ENZYME REGULATION DURING DEVELOPMENT AND AGING

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Summary

Glutamine synthetase and ornithine aminotransferase activities have been shown to decrease in aging rats, but the decline varies in degree for the two enzymes in liver and kidney. The responsiveness of the two liver enzymes to glucagon and cyclic AMP demonstrates a similarity in enzyme regulation between developing and aging rats and a contrast in enzyme regulation between these animals and young adults. The results are interpreted as showing a reversion in certain aspects of enzyme regulation during aging to those prevailing in the perinatal state.

Introduction

Earlier studies on decrease in enzyme activities associated with aging have been made with mammalian erythrocytes because of their ease of fractionation into different age groups. Most of these studies involved enzymes of carbohydrate metabolism (1-3). However, changes accompanying aging of one kind of cells may not represent the derangement arising from aging of an organism. Recently, enzymes in other metabolic pathways have received attention and a decline of their activities in aging mammals has consistently been observed (4-8). One enzyme that has been studied in some detail in connection with aging is aldolase. Gershon and Gershon (9) reported a decline of liver aldolase activity in aged mice and showed that the decline resulted from formation of inactive enzyme molecules.

Comparison of basal enzyme activities in tissues of animals representing two physiologically different states may not reveal the potentiality of the enzymes to respond when challenged by a stress. For instance, many rat hepatomas had basal glutamine synthetase activity similar to that of normal liver, but the response of the enzyme in the hepatomas to hormonal and nutritional stress was quite different from that in normal liver (10). Hence, it would be informative to study not only the change in the basal activities of enzymes during

aging, but also the capability of the enzymes to respond to a given metabolic stimulus. In this communication, we report our observations on two enzymes in amino acid metabolism, glutamine synthetase (EC 6.3.1.2) and ornithine aminotransferase (EC 2.6.1.13).

Materials and Methods

Animals-- Rats of the Sprague-Dawley and Fischer 344 strains were used. Sprague-Dawley rats were obtained from Spartan, Inc. (Haslett, Mich.); Fischer rats, from Charles River Labs. (Wilmington, Mass.) through the National Institute on Aging.

Hormonal administration-- Glucagon (Eli Lilly, ampoules) and cyclic AMP (Sigma Chemical Co., dissolved in 0.9% NaCl and adjusted to pH 7.4) were injected subcutaneously at 1.5 and 50 μg, respectively, per g body weight at 12-hr intervals for 24 hr, the last injection being 12 hr before enzyme assay.

Enzyme assay-- Tissue homogenates prepared in 0.15 M KCl - 0.01 M Tris buffer, pH 7.0, were used for enzyme assays. Glutamine synthetase activity was assayed as before (11), but with the addition of an ATP-regenerating system (12). The enzyme activity is expressed as μmoles of γ-glutamylhydroxamate formed/ g tissue/ hr. Ornithine aminotransferase activity was assayed according to Herzfeld and Knox (13). The enzyme activity is expressed as μmoles of glutamate γ-semialdehyde formed/ g tissue/ hr.

Protein determination-- The procedure of Lowry et al. (14) was used with bovine serum albumin as the standard.

Results

In the two tissues we used, liver and kidney, the protein concentration showed variations with age. Thus, in the three groups of 3-, 16-, and 28-month old male Fischer rats, the liver protein values were 193 ± 14, 242 ± 29, and 181 ± 12 mg/ g liver, respectively, and the kidney protein values were 201 ± 15, 193 ± 17, and 149 ± 8 mg/ g kidney, respectively. Hence, the protein concentrations in both liver and kidney of 28-month old rats were lower than those of 16-month old rats.

Liver glutamine synthetase activity showed a definite decline with age in both strains of the rat. Table 1 summarizes the findings. The activity in 14- and 16-month old rats had decreased to about 53% of that in 3-month old rats. Possibly, the loss in activity had begun some time before the animals were 14 months old. The enzyme activity continued to decline so that in 28-month old rats it was only 32% of that in young adults. However, the kidney enzyme did not show any significant lowering until the animals had attained old age. The life span of the rat is about 3 years. Hence, 28 months in the rat may be considered as equivalent to 70 years of human life.

Liver ornithine aminotransferase activity appeared to be less affected by

Table 1.   Decline of glutamine synthetase and ornithine aminotransferase
activities in liver and kidney of aging male rats

Age of rats (strain)$^\alpha$	Synthetase activity		Aminotransferase activity	
	Liver	Kidney	Liver	Kidney
3 months (S-D)	260 ± 28$^\beta$	86.5 ± 3.1	123 ± 17	187 ± 25
14 months (S-D)	138 ± 33	63.0 ± 16.0	124 ± 31	126 ± 19
3 months (F)	339 ± 47	69.9 ± 9.1	102 ± 10	175 ± 45
16 months (F)	179 ± 23	63.8 ± 6.5	91.5 ± 20.6	172 ± 13
28 months (F)	110 ± 17	38.4 ± 6.2	67.5 ± 6.9	186 ± 26

$^\alpha$Abbreviations used: S-D, Sprague-Dawley; F, Fischer 344.
$^\beta$Standard deviation.

aging than liver glutamine synthetase activity. The enzyme activity remained
unchanged throughout most of the life of the rat and showed only a 34% decrease
at 28 months of age. On the other hand, kidney ornithine aminotransferase ac-
tivity was not affected at all by aging. In fact, the specific activity increased
since the protein concentration decreased in aged rats as noted above.

In order to be certain that lower activities observed in aging rat tissues
were not due to rapid deterioration of the enzymes in vitro, we assayed the en-
zyme activities again in the homogenates after being kept at 4° for 24 hours.
Compared with the activities in fresh homogenates, glutamine synthetase activi-
ty declined 12 and 17% in 24 hours in liver homogenates of 3- and 28-month old
rats, respectively, while ornithine aminotransferase activity decreased 22 and
24% in kidney homogenates of the same animals. Hence, the two enzymes from
aged rats were as stable in vitro as those from younger rats.

Determination of the basal activity of an enzyme often does not reveal the
potentiality of the enzyme to respond to a given stimulus when challenged.
Therefore, we did experiments to determine the responsiveness of the two liver
enzymes to metabolic modulations. Moreover, we wanted to compare the re-
sponses seen in aging and developing rats with those seen in young adults,
which we used as a frame of reference.

Table 2.   Non-responsiveness of liver glutamine synthetase in neonatal
and aged male rats to glucagon

Age of rats (strain)	Synthetase activity		
	Control	Glucagon	% Change
10 days$^{\alpha}$ (S-D)	75.0 ± 2.2	77.2 ± 3.4	+ 2.9
3 months (S-D)	269 ± 30	199 ± 8	− 26.0
3 months (F)	339 ± 47	229 ± 29	− 32.4
28 months (F)	106 ± 15	114 ± 22	+ 7.5

$^{\alpha}$Age in days refers to age at end of the experiment; the control and ex-
perimental groups were litter mates of both sexes.

Table 2 shows the response of liver glutamine synthetase to glucagon in
neonatal, young adult, and aged rats. We can see that the hormone lowered the
enzyme activity in young adults by about 30%, but it had no effect on the en-
zyme activity in either neonatal or aged rats. Apparently, the aged rat has as-
sumed a mode of regulating glutamine synthetase by departing from that found in
the young adult rat and returning to that observed in the developing rat. Con-
tinued injections of glucagon for 3 days lowered the liver enzyme activity further,
although the enzyme activity in kidney, brain, and testis was not appreciably
affected by the hormone (12). Addition of glucagon to the reaction mixture did
not affect the enzyme activity, nor did injection of cyclic AMP evoke the gluca-
gon effect. The mechanism underlying this effect deserves further study.

We also investigated the effect of cyclic AMP administration on liver orni-
thine aminotransferase. Table 3 presents the results. The developmental for-
mation of this liver enzyme shows a drastic decline in activity on the 10th day
of life. Herzfeld and Greengard (15) have observed a similar decline in the en-
zyme activity with a somewhat different magnitude on the 8th day of life of the
rat. We can see that, between the age of 5 and 15 days, cyclic AMP raised liver
ornithine aminotransferase 50-100% above the control levels. By the time the
animals were 20 days old, however, the enzyme was unresponsive to the cyclic
nucleotide, as was the enzyme in young adult rats. Furthermore, as the animals
grew older, the neonatal pattern of regulation reemerged so that liver ornithine

Table 3.   Over-responsiveness of liver ornithine aminotransferase in neo-
natal and aging female rats to cyclic AMP

Age of rats[α]	Aminotransferase activity		
	Control	Cyclic AMP	% Change
5 days	16.5 ± 0.4	23.8 ± 1.0	+ 44.2
10 days	6.6 ± 1.1	13.3 ± 1.1	+ 102
15 days	26.2 ± 2.5	40.4 ± 3.9	+ 58.9
20 days	78.4 ± 4.4	84.3 ± 3.2	+ 7.5
3 months	289 ± 17	300 ± 34	+ 3.8
16 months	177 ± 34	354 ± 35	+ 100

[α]All rats were of the Sprague-Dawley strain. Age in days refers to age
at end of the experiment. Each age group of neonatal rats was from a sepa-
rate litter, but the control and treated rats of the same age were litter mates
of both sexes.

aminotransferase became fully responsive to cyclic AMP again in 16-month old
rats.

Discussion

The results presented herein show that the two enzymes, glutamine synthe-
tase and ornithine aminotransferase, followed two patterns of decline in liver of
aging rats. On the one hand, glutamine synthetase activity already showed a
significant decline when the animals were 14 months old and the decrease inten-
sified with advancing age. On the other hand, ornithine aminotransferase ac-
tivity did not show a significant decline until the animals reached 28 months of
age. Moreover, for the same enzyme, the magnitude of decrease was greater in
liver than in kidney. For this reason, ornithine aminotransferase activity in kid-
ney showed no discernible decline with age. We are currently studying the mech
anism responsible for the decrease in these two enzymes in aging rats.

In the present study, we have observed that the competence of aging ani-
mals to respond to, or to accommodate, a given stimulus also shows deviation
from that of young adults. Thus, the response of the two enzymes to glucagon
and cyclic AMP in aging rats is different from that in young adults, but is simi-

lar to that in neonatal rats. If ontogenic development involves programmed elaboration of its biochemical control machinery through enzymic differentiation (16), then aging would represent an accumulation of multiple failures by the organism to maintain this differentiation. Apparently, the aging phenomenon can be looked upon as an altered enzyme regulation. In a way, the aging organism does not develop a new regulatory mechanism, but repossesses what it once used in the neonatal state. We consider this shift in enzyme regulation during aging from that prevalent in the early adulthood to that operative in the neonatal state as a step backward, since the aging organism has lost its ability to maintain a more elaborate metabolic control machinery in exchange for a less elaborate regulatory apparatus. This reversion or return to the infantile regulation triggers aging. Accordingly, rejuvenation may be considered a process in which such a reversion has been prevented or postponed.

Earlier, we proposed a reversion hypothesis to explain the apparent coincidence in enzyme regulation between perinatal and neoplastic tissues and the apparent contrast in enzyme regulation between adult and neoplastic tissues (17, 18). Admittedly, the proposed scheme is simplistic, but the central idea behind it has been consistent with experimental findings. More recently, this concept has received support from a study of isozyme patterns in rat hepatomas by Weinhouse (19), who reported that normal adult pattern of a number of liver isozymes "is reversed" to the primordial isozyme pattern in hepatomas. In the present study, we have shown a similar phenomenon of reversion of enzyme regulation in aging rats to that in developing rats. Perhaps, the physiological process of aging and the pathological state of neoplasia adopt certain altered mechanisms common to both of them.

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