Age-dependent change in the ability of protein synthesis by rat liver microsomes-significance of 2 associated factors

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Summary. An activator and an inhibitor of polypeptide synthesis were isolated from microsomes of rat liver. The young rat contained a higher level of the activator but a lower level of the inhibitor than the aged rat. The significantly higher ratio of the specific activity of the activator to inhibitor in the young microsomes helps explain the higher capacity for protein synthesis of these microsomes as compared to the microsomes of the aged animal.

The ability of protein synthesis by liver microsomes of young mice and rats is higher than that of old animals[2,3]. The ribosomes, however, have comparable protein-synthesizing capabilities. This suggests that microsomes contain substances (probably removed during ribosome preparation) which influence protein synthesis. We have observed that rat liver microsomes contain 2 'factors', one of which stimulates (an activator) and the other inhibits (an inhibitor) cell-free polypeptide synthesis. The level of the activator in young microsomes is higher than in old microsomes, whereas the reverse is true for the inhibitor. These observations, which help explain the higher activity of young microsomes, form the basis of this communication.

Materials and methods. Male Wistar rats were used. Ribosomes, microsomes, aminoacyl transferase and pH 5 enzyme were prepared essentially by published methods[4,5]. All preparations were stored in liquid N_2. The RNA[6]: protein[7] ratios of adult microsomes and ribosomes had mean values of 0.2 and 0.7 respectively. The cell-free polypeptide synthesizing system (ribosomes and enzymes were from adult liver) contained in a final

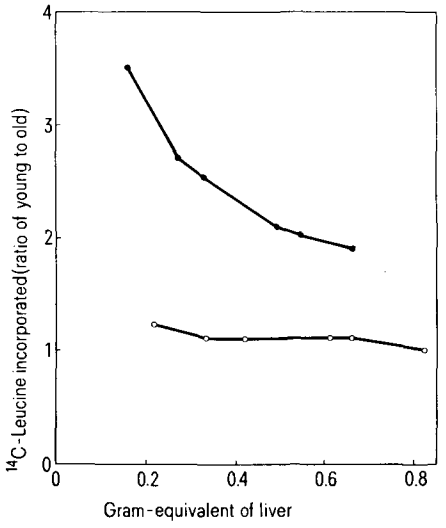

Fig. 1. Comparison of the activities of young and aged rat liver microsomes as well as ribosomes. Each point in the graph shows the ratio of the amount ^{14}C-leucine incorporated by 'young' (3-week-old) to 'aged' (1-year-old) liver microsomes (-●-●-) or ribosomes (-○-○-). The amino acid incorporating system was the same as described in the text, except that the ribosome was replaced by test microsome or ribosome. The g-equivalents of liver corresponding to microsomes or ribosomes, are represented in abscissa. The amount of microsomes (in terms of RNA) obtained from equal amounts of livers of the young and aged rats was comparable. For example, 17 g of young and 16 g of aged liver yielded 13 and 14 mg of RNA (microsomes) respectively.

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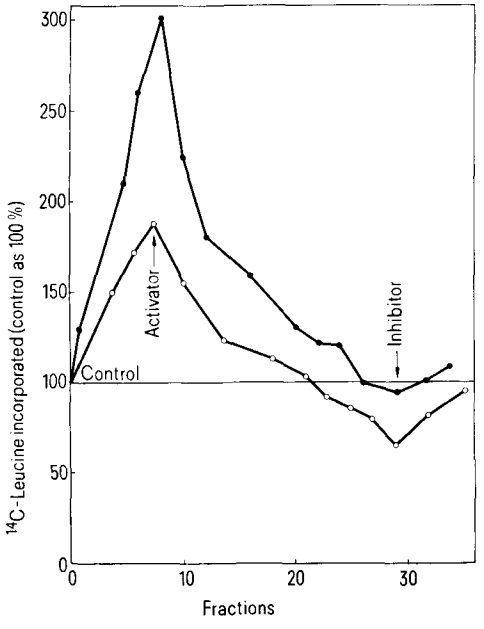

Fig. 2. Separation of activator and inhibitor of protein synthesis from young and aged rat liver microsomes. Microsomes equivalent to 22 g of liver of either 'young' or 'aged' rats were extracted, about 8 ml extract (= 20 mg protein) separated by filtration through sephadex G-200 gel column (dimension = 20 × 1.8 cm) into different fractions and 0.2 ml of fractions tested for their effects on ^{14}C-leucine incorporation in the cell-free system (see text for details). Protein in the aliquot having maximum activator activity contained 1.5%, while the corresponding aliquot with maximum inhibitor activity represented 0.7% of total protein passed through the column. The 'void volume' of the column, as measured by exclusion of dextran blue 2000 (Pharmacia Fine Chemicals, Sweden) was 12.1 ml. The maximum activator and inhibitor activities were associated with fractions corresponding to elution volumes of 14.3 and 30.8 ml respectively. ^{14}C-leucine incorporated in presence of fraction from young (-●-●-) and aged (-○-○-) rats, are shown as percent of control (buffer medium replacing fraction). The radioactivity/min measured in the control (100%) = 7.5 pmoles of ^{14}C-leucine incorporated.

volume of 0.5 ml: 60 mM tris-HCl (pH 7.4), 1 mM dithiothreitol, 50 mM KCl, 4 mM $MgCl_2$, 0.3 mM GTP, 1 mM ATP, 5 mM phosphoenolpyruvate, 10 μg pyruvate kinase, pH 5 enzyme (600 μg protein), amino acyl transferases [200 μg protein of 35–65% $(NH_4)_2$ SO_4 fraction], 2 μmoles of ^{14}C-L-leucine (sp. act. 280 mCi/mmole, New England Nucl. Corp.) and 4 mM of each of 19 L-amino acids except leucine. The reaction was started by ribosomes ($10A_{260\,nm}$ units) and run for 15 min at 37 °C (linear for this time). Polypeptide synthesized was measured by the radioactivity incorporated into trichloracetic acid-insol. material as described before[8]. Fractions of microsomal extracts, tested in the above system, were obtained as follows: Microsomes were sonicated (M.S.E. sonicator, 2 amp. current output, 1 min at 0 °C) in a medium (microsomes equivalent to 1 g liver/0.6 ml of 5 times diluted buffer, pH 7.4 containing 20 mM tris-HCl, 1 mM dithiothreitol, 50 mM KCl and 4 mM $MgCl_2$). An aliquot (20 mg protein) of the clear sonicate (obtained by centrifuging at 105,000 × g for 90 min at 4 °C) was than chromatographed on a sephadex G-200 (Pharmacia) gel column (20 × 1.8 cm) and elution of fractions being done by the same buffer. 40 drops were collected per fraction and 0.2 ml of suitable fractions tested for their effects in the cell-free system. The ages of the 'young' and 'aged' rats were 3 weeks and 1 year respectively.

Results and discussion. Figure 1 compares between young and aged rats the rates of ^{14}C-leucine incorporation by the liver microsomes as well as ribosomes. Each point gives the ratio of the values for radioactivity incorporated by the sample from the young rat to that by the sample from the aged animal; a value greater than 1 signifies an activity higher for the test material from the young as compared to that from the aged. It is seen that at all concentrations tested, the young microsomes have a higher capacity for polypeptide synthesis than the aged microsomes, the difference being specially marked at the lower concentrations. The ribosomal activities in the 2 age-groups are, however, comparable. Figure 2 shows the effects of fractions from the extracts of microsomes of young and aged rats, on cell-free polypeptide synthesis. It is observed that the microsomes of both young and aged rats contain an activator as well as an inhibitor. But in the young, the level of the activator is conspicuously higher and that of the inhibitor lower than the corresponding levels in the aged.

The nature of the 2 'factors' is yet to be clarified. But a role in physiological regulation is expected, in view of the fact that these are associated with microsomes which are the sites of cellular protein synthesis. We[3] found the specific activity (per 100 μg protein) of the activator to that of the inhibitor to be 5–10 in the young microsomes as against only 1 in the aged. The higher ratio in the young is in keeping with their higher capacity to synthesize protein.

Hoagland et al.[9] found in liver microsomes a heat-labile inhibitor whose effect was antagonized by GTP. The inhibitor, described here, is heat-stable (90 °C for 5 min) and its activity, in our hands, is not counteracted even by a 5fold increase of GTP. The inhibitor was tested in this study by using the rat liver system requiring 4 mM Mg^{+2}. It has since been revealed[10] that the same inhibitor is twice as effective in inhibiting ^{14}C-phenylalanine incorporation in a polyuridylic acid (poly U)-dependent polyphenylalanine synthesizing system with 10 mM Mg^{+2}. Using this system, we found[11] that the site of action of the inhibitor lies in the step leading to aminoacylation of tRNA, a reaction prerequisite for protein synthesis. The activator, on the other hand, was found to be heat-labile and lost 50–60% of its stimulatory activity in 72 h even when kept frozen at −20 °C[12]. It showed maximum effect in the rat liver system at 4 mM Mg^{+2}, being totally ineffective in the poly U-directed polyphenylalanine synthesizing system under optimal condition (10 mM Mg^{+2}). Interestingly, these characteristics are reminiscent of those ascribed originally by Miller and Schweet[13] to reticulocyte ribosomal extract known to contain protein initiation factors. Work is underway to delineate the site of action of the activator. This is of particular interest, since the same activator appears also to be implicated in changes associated with other physiological conditions, such as hormone action. Thus, administration of glucocorticoids to adrenalectomized rats induced an increase in the level of the activator in the liver microsomes and concomittantly led to an increased ability for protein synthesis by those microsomes[12].

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Light evoked release of radioactivity from rabbit retinas preloaded with (^{3}H)-GABA[1]

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Summary. Light flashes evoke an increased release of radioactivity in vitro from rabbit retinas preloaded with (^{3}H)-GABA in vivo. Constant light does not affect the release. No light evoked release can be demonstrated from the glia. Pentobarbitone and AOAA depress the evoked release. The results are consistent with GABA being a retinal neurotransmitter, most likely in a class of amacrines.

There is now increasing evidence that γ-aminobutyric acid (GABA) functions as a neurotransmitter in the vertebrate retinas. It occurs naturally in retinal tissue[2–4] and a high affinity uptake system[5–7] and enzyme systems for its formation and metabolism have also been demonstrated[4, 8–10]. In rabbit retina, exogenous GABA is taken up in vivo in cells that have, in the main, the location and distribution of amacrine cells, but also in some ganglion cells[11, 12]. Moreover GABA has an inhibitory effect on the firing of retinal ganglion cells[13–16], and the GABA inhibitors picrotoxin, bicuculline and N-methyl bicuculline affect retinal function[14, 17, 18].