Effect of Age Upon Guanyl Cyclase, Adenyl Cyclase, and Cyclic Nucleotide Phosphodiesterases in Rats[1] (37326)

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The secretion and action of most of the hormones and many metabolic processes have been shown to be interrelated to levels and actions of cAMP[2] (1). There are relatively few reports of corre'ations of age with tissue levels of cAMP or with the enzymes involved in the synthesis (via adenyl cyclase) or degradation (via phosphodiesterases) of this cyclic nucleotide. Moreover, we have found no reports of correlations of age with tissue levels of cGMP[2] or with enzymes involved in its synthesis (via guanyl cyclase) or degradation (via phosphodiesterases).

While studying assay procedures to measure the activities of guanyl cyclase, cGMP PDE[2], adenyl cyclase, and cAMP PDE, it became apparent that the age of the animals from whose tissues the enzymes were prepared influenced these enzyme activities. This paper reports results of assays of these 4 enzymes in liver, heart and skeletal muscle and lung of rats from 26 to 300 days old. The data show marked decreases in enzyme activities associated with aging.

Materials and Methods. In an effort to avoid many biochemical reactions that could influence erroneously our enzyme assays, we applied procedures for rapid sacrifice of the rats (without drug use), and immediate assay of tissue homogenates or freezing of the tissues. We desired to use whole homogenate as much as would permit satisfactory results. The shortest possible assay incubation times were used to measure sufficient activity of the 4 enzymes because many subsidiary changes develop that influence enzyme activity (unpublished observations).

Eight groups of healthy male Wistar rats of various ages were obtained from Simonsen Laboratories, Gilroy, California, housed for 4 days at 76°F, and maintained on Purina chow. The ages and weights (median) were as follows: (a) 26 days, 55 g; (b) 45 days, 160 g; (c) 70 days, 380 g; and (d) 300 days, 600 g. At 5:00 PM the evening before sacrifice, Purina chow was replaced by granulated sucrose until 6:30 AM; no food was permitted for the final 3 to 4 hr of life. Sucrose was chosen to replace regular food because of its more rapid absorption and assimilation. This dietary change was applied to decrease hormonal and other changes produced by fasting or recent food ingestion.

At approximately 10:00 AM, the rats were sacrificed by rapid cervical and abdominal transections and specimens of liver, heart, muscle, and lung were removed. Tissue removal required 45 sec from the time the rat was lifted from the cage, except for muscle which required one more minute. About 400 mg of the posterior left lobe of the liver were used for all experiments. Lungs were severed close to the hilum and any with evidence of hemorrhage were discarded. The great vessels of the heart were severed at the myocardial junction, and all of the heart was used for homogenization. Muscle was taken from a thigh, excluding as much tendon and fascia as practical. Each tissue was rinsed in about 100 ml of 0.32 *M* sucrose (4°) immedietaly after removal. Lung, heart and muscle specimens were quick-frozen in liquid nitrogen and stored at

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[2] cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; PDE, phosphodiesterase; AC, adenyl cyclase; GC, guanyl cyclase.

—70°.

Liver was homogenized immediately in 3 ml of 0.32 *M* sucrose (4°) in a motor driven Potter–Elvehjem homogenizer with teflon pestle (2600 rpm; 8 strokes).

Some major aspects of the measurement of enzyme activities are presented below. Adenyl cyclase was measured using 0.05 ml of homogenate and assays of guanyl cyclase, cAMP PDE and cGMP PDE activities were performed after appropriate dilutions in buffers used for their respective assays. Enzyme dilutions used demonstrated linear kinetics with selected incubation times and protein concentrations. Cyclic AMP, PDE, and cGMP PDE activities were measured according to Thompson and Appleman (2). Adenyl cyclase and guanyl cyclase activities were measured using modifications (5) of the methods of Ramachandran (3) and White and Zenser (4) reported elsewhere.

Substrates consisted of 100 μM GTP for guanyl cyclase, 50 μM ATP for adenyl cyclase, 10 μM cGMP for cGMP PDE, and 0.125 μM cAMP for cAMP PDE. The incubation phases of the adenyl and guanyl cyclase assays were begun within 45 sec of initiating rat sacrifice; that for phosphodiesterase assays was begun within 1.5 min. Incubation (30°) times were 2.5 min for adenyl cyclase, 3.0 min for guanyl cyclase, and 3.0 min for each phosphodiesterase assay.

Assay of enzyme activities in heart, lung, and skeletal muscle were performed similarly to that for liver with a few modifications. These tissues were weighed and then powdered by crushing in a tightly fitted stainless steel mortar, cooled with liquid nitrogen. Each powdered tissue was homogenized in 0.32 *M* sucrose (approximately 500 mg/3 ml) at 4° using a motor-driven Duall type homogenizer with teflon pestle (2600 rpm; 8 strokes) and enzyme activities were determined.

Very low levels of guanyl cyclase activity in homogenates of heart and muscle necessitated use of supernatants derived from centrifugation at 4000*g* for assay; 85% of the total guanyl cyclase activity was found in these supernatants.

Increased activity and reproducibility in guanyl cyclase assays of heart and skeletal muscle were achieved with the use of 100 mg of dry neutral alumina per 3 ml of homogenate prior to centrifugation and a GTP regenerating system similar to the ATP regenerating system for adenyl cyclase assay. The products of guanyl and adenyl cyclase assay of lung, heart, and skeletal muscle were verified in a manner identical to liver tissues (5).

All protein determinations were by the Lowry method (6). We found no appreciable difference in levels of enzyme activities whether the assays were conducted immediately after removal of tissue, or after quickly freezing and storing the tissue at —70° for at least 2 months; an exception consisted of cGMP PDE in muscle, which showed loss of activity.

Results. The rate of actions of guanyl cyclase, adenyl cyslase, cGMP PDE, and cAMP PDE differed markedly in liver, lung, heart, and skeletal muscle (Fig. 1). The activity of each enzyme in any one tissue

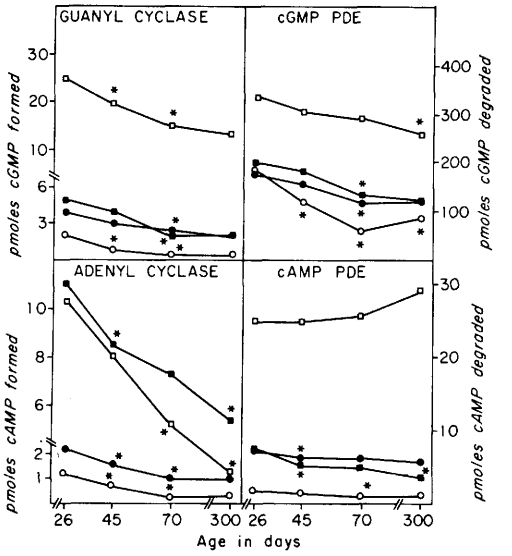

FIG. 1. Each point is the average of the values obtained from assays (2) in 8 rats, and indicates the pmoles of cGMP, or cAMP, formed or degraded, per min/mg protein. An asterisk signifies that the level at that point is significantly different by the Student *t* test ($p < 0.05$) from that of the preceding point. ● Liver; □ lung; ■ heart; ○ muscle.

was less ($p < 0.05$) in rats aged 70 days or 300 days than in those aged 26 days, except for cAMP PDE in lung which did not change. In each of the 4 tissues, a progressive decrease occurred with aging, with decrements usually greater in the 26–45 day and 45–70 day groups than from the 70–300 day groups.

In each of the age groups the lung had more than 4 times the guanyl cyclase activity as the other 3 tissues. Heart and liver had comparable amounts, and much more than muscle. The decline with age was at a comparable rate in all 4 tissues.

In each of the 4 age groups, heart and lung appeared to have several times the adenyl cyclase activity as liver or muscle (Fig. 1), and showed faster rates of decline of activity with aging.

At all ages, cAMP PDE activity was much greater in lung than in the other 3 tissues. Muscle activities were similar to those of heart and liver in the 26-day-old rats, but much less in the other age groups.

cAMP PDE activity in lung in each age group was more than 3 times that in the other tissues. With aging there was no change in the level of this activity in lung and only a slight decrease in the level in the other tissues. In Table I comparisons are shown of the changes in the 4 enzyme activities in each of the 4 tissues. This Table also shows the changes with aging in the ratio of each cyclic nucleotide-synthesizing activity to the activity of the respective phosphodiesterase. In most instances, each of the 4 enzyme activities of the 4 tissues was less ($p < 0.05$) at 70 and 300 days than at 26 days. Using the results from theses 3 age groups for calculations, there apparently were decreases in the ratios of guanyl cyclase to cGMP PDE, and of adenyl cyclase to cAMP PDE in each tissue (Table I). Most of the decrease occurred between Days 26 and 70.

In liver, both the guanyl cyclase and adenyl cyclase activities decreased comparably with aging; cGMP PDE decreased more than cAMP PDE (Table I). The ratio of adenyl cyclase to cAMP PDE (AC:cAMP PDE) decreased sooner and to a greater extent than the ratio of guanyl cyclase to cGMP PDE (GC:cGMP PDE) (Table I).

TABLE I. Activities of Enzymes Synthesizing and Hydrolyzing cGMP and cAMP.

Tissue	Age (days)	Guanyl cyclase[a]	cGMP PDE[b]	GC: cGMP PDE	Adenyl cyclase[a]	cAMP PDE[b]	AC: cAMP PDE
Liver	26	3.8 ± 0.8	190 ± 47	0.020	2.2 ± 0.3	7.0 ± 0.5	0.31
	45	3.0 ± 0.9	184 ± 32	0.016	1.6 ± 0.1*	6.1 ± 0.5*	0.26
	70	2.4 ± 0.7*	112 ± 21*	0.021	1.0 ± 0.1*	6.0 ± 0.5	0.17
	300	2.0 ± 0.3	128 ± 24	0.016	1.0 ± 0.1	5.7 ± 0.6	0.17
Lung	26	25 ± 3	332 ± 33	0.075	10 ± 2	25 ± 6	0.40
	45	20 ± 3*	306 ± 47	0.065	8.0 ± 2	25 ± 8	0.32
	70	15 ± 1*	288 ± 38*	0.052	5.3 ± 0.8*	26 ± 6	0.20
	300	14 ± 2	259 ± 44	0.054	3.6 ± 0.5*	29 ± 8	0.10
Heart	26	4.9 ± 0.8	195 ± 13	0.025	11 ± 3	7.1 ± 0.6	0.15
	45	4.0 ± 0.8	183 ± 13	0.021	8.5 ± 2*	5.3 ± 0.5*	0.16
	70	1.9 ± 0.5*	138 ± 35*	0.014	7.3 ± 1	4.9 ± 0.4	0.15
	300	2.1 ± 0.6	122 ± 15	0.017	5.4 ± 1*	3.7 ± 0.6	0.15
Muscle	26	2.1 ± 0.8	188 ± 20	0.011	1.2 ± 0.3	2.1 ± 0.2	0.56
	45	1.0 ± 0.3*	122 ± 37	0.008	0.7 ± 0.3*	1.8 ± 0.4	0.41
	70	0.4 ± 0.07*	63 ± 10*	0.006	0.2 ± 0.1*	1.4 ± 0.3*	0.15
	300	0.5 ± 0.02	87 ± 21	0.006	0.2 ± 0.1	1.4 ± 0.2	0.18

[a] Picomoles cGMP or cAMP formed/min/mg protein.

[b] Picomoles cGMP or cAMP hydrolyzed/min/mg protein. Each number is the average of results of enzyme assays on each organ from 8 rats. The standard deviation for each group is indicated. Each asterisk designates a significant difference ($p < 0.05$) from the preceding age group.

In heart, aging caused comparable decreases in guanyl cyclase and adenyl cyclase activities (Table I). The decrease in cAMP PDE was somewhat faster than that for cGMP PDE. The ratio of GC:cGMP PDE decreased much more than AC:cAMP PDE.

In lung, aging was associated with a marked decrease in adenyl cyclase activity, but with no change in cAMP PDE (Table I). Guanyl cyclase and cGMP PDE activities both decreased with aging but the former to a greater extent.

In muscle, guanyl cyclase and adenyl cyclase activities decreased significantly with aging and to a greater degree than the levels of cGMP PDE and cAMP PDE (Table I). Therefore, our assays appear to indicate a greater decline in the rates of synthesis of cGMP and cAMP than in their rates of degradation.

Discussion. The following orders of enzyme activities were found in the rat tissues of each of the age groups: (a) guanyl cyclase, lung>>heart and liver > muscle; (b) adenyl cyclase, heart and lung>> liver > muscle; (c) cGMP PDE, lung>> heart and liver > muscle; (d) cAMP PDE, lung >> heart and liver > muscle. The order of levels of guanyl cyclase that we found is similar to that reported by others (7, 8). The rate of synthesis of cAMP activity was reported (8) to be about equal in lung and heart, and much higher than in liver. Assays of cGMP levels showed (9): lung >>heart, muscle, or liver. Assays of cAMP revealed (9): lung>> muscle > liver or heart.

Assay conditions used in our experiments were not designed to measure the maximum velocity of these enzymes in most cases. Using high enzyme concentration and substrate levels below the reported Michaelis–Menten constant for most of these enzymes, we were able to measure enzyme activities with very short incubation times, thus minimizing *in vitro* artifacts. If there are any changes in the affinity of these enzymes (which is quite unlikely), these would be expressed in our velocity determinations. Further studies on the interesting aspect of the mechanism and possible causitive factors for the observed changes in enzyme velocity are currently in progress. Also assays using low substrate concentrations with homogenate preparations risk possible influences from endogenous substrate (*e.g.*, adenyl cyclase). However, our calculations based on known substrate concentrations indicate that changes in tissue levels of substrate to account for the changes seen in enzyme activities are far greater than practically possible.

Because of relatively recent advances in our knowledge of cAMP PDE and cGMP PDE kinetic properties and the subsequent necessity for assay at the substrate concentrations used for these studies, we cannot compare our cAMP PDE results with those of other aging studies. There are no reports of comparative studies on cGMP PDE activities.

Under the conditions of our assays, both the synthesis and degradation of cGMP and cAMP decreased with aging but the synthesis declined more rapidly. In heart, no decrease in cAMP enzyme ratios was apparent. The physiologic significance of the pattern of change in all 4 tissues is not known. Although unlikely, large redistributions of protein composition could influence enzyme activity calculations in this study. Whether there are net decreases in cGMP and cAMP concentrations in these 4 tissues with aging also remains to be shown.

Questions posed by these observations are: (a) What metabolic alterations of aging produce the changes in cyclic nucleotide metabolism? (b) What are the effects of cyclic nucleotides upon aging? There are numerous aspects of aging that could influence the levels and activities of the cyclic nucleotides, including various changes in hormone activities, but the main determinant appears to be the genetic pattern, since it controls the size, number and activities of all cells, including the production, metabolism and actions of hormones and cyclic nucleotides. Hormones, however, influence the amount and type of DNA, RNA, protein synthesis, lipid synthesis, membrane permeability, and cyclic nucleotides; cyclic nucleotides affect patterns of DNA, RNA, protein synthesis, and hor-

mone activities. Although aging, nucleotide metabolism and hormone activities appear to have many interrelationships, data are meager.

Only a few reports correlate age changes with activities of adenyl cyclase and phosphodiesterase, and there are none that correlate guanyl cyclase activities, or the two types of phosphodiesterase activity, with aging.

Adenyl cyclase activity in fetal rat liver has been reported (10) to attain adult levels by the time of birth. In muscle it increased more slowly, but reached adult levels by 20 days. Phosphodiesterase activities in liver and muscle were as high at birth as during adulthood. Liver adenyl cyclase responses to epinephrine or glucagon have been shown to decrease with age (11). Adenyl cyclase responses in fat cell homogenates from rats weighing 150–175 g were no greater than in those from rats weighing 350–400 g (12). The effectiveness of glucagon upon this activity was far less in the older group, while there was not much change in the response to epinephrine. Using either isolated fat cells or fat cell homogenate, norepinephrine caused much less increase in adenyl cyclase activity in rats aged 18–24 week than in ones 5–6 or 10–12 weeks (13).

In conclusion, changes in nucleotide metabolism and in hormone activities are associated with aging, but correlations of their interrelationships are few (14–20).

Summary. Guanyl cyclase, adenyl cyclase, cyclic 3′,5′-adenosine monophosphate phosphodiesterase (cAMP PDE), and cyclic 3′,5′-guanosine monophosphate phosphodiesterase (cGMP PDE) activities in heart, lung, liver, and skeletal muscle of rats 26, 45, 70, and 300 days of age were measured. Decreases in each of these enzyme activities, except for that of cAMP PDE in lung, occurred with aging.[3] The most rapid declines in activity occurred within 70 days. The activities of the cyclases decreased more rapidly than those of the phosphodiesterases. Therefore, in our experiments, the rates of synthesis of the cyclic nucleotides declined faster than the rates of degradation with aging. Guanyl cyclase activity had greater decreases in lung homogenate than in other tissues, and adenyl cyclase activity decreased much faster in heart and lung than in liver and skeletal muscle. cGMP PDE and cAMP PDE activities were much greater in lung than in other tissues at all ages. It is not known how or to what extent cyclic nucleotide metabolism might contribute to aging, or by what mechanisms alterations in the metabolism of such compounds are produced with, or by, aging. However, numerous changes in metabolic and endocrine activities are known to occur at different intervals of the lifespan, especially during gestation, maturation, and senescence, and may be related to the decline in activity of these cyclic nucleotide synthetic and degradative enzymes.

[3] We use "aging" to signify increasing age. All of the rats studied were less than one year of age; the first 3 age groups were rapidly growing and maturing. While the average lifespan of this type of rat is about 3–4 years, in some ways they appear to mature relatively much faster than man. For example, the 45-day-old rats have already attained puberty.

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