Hormone-Sensitive Fat Cell Adenylate Cyclase in the Rat

INFLUENCES OF GROWTH, CELL SIZE, AND AGING

BARRY COOPER and ROBERT I. GREGERMAN

From the Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland 21224

A B S T R A C T The possibility has been explored that decreases of adenylate cyclase may explain diminished hormone sensitivity of adipose tissue with aging. Isolated cells were prepared from epididymal fat pads of rats 1-, 2-, 6-, 12-, and 24-mo-old, fixed in OsO_4, and sized and counted with a Coulter apparatus. Adenylate cyclase was assayed in cell membranes (ghosts) using [α-^{32}P]ATP as substrate and expressed as cyclic[^{32}P] AMP/10 min per mg protein or per 10^6 cells. Enzyme activity was determined for the basal state and in the presence of varying concentrations of glucagon, ACTH, epinephrine, and fluoride. Basal activity per cell increased threefold between 1 and 2 mo with a comparable increase in cell surface area, suggesting synthesis of enzyme along with new cell membrane. Although epinephrine stimulated adenylate cyclase 8-fold and fluoride 12-fold throughout the life-span of the rat, stimulated activity paralleled basal levels, decreasing 60% between 2 and 24 mo per mg protein and 40% between 6 and 24 mo per cell. Glucagon stimulated adenylate cyclase 4.5-fold relative to basal in the 1-mo-old rat, but its effect then rapidly decreased and was absent by 12 mo. The fourfold stimulation by ACTH noted in the 1-mo-old animals decreased gradually with age but was still twice basal at 24 mo. Since no significant change of cell size occurred after 6 mo, diminished hormone sensitivity with senescence cannot be related to cell size. Similar age-related patterns of hormonal activation were evoked by 5'-guanylyl-imidodiphosphate [GMP-P(NH)P], a nucleotide analogue which increased both basal- and hormone-activated enzyme at all ages studied. Dose-response curves to hormones, fluoride, and GMP-P(NH)P were not affected by age.

High Mg^{++} (50 mM) in the presence of GMP-P-(NH)P stimulated adenylate cyclase to levels greater than with fluoride, but a similar loss of activity with aging was still observed. Loss of hormone receptors may partially explain the age-related decreases of glucagon and ACTH-sensitive adenylate cyclase, but decreased basal-, epinephrine-, fluoride-, and GMP-P-(NH)P-stimulated responses suggest loss of the catalytic component of the adenylate cyclase enzyme complex in the aging fat cell membranes.

INTRODUCTION

In the past, most studies of aging and hormones have dealt with effects of hormones on the processes of aging or, more often, with effects of aging on hormone secretion and metabolism (1). More recently, however, effects of aging on hormone responsiveness or sensitivity have come under investigation. A number of postmaturational changes of this type have now been identified. In view of the variety of mechanisms involved in the actions of hormones of different types, it is not surprising that a mixed picture has emerged (2). For the many hormones whose mechanism of action involves activation of adenylate cyclase, relatively little information on age relationships is as yet available. To extend our information in this area, and with the hope of being able eventually to relate the findings to physiologic events, we have undertaken studies of adenylate cyclases in a variety of animal and human tissues.

This paper describes the effects of growth, maturation, and senescence on fat cell adenylate cyclase of the rat. Data already available have suggested effects of aging on adenylate cyclase in several other tissues (3, 4), as well as on the adenylate cyclase-mediated lipolytic responses to several hormones. Because of the influence of fat cell size, per se, on hormone responsive-

ness, we have measured adenylate cyclase activity in relation to cell size and number, as well as to the protein content of the fat cell membranes. Finally, we have assayed the hormone-sensitive enzyme responses over a wide range of hormone concentrations and under several assay conditions to be reasonably certain that adenylate cyclase activity was being measured under conditions which accurately reflected membrane content of the enzyme.

METHODS

Characteristics of the animals used. All studies used male Wistar rats from an outbred strain raised in the Gerontology Research Center. A 6-mo-old rat is considered a young adult and a 12-mo-old rat approximates middle age. In this colony mortality is very low until about 20 mo. By 24 mo the colony exhibits a 50% mortality, and animals at this age are considered to be senescent. Other characteristics of this rat colony have recently been described (5). All animals were maintained on a standard ad lib. diet (NIH open formula) purchased from Allied Mills, Inc., Chicago, Ill.

Preparation of fat cell ghosts. Animals were rendered unconscious with carbon dioxide and the epididymal fat pads rapidly removed. Isolated fat cells were then prepared by treatment with collagenase (Worthington Biochemical Corp., Freehold, N. J.; type I) as described by Rodbell (6). Plasma membranes or "ghosts" were prepared from the cells by a minor modification of the method of Harwood and Rodbell (7) in which 1 mM dithiothreitol replaces mercaptoethanol in the lysing and suspending media. Since it was inconvenient both to prepare ghosts from several animals and to perform the enzyme assays on the same day, the ghosts were frozen and stored in liquid nitrogen (−196°C) until immediately before assay. All enzyme assays were performed 24–72 h after freezing. There was no loss of activity of basal or stimulated states over this time interval for a wide range of hormone concentrations. Limited observations indicated that adenylate cyclase activity stored under these conditions was stable for up to at least 30 days, except for the epinephrine-stimulated enzyme which lost 20% of its activity after 7 days and 70% after 14 days.

Determination of fat cell size. Isolated fat cells were fixed in osmium-tetroxide buffer as described by Hirsch and Gallian (8) for whole tissue shreds and modified by Cushman[1] for isolated fat cells. In this procedure 0.5 ml of a 30% cell suspension in 4% Krebs-albumin buffer was added to 2 ml of OsO_4-0.05 M collidine-HCl buffer, pH 7.4, and incubated for 1 h at 37°C. An additional 25-ml osmium-collidine buffer was then added and fixation continued at 37°C for an additional 24–48 h. The fixed cells were isolated with the aid of 25 and 250-μm nylon filters (Tobler, Ernst, and Traber, Inc., New York) as originally described (8). Cell number was calculated with a model ZBI Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.) and median cell volume and diameter determined with the Coulter Channelyzer (model C-1000) and Distribution Plotter (XY Recorder II).

Assay of adenylate cyclase activity. Enzyme activity was measured by the method of Salomon et al. (9). This is a labeled substrate assay using [α-^{32}P]ATP as substrate

with measurement of cyclic[^{32}P]AMP (cAMP)[2] product. The method employs a two-column technique using sequentially Dowex 50 and neutral alumina to separate the labeled cAMP product from the ATP substrate. With this method the reagent blanks were very low. With some batches of [α-^{32}P]ATP the blanks were zero and were never in excess of 20 dpm. The standard assay mixtures contained 1.5×10^6 dpm [α-^{32}P]ATP (New England Nuclear, Boston, Mass.), 10–30 Ci/mM; 1.6 mM ATP (Sigma Chemical Co., St. Louis, Mo.) 25 mM Tris-HCl (pH 7.4); 5 mM $MgCl_2$; 2 mM cAMP (Sigma); 0.1% albumin (Pentex Biochemical, Kankakee, Ill. fraction V); 10 mM theophylline (Sigma); and an ATP-regenerating system consisting of 20 mM creatine phosphate and 1 mg/ml creatine kinase (Sigma). [^{3}H]cAMP (New England Nuclear) was included in the incubation medium to monitor possible loss or destruction of the [^{32}P]cAMP product by phosphodiesterase. Deviations from these standard assay conditions are noted wherever applicable in the Results. Incubation mixtures contained a final volume of 50 μl. Reactions were initiated by addition of 20 μl of suspended ghosts (40–80 μg protein) and continued for 10 min at 30°C. Enzyme activity was linear within this range of protein concentrations and time. Reactions were terminated by the addition of sodium lauryl sulfate containing excess carrier ATP and cAMP, followed by heating for 5 min in a boiling water bath. Approximately 10^4 dpm of [^{14}C]cAMP (Schwarz/Mann Div., Orangeburg, N. Y.) was added immediately after the stopping solution to monitor column losses. The recoveries for [^{3}H] and [^{14}C]cAMP were very nearly the same in all experiments, indicating absence of appreciable phosphodiesterase activity under our experimental conditions. All data were expressed as nmol cAMP/mg ghost protein per 10 min or nmol cAMP/10^6 cells per 10 min. Determination of ghost protein was by the method of Lowry et al. (10).

Hormones and activators included L-epinephrine bitartrate (Sigma); porcine ACTH (Calbiochem, San Diego, Calif., grade B); glucagon (Sigma); sodium fluoride (Fisher Scientific Co., Pittsburgh, Pa.) and 5'-guanylyl-imidodiphosphate [GMP-P(NH)P]; (ICN Pharmaceuticals Inc., Cleveland, Ohio). Epinephrine stock solutions (50 mM) were stable for several weeks when stored in the dark at pH 1.5, 0–5°C. Solutions of ACTH (5 mM) and glucagon (0.25 mM) were stable when frozen for several weeks when compared to the stimulatory effect of fresh solutions. Fresh stock solutions of GMP-P(NH)P (5 mM) were prepared each week and stored frozen (−20°C).

Statistical analysis of the results. Early in the work it became apparent that some day-to-day variability in the results was related to variability in the assay itself or to the method of preparation of the ghosts. Accordingly, each day's experiment included animals from every age group so that ghosts were prepared under identical conditions from all animals sacrificed on a given day. Enzyme assays were subsequently and simultaneously performed with ghosts prepared from each such group of animals. By these means we avoided introduction of bias which might otherwise have occurred had animals of different ages been studied at different times. Moreover, this experimental design allowed comparison of animals of different ages by statistical analysis using the paired t test (e.g., 2 vs. 6 mo; 6 vs. 12 mo, etc.) and minimized the problem of interassay variation. Ghosts from 1- and 2-mo-old rats were prepared by pooling epididymal fat from three to six animals of the same age.

---

[2] *Abbreviations used in this paper:* cAMP, cyclic[^{32}P]-AMP; GMP-P(NH)P, 5'-guanylyl-imidodiphosphate.

---

[1] Personal communication.

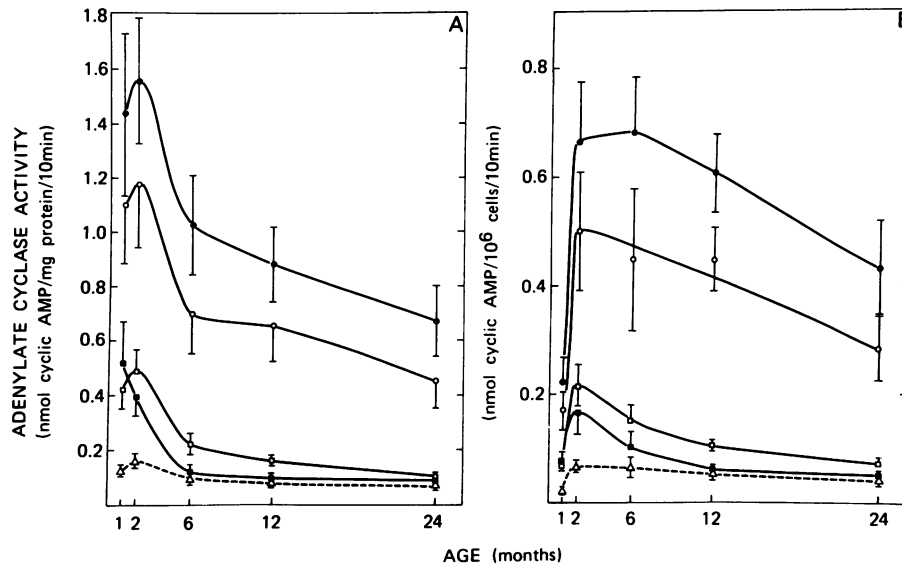FIGURE 1 Relationship of age to hormone- and fluoride-stimulated rat fat cell adenylate cyclase activity per milligram ghost protein (A) and per 10^6 cells (B). Results are mean±SEM of nine experiments. Additions were none (△--△); epinephrine, 1 mM (○—○); ACTH, 0.1 mM (□—□); glucagon, 0.01 mM (■—■); and fluoride, 5 mM (●—●). Similar patterns were seen at lower concentrations of hormones and fluoride. Statistical significance of age differences in enzyme activity are not readily appreciated by inspection of the standard errors since these values included interassay variability as described in the text. Statistical significance was determined by paired t tests for animals of different ages (see Methods).

The pooled preparations were necessary because of the small amount of fat present in each pad. An n of 1 was assigned to each such pool. Individual rats were used to prepare ghosts from the older animals.

Later in the work in a single experiment we compared enzyme activities of ghosts prepared simultaneously with three different batches of collagenase. This experiment clearly showed two to threefold differences in basal- and hormone-sensitive responses. Collagenase is therefore a major contributor to interassay variability and probably accounts for differences in basal activity between the early experiments and the later studies using GMP-P(NH)P. In our current work, not reported here, all cell preparations are made using collagenase from a single pretested batch thus eliminating this source of variability.

The standard errors shown in the Figures are for reference only and are not used in comparing the significance of differences between groups of animals. For reasons presented above, all such comparisons are made by paired t analysis.

RESULTS

Age-related changes of adenylate cyclase. The relationship of age to hormone and fluoride activation of rat fat cell adenylate cyclase per milligram protein is shown in Fig. 1A. Levels of activity were highest during the first 2 mo, the period of the most rapid cell growth (Figs. 1A and 2). After 2 mo, activity dropped rapidly until about 6 mo and more slowly thereafter throughout the remainder of the life-span. Despite a considerable increase of cell size between 1 and 2 mo, hormone-

sensitive enzyme activities were essentially the same at these ages. A 25% increase of basal activity between 1 and 2 mo was significant ($P < 0.01$). The decline in basal activity for 2 vs. 24 mo was significant ($P < 0.01$) as were the decreases of hormone-sensitive cyclase: epinephrine ($P < 0.005$); ACTH ($P < 0.001$); glucagon ($P < 0.005$). Similarly, activation by fluoride was also decreased ($P < 0.005$). This age-related pat-
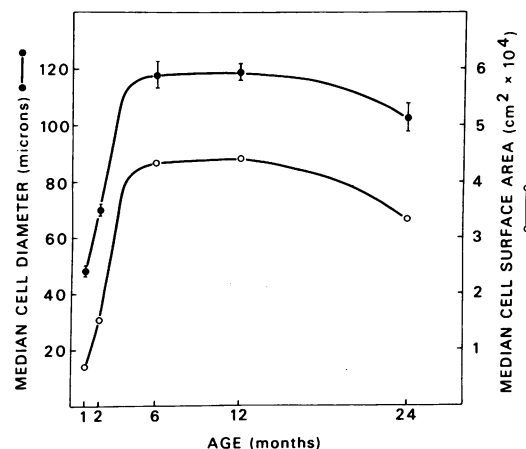

FIGURE 2 Effect of age on median diameter (●—●) and surface area (○—○) of osmium-fixed fat cells. Results are mean±SEM for nine experiments.

tern of enzyme activation was also evident with sub-maximal hormone and fluoride concentrations. Since senescence in the rat appears to occur between 12 and 24 mo of age, we were especially interested in altered adenylate cyclase responses over this interval. Epinephrine- and ACTH-activated adenylate cyclase (activity/milligram protein) decreased over this age range ($P < 0.02$ and $P < 0.005$, respectively). The 20% decrease in mean levels of basal adenylate cyclase between 12 and 24 mo was not statistically significant.

Activity per unit (10^6) of cells is shown in Fig. 1B. There was a threefold increase in basal enzyme activity between 1 and 2 mo of age ($P < 0.01$); thereafter, mean basal values decreased but statistical significance was not demonstrated between 2 and 24 mo. Epinephrine-, ACTH-, and fluoride-stimulated adenylate cyclase showed threefold increases over basal activity between 1 and 2 mo ($P < 0.02$); glucagon-stimulated adenylate cyclase increased by twofold over this early period ($P < 0.02$). Thereafter, glucagon responsiveness rapidly diminished. Although glucagon still produced a 50% increase of activity at 6 mo, by 12 mo, no increase was demonstrable. In contrast, epinephrine ($P < 0.02$), ACTH ($P < 0.005$), and fluoride ($P < 0.005$) activation declined only gradually and was still considerable even at 24 mo. Similar results were found using hormones and fluoride at submaximal concentrations.

Comparison of age-related changes of adenylate cyclase; activity per milligram protein vs. activity per cell. The major difference between enzyme activity expressed per milligram protein compared with activity per cell (Figs. 1A vs. 1B) was the threefold increase in basal- and stimulated-adenylate cyclase activity per cell occurring between 1 and 2 mo with no significant difference in activity expressed per milligram protein between these ages. One must conclude that the increase of activity was due to an increase of total cell membrane. Fig. 2 shows the age-related alteration of fat cell diameter for the rats used in our studies. Cell diameter increased from 48 to 70 μm between 1 and 2 mo. Such a change represents a 3-fold increase of cell volume and a 2.1-fold increase of cell-surface area. The latter parameter probably reflects more closely the increase in cell membrane. The data suggest that during maturation the enlarging fat cells synthesize membrane protein and adenylate cyclase in the same proportion accounting for the constant enzyme activity/milligram protein and the threefold increase in total activity/cell over this same 4 wk time period.

The changes which occur during completion of maturation between 2 and 6 mo are not readily explained. During this interval cell diameter increased from 70 to nearly 120 μm with a corresponding 2.8-fold increase in cell-surface area. For the epinephrine-sensitive cy-

clase and the fluoride-sensitive activity, no change was seen in terms of activity per cell. Nonetheless, activity per milligram ghost protein decreased sharply, suggesting that maturation of the membrane over this interval involved accumulation of membrane protein without comparable change of total adenylate cyclase activity. With regard to the postmaturational decreases in enzyme activity that occurred between 6 and 24 mo (Fig. 1), the loss of adenylate cyclase activity over this time span cannot be related to cell size which was constant over this age interval (Fig. 2). This loss probably represents an age-related alteration of the cell membrane's content of adenylate cyclase.

Relation of age to the ratio of hormone- (and fluoride-) stimulated activity to basal activity. We have examined ratios of stimulated to basal activities as possibly better reflecting age-related changes of hormonal "sensitivity". Fig. 3 shows such ratios or "relative" hormone activations for glucagon, ACTH, and epinephrine. For glucagon there was 4-fold stimulation over basal levels in the 1 mo rat with 2.5-fold activation at 2 mo, and only 1.3-fold stimulation at 6 mo; by 12 and 24 mo glucagon failed to stimulate the enzyme significantly over basal levels. A loss of ACTH sensitivity with maturation and aging is also seen. At 1, 2, 6, 12, and 24 mo of age, there was 4.0-, 3.5-, 2.9-, 2.3-, and 1.9-fold stimulation of basal levels, respectively. In contrast to glucagon, this loss of sensitivity to ACTH was more gradual with time; even the 24-mo-old-senescent rat showed considerable enzyme activation by ACTH.

For epinephrine and fluoride age-related changes of stimulated to basal enzyme activities were not demonstrable at any concentration tested. The highest epinephrine concentration (1 mM, Fig. 3) stimulated the activity approximately eightfold in the 2-, 6-, 12-, and 24-mo-old rat; the 9.7-fold activation in the 1-mo-old animal was not significantly greater. Epinephrine at 0.1 mM activated the enzyme four to fivefold and 0.01 mM stimulated approximately twofold at all ages. 5 mM fluoride produced 12-fold activation of adenylate cyclase regardless of age. These results suggest that the age-related loss of epinephrine- and fluoride-stimulated activity per milligram ghost protein and/or per cell (Fig. 1) could reflect changes of basal adenylate cyclase levels rather than alteration of epinephrine or fluoride "sensitivity" of the enzyme. However, the loss of activity noted in the presence of glucagon or ACTH was clearly greater than be can explained by changes of basal activity alone.

Activation of adenylate cyclase by GMP-P(NH)P. This nucleotide analogue of GTP has been recently shown to enhance both basal- and hormone-activated adenylate cyclase in various tissues, including the en-

zyme from fat cells (11–14). Fig. 4A shows that 0.1 mM GMP-P(NH)P produced twofold stimulation of basal adenylate cyclase activity per milligram protein at all ages studied. From the peak at 2 mo to that at 24 mo there was a 60% loss of activity ($P < 0.02$). The age-related patterns of GMP-P(NH)P-augmented glucagon and epinephrine activities were very similar to those seen in the absence of the nucleotide (cf. Fig. 1). Even in the presence of GMP-P(NH)P glucagon activation was rapidly lost by 6 mo. Fig. 4B illustrates the effect of GMP-P(NH)P on basal- and hormone-stimulated adenylate cyclase with activity expressed per 10^6 cells. These results when compared to those in Fig. 1B show patterns of activation similar to those without added nucleotide. Basal activities were increased by GMP-P(NH)P about twofold at all ages studied. There was a fourfold increase ($P < 0.01$) of basal- and epinephrine-stimulated adenylate cyclase between 1 and 2 mo with a 30% decrease ($P < 0.05$) of epinphrine-stimulated enzyme between 6 and 24 mo. Glucagon activation was 2.7-fold at 1 mo and 2-fold at 2 mo, but there was no longer significant glucagon activation by 6 mo of age (Fig. 4B). We also studied the age-related activation of the enzyme by GMP-P(NH)P at high Mg^{++} concentration (50 mM), compared to 5 mM Mg^{++} concentration in our standard assay conditions. These incubation conditions maximize adenylate cyclase activity in fat cell membranes (15). As noted in Fig. 4, age-related patterns of enzyme activation were similar to that seen with epinephrine (Figs. 1 and 4) and with fluoride (Fig. 1).
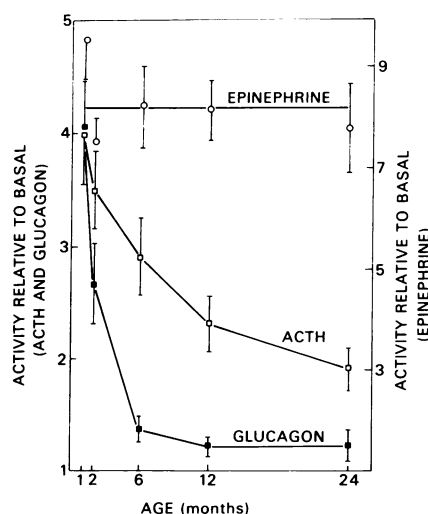

FIGURE 3 Relationship of age to the ratio of stimulated to basal activity for hormone-sensitive adenylate cyclase in rat fat cell ghosts. Additions include glucagon, 0.01 mM (■—■); ACTH, 0.1 mM (□—□); and epinephrine, 1 mM (○—○). Results are mean±SEM for nine experiments.

DISCUSSION

Previous studies on age- or growth-related alterations of fat cell responses to hormones in the rat have not always considered the well-described rapid increase of cell size and number during the first 4 mo of age (16). In these investigations the base lines used for responses such as lipolysis have often included clearly
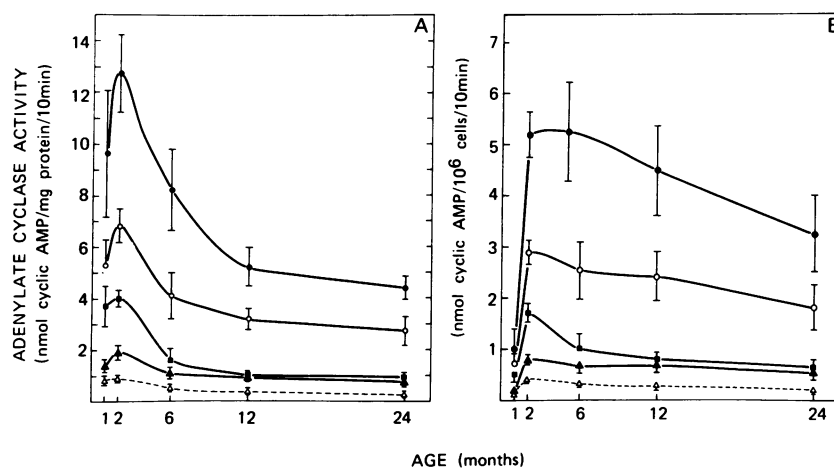

FIGURE 4 Effect of GMP-P(NH)P on activation of rat fat cell adenylate cyclase per milligram ghost protein (A) and per 10^6 cells (B). Standard assay conditions were used in the presence of 1.0 mM ATP. Additions include none (△—△); GMP-P(NH)P, 0.1 mM (▲—▲); GMP-P(NH)P, 0.1 mM + epinephrine, 1 mM (○—○); GMP-P(NH)P, 0.1 mM + glucagon, 0.01 mM (■—■); GMP-P(NH)P, 0.1 mM + MgCl₂, 50 mM (●—●). Results are mean±SEM of six experiments (four experiments with GMP-P(NH)P, 0.1 mM + MgCl₂, 50 mM). The higher basal values in these experiments is probably attributed to the collagenase used (see Methods).

unsatisfactory parameters such as tissue weight or triglyceride content. Cell protein content, cell number, and parameters related to cell size (diameter, volume, surface area) are much more meaningful. As shown in our present studies, combinations of these base lines can be even more revealing. Our present expression of adenylate cyclase activity in terms both of ghost membrane protein and of activity per cell, as well as the estimation of cell size, has allowed us to make at least a partial differentiation between age-related changes of hormone-sensitive cyclase and changes attributable to cell size, per se. The present work clearly reveals differences in the patterns of age-related changes of adenylate cyclase responses for the several hormones studied: glucagon, epinephrine and ACTH.

The most striking age-related loss of hormone-sensitive fat cell adenylate cyclase was seen with glucagon. While this hormone activated the enzyme of young animals (4.5-fold, 1 mo; 2.6-fold, 2 mo), it does not appreciably activate the enzyme in animals of 6, 12, or 24 mo (Fig. 3). Since this rapid loss of glucagon activation of adenylate cyclase occurred over an age interval in which cell size was rapidly increasing, this altered enzyme sensitivity may be secondary to physical alterations of the membrane (in turn related to expanding cell size) or be secondary to other cell changes associated with growth and maturation in the rat. The only prior study reporting a loss in glucagon activation of fat cell adenylate cyclase was by Manganiello and Vaughan (17). Although the ages of their rats were not specified, they noted a loss of enzyme activation by glucagon in the fat cell particulate fractions from 400-g rats compared to those from 100 to 125 g. Presumably the larger rats were 3–4 mo or older and the younger animals were about 1 mo. The very few animals studied precluded any statistical analysis.

In a relevant study of glucagon-stimulated lipolysis in isolated fat cells, Livingston et al. (18) compared glycerol release by cells obtained from 130–180 vs. 400–450-g rats. Activity was expressed per number of cells estimated from measurement of DNA. Glucagon response by large cells was only 20% of that seen with the small cells. Moreover, there appeared to be 50% fewer glucagon receptors on the large cells, as measured by membrane binding of ^{125}I-glucagon. Thus, at least part of the diminished glucagon responsiveness may be related to receptor loss. In contrast to the rat, human fat has been found unresponsive to glucagon as a lipolytic agent (19). Such observations, however, may be a result of the use of fat from adults. If age- and/or size-related loss of glucagon-sensitive adenylate cyclase occurs in man as in the rat, fetal or preadolescent human fat may yet prove to be glucagon responsive.

While age-related loss of the glucagon-sensitive fat cell adenylate cyclase is now clearly demonstrated, such changes in the catecholamine-sensitive cyclase are more complicated. We found that the age-related decreases in epinephrine-sensitive enzyme paralleled the fall in basal levels, resulting in a constant ratio of epinephrine sensitive: basal throughout the life-span of the rat. Forn et al. (20) studied activation by norepinephrine in homogenates of isolated fat cells and reported a 65% decline of stimulated activity per cell between 3 and 6 mo of age; older animals were not studied. Their data for the interval between 3 and 6 mo demonstrated an age-related loss of basal- and catecholamine-sensitive adenylate cyclase per cell that we noted only much later, between 6 and 24 mo. Other workers have noted little change of epinephrine-stimulated adenylate cyclase activity per milligram particulate protein (17) or per cell (21) between small and large rats of unspecified age.

In studies of catecholamine-stimulated lipolysis, several groups have reported decreased lipolysis by catecholamines in aged rats both in vivo and in vitro (22–24). However, recent studies in the rat which considered age-related variation in triglyceride content with cell size noted little change in catecholamine stimulation of lipolysis when the data were expressed per cell rather than per milligram triglyceride (21, 25, 26). Nonetheless, neither very young (1 mo) nor senescent (or even > 16 mo) rats were used in any of these reports. With human fat cells norepinephrine-induced lipolysis per cell diminished with increasing age in nonobese adult subjects (27). James et al. (28) found that basal- and epinephrine-activated lipolysis per gram lipid decreased with age, although activity per cell was not determined.

We are not aware of any previous reports on age-related effects of ACTH on fat cell adenylate cyclase. Our results showed a gradual decrease over the adult portion of the life-span, even as cell size is no longer changing. This pattern of loss contrasts in its timing to the loss of glucagon sensitivity which occurred during maturation. Data concerning the age-related lipolytic effects of ACTH on adipose tissue are less extensive than studies with catecholamines or glucagon. One study noted a decrease of ACTH-stimulated lipolysis in 1-yr-old rats compared to those 50 days old (26) while another report found that ACTH-stimulated lipolysis was proportional to cell-surface area in rats of unspecified age (29). In contrast to the rat, adult human fat has been found insensitive to ACTH as a lipolytic hormone (30).

While the age-related loss in ACTH- and glucagon-sensitive enzyme may reflect loss of hormone receptors, the parallel decreases in basal, fluoride, and epinephrine responses probably indicates loss of the catalytic com-

ponent of the enzyme complex. Fluoride enhances adenylate cyclase by a direct effect on the enzyme independent of membrane receptors (7). In addition, GMP-P(NH)P acts as an allosteric regulator of the enzyme and enhances hormone-sensitive adenylate cyclase by binding to the transducer component of the enzyme complex (15). Under conditions which currently best measure total available enzyme [50 mM Mg^{++}, 0.1 mM GMP-P(NH)P] independent of hormone activation, a similar age-related loss in enzyme activity was noted. The similarity of these results under varying experimental conditions and with activators not involving hormone receptors strongly suggests that some component of the enzyme complex other than receptors is being lost with aging. Documentation of actual enzyme loss awaits methods which unequivocally measure total available enzyme.

While the present studies define a range of age-related effects on hormone-sensitive adenylate cyclase, one must be cautious in attempting to correlate age changes of enzyme activation with overall metabolic responses such as lipolysis. As already shown in a few studies, phosphodiesterase activity appears to increase with aging and/or enlarging cell size in the rat (20, 31). Thus, the effect of a hormone's action may be grossly altered at some point distant to its effect on adenylate cyclase and the cAMP produced, which may in turn depend upon phosphodiesterase activity. More subtle effects may relate to intracellular compartmentalization of cAMP. These considerations emphasize the need for correlative studies in intact cells of adenylate cyclase activity, phosphodiesterase, intracellular cAMP, and overall physiologic or metabolic responses.

ACKNOWLEDGMENTS

REFERENCES

1. Gregerman, R. I., and E. L. Bierman. 1974. Aging and hormones. *In* Textbook of Endocrinology. R. H. Williams, editor. W. B. Saunders Co., Philadelphia. 5th edition. 1059–1070.
2. Roth, G. S., and R. C. Adelman. 1975. Age related changes in hormone binding by target cells and tissues: possible role in altered adaptive responsiveness. *Exp. Gerontol.* 10: 1–11.
3. Walker, J. B., and J. P. Walker. 1973. Properties of adenylate cyclase from senescent rat brain. *Brain Res.* 54: 391–396.
4. Williams, R. H., and W. J. Thompson. 1973. Effect of age upon guanyl cyclase, adenyl cyclase, and cyclic nucleotide phosphodiesterases in rats. *Proc. Soc. Exp. Biol. Med.* 143: 382–387.

5. Lakatta, E. G., G. Gerstenblith, C. S. Angell, N. W. Shock, and M. L. Weisfeldt. 1975. Prolonged contraction duration in aged myocardium. *J. Clin. Invest.* 55: 61–68.
6. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* 239: 375–380.
7. Harwood, J. P., and M. Rodbell. 1973. Inhibition by fluoride ion of hormonal activation of fat cell adenylate cyclase. *J. Biol. Chem.* 248: 4901–4904.
8. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* 9: 110–119.
9. Salomon, Y., C. Londos, and M. Rodbell. 1974. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* 58: 541–548.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
11. Harwood, J. P., H. Löw, and M. Rodbell. 1973. Stimulatory and inhibitory effects of guanyl nucleotides on fat cell adenylate cyclase. *J. Biol. Chem.* 248: 6239–6245.
12. Lefkowitz, R. J. 1974. Stimulation of catecholamine-sensitive adenylate cyclase by 5'-guanylyl-imidodiphosphate. *J. Biol. Chem.* 249: 6119–6124.
13. Londos, C., Y. Salomon, M. C. Lin, J. P. Harwood, M. Schramm, J. Wolff, and M. Rodbell. 1974. 5'-guanylyl-imidodiphosphate, a potent activator of adenylate cyclase systems in eukaryotic cells. *Proc. Natl. Acad. Sci. U. S. A.* 71: 3087–3090.
14. Spiegel, A. M., and G. D. Aurbach. 1974. Binding of 5'-guanylyl-imidodiphosphate to turkey erythrocyte membranes and effects on β-adrenergic-activated adenylate cyclase. *J. Biol. Chem.* 249: 7630–7636.
15. Rodbell, M., M. C. Lin, Y. Salomon, C. Londos, J. P. Harwood, B. R. Martin, M. Rendell, and M. Berman. 1974. The role of adenine and guanine nucleotides in the activity and response of adenylate cyclase systems to hormones: evidence for multi-site transition states. *Acta Endocrinol.* 77(Suppl. 191): 11–37.
16. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* 10: 77–82.
17. Manganiello, V., and M. Vaughan. 1972. Selective loss of adipose cell responsiveness to glucagon with growth in the rat. *J. Lipid Res.* 13: 12–16.
18. Livingston, J. N., P. Cuatrecasas, and D. H. Lockwood. 1974. Studies of glucagon resistance in large rat adipocytes: ^{125}I-labeled glucagon binding and lipolytic capacity. *J. Lipid Res.* 15: 26–32.
19. Burns, T. W., and P. Langley. 1968. Observations on lipolysis with isolated adipose tissue cells. *J. Lab. Clin. Med.* 72: 813–823.
20. Forn, J., P. S. Schonhoffer, I. F. Skidmore, and G. Krishna. 1970. Effect of aging on the adenyl cyclase and phosphodiesterase activity of isolated fat cells of the rat. *Biochim. Biophys. Acta.* 208: 304–309.
21. Hartman, A. D., A. I. Cohen, C. J. Richane, and T. Hsu. 1974. Lipolytic response and adenyl cyclase activity of rat adipocytes as related to cell size. *J. Lipid Res.* 12: 498–505.
22. Jelínková-Tenorová, M., and Z. Hruza. 1963. The effect of epinephrine on fat metabolism in old rats. *Gerontologia.* 7: 168–180.
23. Jelínková, M., E. Stuchlíková, and M. Smrž. 1970. The effect of theophylline and adrenaline on the lipolytic

response of rats of different age. *Exp. Gerontol.* **5**: 257–260.

24. Nakano, J., A. C. Gin, and T. Ishii. 1971. Effect of age on norepinephrine-, ACTH-, theophylline-, and dibutryl cyclic AMP-induced lipolysis in isolated rat fat cells. *J. Gerontol.* **26**: 8–12.

25. Reardon, M. F., R. B. Goldrick, and N. H. Fidge. 1973. Dependence of rates of lipolysis, esterification, and free fatty acid release in isolated fat cells on age, cell size, and nutritional state. *J. Lipid Res.* **14**: 319–326.

26. Miller, E. A., and D. O. Allen. 1973. Hormone-stimulated lipolysis in isolated fat cells from "young" and "old" rats. *J. Lipid Res.* **14**: 331–336.

27. Berger, M., H. Preiss, C. Hesse-Wortmann, and F. A. Gries. 1971. Age dependence of fat cell size and lipo-

lytic activity in human adipose tissue. *Gerontologia.* **17**: 312–322.

28. James, R. C., T. W. Burns, and G. R. Chase. 1971. Lipolysis of human adipose tissue cells: influence of donor factors. *J. Lab. Clin. Med.* **77**: 254–266.

29. Zinder, O., and B. Shapiro. 1971. Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells. *J. Lipid Res.* **12**: 91–95.

30. Burns, T. W., P. E. Langley, and G. A. Robison. 1971. Adrenergic receptors and cyclic AMP in the regulation of human adipose tissue lipolysis. *Ann. N. Y. Acad. Sci.* **185**: 115–128.

31. De Santis, R. A., T. Gorenstein, J. N. Livingston, and D. H. Lockwood. 1974. Role of phosphodiesterase in glucagon resistance of large adipocytes. *J. Lipid Res.* **15**: 33–38.