were then placed in ice-cold saline (0.9% NaCl). The tissues were blotted dry, weighed, and homogenized in 4 vol. of ice-cold buffer A (20 mM-Tris, pH 7.5, 1 mM-dithiothreitol, 35 mM-sucrose, 0.1 mM-EDTA) containing 125 mM-KCl. A portion of the homogenate was diluted 1:10 in buffer A containing 25 mM-KCl, and 2 vol. of SDS sample buffer [0.143 M-Tris, pH 6.8, 29% (v/v) glycerol, 2.9% (w/v) SDS, 0.014 M- β -mercaptoethanol and 0.23% (w/v) Bromophenol Blue] was added. The samples were then incubated at 100 °C for 3 min. A second portion of the homogenate was diluted 1:25 in buffer A containing 25 mM-KCl and was stored frozen at –80 °C until analysed for protein content [12]. The

Abbreviations used: eIF-2, eukaryotic initiation factor 2; poly(A)⁺ RNA, polyadenylated RNA.

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remainder of the homogenate was stored at -80°C until analysed for RNA content [13].

Measurement of protein synthesis *in vivo*

Rates of protein synthesis *in vivo* were estimated by using the flooding-dose method as described previously [14,15]. Animals were anaesthetized (Nembutal; 50 mg/kg body wt.), an incision was made in the neck, and polyethylene catheters (PE-50 tubing) were inserted into the carotid artery. A bolus infusion of L-[^3H]phenylalanine ($0.2\ \mu\text{Ci}/\text{ml}/\mu\text{mol}$, $30\ \mu\text{Ci}/100\ \text{g body wt.}$; $1\ \text{ml}/100\ \text{g body wt.}$) was delivered over a 10–15 s interval via the jugular vein [14,15]. At 2, 6 and 10 min after injection of the radioisotope, an arterial blood sample (1 ml) was withdrawn through the catheter in the carotid artery for measurement of plasma phenylalanine concentration and radioactivity. Immediately after removal of the last blood sample, tissues were removed in the order kidney, spleen, liver, lung and brain, frozen at the temperature of liquid N_2 , and weighed. The exact time of removal of each tissue was recorded.

The frozen tissue was powdered under liquid N_2 , and a portion was used to estimate the rate of incorporation of [^3H]phenylalanine into protein. Approx. 0.5 g of powdered tissue was homogenized in 2 ml of ice-cold 3.6% (w/v) HClO_4 and centrifuged at $10000\ g$ for 11 min at 4°C . The supernatant was decanted, and the pellet was washed a minimum of five times with 3.6% HClO_4 to remove any acid-soluble radioactivity. The pellet was sequentially washed with acetone, chloroform/methanol (1:1, v/v) and water. The pellet was dissolved in 0.1 M-NaOH, and samples were assayed for protein [16], with crystalline BSA as a standard. Another sample was assayed for radioactivity by liquid-scintillation spectrometry with corrections for quenching (d.p.m.). Count rates varied depending on tissue size but were typically 2000–3000 d.p.m.

The specific radioactivity of the plasma phenylalanine was measured by h.p.l.c. analysis [17,18] of supernatants from trichloroacetic acid extracts of plasma. Samples were chromatographed on an Alltech Econosphere C_{18} ($5\ \mu\text{m}$) column with a Beckman model 450 h.p.l.c. apparatus equipped with a Beckman 171 Radioisotope Detector. The specific radioactivity was calculated by dividing the amount of radioactivity in the peak corresponding to phenylalanine by the concentration of the amino acid in the same fraction. The specific radioactivities from the three time points were averaged [19].

Rates of protein synthesis were calculated as described previously [14,15] by using the mean specific radioactivity of the plasma phenylalanine as the precursor. The assumption in using this technique to estimate the rate of protein synthesis *in vivo* is that the tissue phenylalanine concentration is elevated to such an extent that any dilution effect of non-radioactive phenylalanine derived from proteolysis on the intracellular specific radioactivity would be minimal. The plasma phenylalanine concentration was increased from approx. $70\ \text{nmol}/\text{ml}$ to $1300 \pm 146\ \text{nmol}/\text{ml}$ at the time of tissue sampling (average of all three conditions). Under the condition of these elevated plasma phenylalanine concentrations, the specific radioactivity of the plasma phenylalanine was assumed to be equal to the specific radioactivity of the tRNA-bound phenylalanine. This assumption has been demonstrated in various isolated perfused organs [20–23].

Determination of eIF-2

The amount of eIF-2 in the tissue homogenates was determined by protein immunoblot analysis using a rabbit anti-rat eIF-2 antibody specific for the β -subunit of rat eIF-2 [24]. Samples that had been boiled in SDS sample buffer were centrifuged in a table-top centrifuge for 3 min and subjected to SDS/PAGE as described previously [25]. In addition to the samples being analysed,

pure eIF-2 (serial dilutions from 27 to 872 ng) was electrophoresed on each gel as a standard. The protein in the gel was then electrophoretically transferred to Immobilon P membrane (Millipore Corp.), and the blot was developed by using alkaline phosphatase-conjugated goat anti-rabbit IgG as described by Kimball *et al.* [24]. Finally, the dried blot was subjected to densitometric scanning with a Beckman DU-8B spectrophotometer. The amount of eIF-2 in each sample was calculated by comparison with the eIF-2 standard curve from that gel [24].

Determination of eIF-2B activity in tissue extracts

eIF-2B activity was measured in postmitochondrial supernatants by a slight modification of the method described previously [26]. Livers and brains were homogenized in 4 vol. of buffer consisting of 20 mM-Tris/HCl, pH 7.5, 125 mM-KCl, 1 mM-dithiothreitol, 35 mM-sucrose and 0.1 mM-EDTA, and the homogenate was centrifuged at $10000\ g$ for 10 min at 4°C . The supernatant was assayed immediately for eIF-2B activity. Briefly, a binary complex consisting of eIF-2 and [^3H]GDP was formed exactly as described previously [27]. A $4\ \mu\text{l}$ or $15\ \mu\text{l}$ sample of supernatant from liver or brain, respectively, was mixed with $40\ \mu\text{l}$ of buffer B (50 mM-Mops, pH 7.4, $209\ \mu\text{M}$ -GDP, 2 mM-magnesium acetate, 100 mM-KCl, 1 mM-dithiothreitol and $200\ \mu\text{g}$ of BSA/ml) and $21\ \mu\text{l}$ of water. The tubes containing this mixture were incubated at 30°C for 2 min, and then the reaction was initiated by addition of $10\ \mu\text{l}$ of eIF-2·[^3H]GDP complex. At either 1 min (liver) or 3 min (brain), 2.5 ml of ice-cold wash buffer was added to each tube and the contents were filtered through a nitrocellulose filter disc. The tube was washed twice with wash buffer and the washes were filtered through the same disc. The filters were dissolved in 7 ml of Filtron X (National Diagnostics), and radioactivity was measured in a liquid-scintillation counter. Under these conditions, approx. 20% (0.3 pmol) of the [^3H]GDP was released from the eIF-2·[^3H]GDP complex. Furthermore, the amount of GDP exchanged was proportional to the amount of supernatant added to the assay.

Determination of amounts of eIF-2 α mRNA

RNA was isolated from liver, lung and brain by a modification of the method described by Chirgwin *et al.* [28]. Tissue ($\leq 7\ \text{g}$) from a single animal (liver) or a combined sample from three animals (lungs, brain) was homogenized in 100 ml of a solution consisting of 4.0 M-guanidine isothiocyanate, 25 mM-sodium citrate, pH 7.0, 0.1 M- β -mercaptoethanol, 0.5% (w/v) *N*-lauroylsarcosine and 0.1% antifoam A, with a Polytron homogenizer. The homogenate was then centrifuged at $5860\ g$ for 15 min at 10°C . The supernatant (5 ml) was layered over 6 ml of a solution of 5.7 M-CsCl and 0.1 M-EDTA, pH 7.0, and centrifuged in a Beckman SW-41 rotor at $100000\ g$ for 20 h at 15°C (four tubes per tissue were centrifuged). After centrifugation, the CsCl solution was aspirated (carefully removing all of the DNA band), and the RNA pellet in each tube was resuspended in $100\ \mu\text{l}$ of TE buffer (0.01 M-Tris, pH 7.5, 0.001 M-EDTA). The tube was washed with a second $100\ \mu\text{l}$ of TE buffer, and the wash was combined with the resuspended pellet. RNA was then precipitated by addition of 0.1 vol. of 2.0 M-sodium acetate and 2.5 vol. of ethanol, followed by overnight incubation at -80°C . RNA was pelleted by centrifugation at 4°C for 15 min in a table-top micro-centrifuge. The pellets were washed twice with $500\ \mu\text{l}$ of 70% (v/v) ethanol and then dried in a Savant Speedvac for 15 min. The pellets were resuspended in $400\ \mu\text{l}$ of RNAase-free water and stored at -80°C . The A_{260}/A_{280} ratio was > 2.0 for all RNA preparations.

Poly(A)⁺ RNA was isolated from total RNA by using a kit (PolyAtract mRNA Isolation System) from Promega. The system uses a biotinylated oligo(dT) probe to hybridize with

poly(A)⁺ RNA in the sample. The hybrids are then captured by using streptavidin coupled to paramagnetic particles. This procedure yields poly(A)⁺ RNA with little or no contamination with 18 S or 28 S rRNA. Briefly, total RNA from three separate preparations was combined and diluted to 1.215 ml with RNAase-free water and then incubated at 65 °C for 10 min. Biotinylated oligo(dT) probe (5 µl) and 30 µl of 20 × SSC (3 M-NaCl/0.3 M-sodium citrate) were added and the sample was cooled to room temperature. The streptavidin magnisphere particles (SA-PMP) were washed three times with 0.5 × SSC, and 0.25 ml of SA-PMP was added to the cooled sample. After a 10 min incubation at room temperature, the particles were collected with a magnetic separator stand and thoroughly washed with 0.1 × SSC. The poly(A)⁺ RNA was eluted with 1 ml of RNAase-free water, and the samples were stored at -80 °C as ethanol precipitates.

The amount of eIF-2α mRNA in the poly(A)⁺ RNA samples was determined by slot-blot analysis. Radiolabelled probes were prepared by using a kit (Multiprime DNA Labelling System) from Amersham. The cDNA for the α-subunit of eIF-2 (pHr-2α) was generously provided by Dr. John W. B. Hershey of the University of California, Davis, CA, U.S.A. [29]. Radiolabelled probes were separated from unincorporated nucleotides before use by gel-filtration chromatography on Pharmacia Nick columns equilibrated in TE buffer. For preparation of the blot, RNA was dissolved in water, and 2.9 vol. of a solution of 69 % (v/v) formamide, 9 % (w/v) formaldehyde and 1 × SSC were added. The mixture was incubated at 68 °C for 15 min, cooled, and 2 vol. of 20 × SSC was added. The RNA was then applied to a nitrocellulose membrane with a slot-blot apparatus (Schleicher and Schuell) and cross-linked to the membrane by exposure to u.v. irradiation in a UV Stratalinker 1800 (Stratagene). Before hybridization, the blot was incubated for 1 h at 42 °C in 6 ml of hybridization buffer [0.05 M-Tris/HCl, pH 7.5, 0.5 M-NaCl, 10 % (w/v) dextran sulphate, 1 % (w/v) SDS, 0.4 % (w/v) BSA, 0.4 % (w/v) polyvinylpyrrolidone, 0.4 % (w/v) Ficoll 400, 50 % (v/v) formamide and 100 µg of salmon sperm DNA/ml] in a Hybridization Incubator (Robbins Scientific). Radiolabelled probe (3 × 10⁷ c.p.m. of pHr-2α or 1.2 × 10⁷ c.p.m. of β-actin probe) was then added to the tube containing the blot and incubation at 42 °C was continued overnight. The blot was then washed twice (20 min each) at room temperature with 2 × SSC, twice (20 min each) at 65 °C with 2 × SSC/1 % SDS, and once for 30 min at 65 °C with 0.2 × SSC/0.1 % SDS. The blot was then exposed to Kodak XAR film for 3–4 h. The autoradiograph was scanned with a Beckman DU-8B spectrophotometer.

RESULTS

Initially, the animals used in these experiments were characterized as to body weight and tissue wet weight, protein content, and RNA content. The body weights of the animals that were 1 month old was 117.7 ± 4.0 g (*n* = 31), 4 months old 402.2 ± 5.0 g (*n* = 30) and 10 months old 579.0 ± 12.6 g (*n* = 33). The wet weight of all of the tissues examined was significantly (*P* < 0.0025) greater in 4-month-old than in 1-month-old animals (Fig. 1a). The increase in tissue mass between 4 and 10 months of age was also significant (*P* < 0.01) in liver, brain and spleen. There was a general trend towards an increase in protein content with increasing age (Fig. 1b). The protein content of liver, brain and lung was significantly (*P* < 0.01) greater in 4-month-old than in 1-month-old rats. However, only the brain exhibited a significant (*P* < 0.005) increase in protein content in 10-month-old compared with 4-month-old animals. The content of RNA was significantly (*P* < 0.01) less in all tissues from 10-month-old than in 1-month-old rats (Fig. 1c). In addition, the RNA content

in liver, brain, kidney and spleen of 4-month-old animals was lower (*P* < 0.01) than in 1-month-old animals. Since rRNA represents 80–90 % of total RNA, the 20–25 % decrease in total RNA observed in each of these tissues suggests that significantly fewer ribosomes are available to synthesize protein in older animals than in 1-month-old animals. Thus, in part, the decrease in protein synthesis in tissues from older animals is due to a decrease in the number of ribosomes.

Although several studies have reported that the rate of protein synthesis decreases with increasing age in rats, the magnitude of the effect varies from one laboratory to another. In particular, the rate of protein synthesis in rat liver is reported to decrease from 10 % to 75 %, depending on the method used to measure protein synthesis and the age and strain of animals used (reviewed in [30]). Therefore the rate of protein synthesis was measured in each of the tissues examined, so that a direct comparison could be made between eIF-2 content and the synthesis rate (Fig. 2). The rate of protein synthesis did not change with increasing age

Fig. 1. Age-related changes in tissue weights and protein and RNA contents

(a) Tissue weight (g). (b) Protein content and (c) RNA content were determined as described in the Materials and methods section. The results represent the mean ± S.E.M. of 8–33 determinations.

Fig. 2. Rate of protein synthesis in tissues from 1-, 4- and 10-month-old rats

The rate of protein synthesis was determined *in vivo* as described in the Materials and methods section. The results are expressed as nmol of phenylalanine incorporated into protein/h per g wet wt. and represent means $\pm$ S.E.M. of either 6 or 7 animals for each age group.

Fig. 3. eIF-2B activity in tissue extracts from 1-, 4- and 10-month-old rats

eIF-2B activity was measured in post-mitochondrial supernatants by the GDP-exchange assay as described previously [26]. The results are expressed as a fraction of the total eIF-2. [3 H]GDP added to the assay and represent means $\pm$ S.E.M. of 7 experiments. Within each experiment, tissue from 3 animals of each age was analysed, and each of the analyses was performed in duplicate.

in either kidney or lung. In contrast, protein-synthesis rates were significantly lower ($P < 0.05$) in liver, spleen and brain at 10 months of age than the rates measured in the corresponding tissues from 1-month-old animals. In addition, the rate of protein synthesis was significantly lower ($P < 0.05$) in spleen from 4-month-old than in 1-month-old animals.

Since eIF-2 activity is significantly decreased in liver [11] and brain [4–6] during aging, and because a decrease in eIF-2B activity could cause a decrease in eIF-2 activity measured in tissue extracts, eIF-2B activity was measured in post-mitochondrial extracts from liver and brain. The activity of eIF-2B in extracts from brain was only approx. 20% of that measured in liver extracts, and the activity declined with increasing age in both tissues (Fig. 3). In particular, the decrease in the amount of eIF-2B activity in extracts of livers from 4- and 10-month-old rats, to 79% and 70% of the activity in extracts of livers from 1-month-old animals, was similar in magnitude to the observed decrease in protein-synthesis rate, to 81% and 68% of control respectively. Likewise, in extracts of brain from 4- and 10-month-old rats eIF-2B activity and the rate of protein synthesis

Fig. 4. eIF-2 content in various tissues from 1-, 4- and 10-month-old rats

The content of eIF-2 in various tissues was determined by protein immunoblot analysis using a rabbit anti-(rat eIF-2) antibody as described in the Materials and methods section. The results represent means $\pm$ S.E.M. of 4–9 experiments. Within each experiment, tissue from 2–3 animals of each age was analysed.

Fig. 5. Correlation of eIF-2 content and protein-synthesis rate in animals 1, 4 and 10 months old

The amount of eIF-2 and the rate of protein synthesis in various tissues were determined as described in the Materials and methods section. Black, white, and white symbols with dots in the centre represent values from 1-, 4- and 10-month-old animals respectively: ●, liver; ■, spleen; ▲, kidney; ◆, lung; ▼, brain.

each declined to similar extents compared with the values observed in 1-month-old control rats. Thus a decrease in eIF-2B activity may partially account for the observed decrease in protein synthesis that occurs during aging.

A second mechanism that could account for a decrease in eIF-2 activity with age would be a fall in the amount of eIF-2 protein in the tissue. Therefore the amount of eIF-2 in spleen, liver, kidney, lung and brain was measured by protein immunoblot analysis using a polyclonal antibody specific for the β -subunit of rat eIF-2 [24]. The amount of eIF-2, expressed relative to total tissue protein, declined with increasing age in all tissues examined (Fig. 4). The decrease in amount of eIF-2 between 1 and 10 months of age was greatest in lung (58%) and least in brain (21%).

An interesting observation was that the amount of eIF-2 in the

Fig. 6. Slot-blot analysis of poly(A)⁺ RNA from liver, lung and brain from 1-, 4- and 10-month-old rats

Poly(A)⁺ RNA was isolated as described in the Materials and methods section. The amount of eIF-2 α mRNA and β -actin mRNA present in each sample was determined by slot-blot analysis as described in the Materials and methods section. For each sample, a series of 1:2 dilutions was prepared and applied to nitrocellulose to ensure that the response was linear. The analysis was repeated twice, with essentially identical results obtained in each case. A representative result is shown for each sample.

Fig. 7. eIF-2 α mRNA content in liver, brain and lung from 1-, 4- and 10-month-old rats

The amount of eIF-2 α mRNA was determined by slot-blot analysis as described in the Materials and methods section. The amount of eIF-2 α mRNA was normalized by using the relative amount of β -actin mRNA for each sample.

various tissues was directly proportional to the rate of protein synthesis (Fig. 5). Least-squares analysis of the data yielded a straight line with a correlation coefficient of 0.89. Thus the tissue that exhibited the highest rate of protein synthesis, i.e. liver from 1-month-old animals, also had the highest eIF-2 content, whereas the tissue with the lowest rates of protein synthesis, i.e. brain, also had the lowest eIF-2 content. These results suggest that eIF-2 content may be rate-limiting for protein synthesis in these tissues.

The amount of mRNA coding for the α -subunit of eIF-2 was measured by slot-blot analysis of poly(A)⁺ RNA isolated from liver, brain and lung (Fig. 6). The blots shown in Fig. 6 were scanned as described in the Materials and methods section. The values obtained by using the eIF-2 α cDNA were normalized by

using data obtained for β -actin, since the level of β -actin mRNA in liver, spleen, and brain is the same in 24-month-old rats as in young animals [31]. There was no consistent trend in changes in the relative abundance of eIF-2 α mRNA in either liver or lung with increasing age (Fig. 7). However, in brain the amount of eIF-2 α mRNA relative to total poly(A)⁺ RNA gradually increased from 1 to 10 months of age. It is noteworthy that the magnitude of the change in a eIF-2 α mRNA content observed in all three tissues was small and was probably not significant.

DISCUSSION

The rate of protein synthesis can be regulated by changes in either the capacity for or the efficiency of protein synthesis. The efficiency of protein synthesis is a reflection of the activity of the individual components of the protein-synthetic machinery. Capacity refers to the quantity of the components involved in the synthesis of protein, which includes ribosomes, mRNA, tRNA, aminoacyl-tRNA synthetases and initiation, elongation and termination factors. In part, the age-dependent decline in protein synthetic rate can be attributed to a decrease in the capacity for protein synthesis, since the content of RNA in all tissues examined fell with increasing age of the animal. However, the decrease in RNA content with increasing age is not of sufficient magnitude to account completely for the diminution of protein synthesis that occurs in liver, spleen or brain, since the decrease in protein-synthesis rate is greater than the decline in RNA content.

The efficiency of translation can be regulated through alterations in the activity of peptide-chain initiation and/or elongation factors. For example, the activities of both eIF-2 and eukaryotic elongation factor 1 (eEF-1) decrease in rat and mouse brain with increasing age [5]. However, in brain, polysomal aggregation diminishes with age [32,33]. This type of shift in the distribution of ribosomes is characteristic of a decrease in the rate of initiation of peptide chains relative to the rate of elongation [34]. Therefore, although elongation may also be decreased, modulation of peptide-chain initiation is probably more important in the age-dependent decline of protein synthesis than is regulation of elongation.

A decrease in eIF-2 activity could be due to a decrease in the tissue content of eIF-2 protein, or a decrease in the activity of eIF-2B, which would lead to an apparent fall in eIF-2 activity, or a specific inhibition of eIF-2 activity. In the present study, there was a downward trend in the amount of eIF-2 with increasing age in all tissues examined. This result suggests that a decrease in the amount of eIF-2 may, at least partially, account for the decrease in protein-synthesis rate that occurs with age in some tissues. Furthermore, eIF-2 content was directly proportional to the rate of protein synthesis, suggesting that the content of eIF-2 is important in regulating the synthesis rate.

The activity of eIF-2B was less in extracts of brain and liver from older rats than in extracts from 1-month-old animals. This decrease could have been due to a specific inhibition of eIF-2B. For example, *in vitro*, the activity of eIF-2B can be modulated by phosphorylation of the ϵ -subunit of the protein by casein kinase II [35]. In addition, eIF-2B activity is inhibited *in vitro* by NADP⁺ [36]. Addition of equimolar amounts of NADPH reverses the inhibition caused by NADP⁺. Alternatively, as discussed above, the activity of eIF-2B can be modulated through changes in the phosphorylation state of the α -subunit of eIF-2. Which, if any, of these mechanisms is involved in the decrease in eIF-2B activity in brain and liver with age is unknown.

It is noteworthy that, although the amount of eIF-2 diminishes with age in each of the tissues examined, the amount of eIF-2 α mRNA does not decrease in parallel with the protein. However, the amount of eIF-2 in liver and brain only decreased by approx.

25%. A decrease in eIF-2 α mRNA of this magnitude might not have been detected in the slot-blot analysis. In contrast, the amount of eIF-2 in lung fell by approx. 60%. A decrease in mRNA of this magnitude would certainly have been detected, since serial 1:2 dilutions of poly(A)⁺ RNA were applied to each blot and a decrease in signal was easily detected at each dilution (results not shown). These results suggest that, at least in lung, regulation of the synthesis of eIF-2 during aging may occur of the translational rather than the transcriptional level. A second possibility that could account for these observations would be if the rate of degradation of eIF-2 was increased in lung from 10- versus 1-month-old rats. However, since the concentration of total protein is increased in 10- compared with 1-month-old rats (Fig. 1), an increase in protein degradation would have to be specific for eIF-2.

Overall, the results presented herein suggest that the diminished rate of protein synthesis in brain during aging may have a multifactorial etiology. It seems likely that the decrease in RNA and eIF-2 concentrations and the fall in eIF-2B activity reported in the present study, in combination with the decrease in eIF-2 and eEF-1 activities reported elsewhere [5], may all play a role in the decremental changes in protein synthesis that occur in brain during aging. Finally, the large decrease in eIF-2 activity in brain of old versus young rats is not due to a decrease in eIF-2 concentration and is probably not due to a decrease in eIF-2B activity. This observation suggests that eIF-2 activity may be regulated by a different, and therefore novel, mechanism in brain during aging.

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