Mechanism of the age-related decrease of epinephrine-stimulated lipolysis in isolated rat adipocytes: β-adrenergic receptor binding, adenylate cyclase activity, and cyclic AMP accumulation

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Abstract β-adrenergic binding ([^{3}H]dihydroalprenolol), adenylate cyclase activity, and cAMP accumulation were measured in adipocytes to investigate whether the mechanism of decreased hormone-sensitive lipolytic response with age was mediated through membrane-associated events. The dose of epinephrine required for half maximal stimulation of glycerol release (ED_{50}) was significantly lower in 2-month-old rats (0.8 ± 0.2 μM) than in mature (6- and 12-month-old) rats (5.2 ± 1.5 and 6.2 ± 1.5 μM, respectively). In 24-month-old rats the ED_{50} (0.7 ± 0.2 μM) was less than in mature rats. Maximum rates of hormone-stimulated glycerol release (per 10^6 cells) was highest in the two mature groups and decreased by 50% in the old rats ($P < 0.01$). Lipolytic changes were independent of cell size. β-adrenergic receptor number (50–90 thousand sites/cell) and affinity (K_D 4–5 nM) were the same in each age group. ED_{50} and maximum level of hormone-stimulated adenylate cyclase activity did not change with age. The ED_{50} of cAMP accumulation of young rats was 3 ± 5 μM compared with 24 ± 4 and 25 ± 5 μM in 6- and 12-month-old rats, respectively. In old rats, the ED_{50} of cAMP accumulation was 2 ± 1 μM ($P < 0.001$ compared with mature rats). Maximally stimulated cAMP levels were the same in old and mature animals. Phosphodiesterase activity in the presence and absence of 10^{-5} M isoproterenol did not change with age. The results suggest that age-related decrease of epinephrine-sensitive lipolysis in old rats may be due to alterations of the lipolytic pathway distal to the receptor-adenylate cyclase complex and the generation of cyclic AMP. —**Dax, E. M., J. S. Partilla, and R. I. Gregerman.** Mechanism of the age-related decrease of epinephrine-stimulated lipolysis in isolated rat adipocytes: β-adrenergic receptor binding, adenylate cyclase activity, and cyclic AMP accumulation. *J. Lipid Res.* 1981. **22:** 934–943.

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Age-related changes in the β-adrenergic lipolytic response have been previously reported, but little evidence concerning mechanisms has been presented (1–4). Yu, Bertrand, and Masoro (1) have shown that catecholamine-induced lipolysis in adipocytes decreased with age, while the age-effect was delayed by dietary restriction. Guidicelli and Pecquery (2) reported a loss of lipolytic response to epinephrine with aging and proposed that this finding was related to a decrease in the number of β-adrenergic receptor sites per cell. Forn et al. (5) reported a decrease of norepinephrine-stimulated lipolysis during maturation in the rat. Decreased adenylate cyclase activity and increased phosphodiesterase were observed and were postulated to explain the age-related alteration of lipolysis.

On consideration of the published reports in this area, the fragmentary nature of the information becomes even more apparent. Changes during maturation have not been differentiated from those of aging in the latter part of the life span (4–8). Lipolysis has sometimes been related to cell triglyceride content rather than to cell number (9). Increasing cell size which occurs during maturation has been equated with changes due to aging (4, 5, 7, 10). Some studies have used only a single submaximal dose of hormone and thus fail to examine the issue of sensitivity to hormone (dose-response, 2). Other work has used doses of hormone that may be supramaximal and result in non-specific stimulation of the lipolytic response (8).

The present investigation of the nature and mechanism of age-related alteration of lipolysis was an attempt to take into account a number of these

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issues and variables. Dose-response relationships along with cell size and number have been determined. Groups of animals have been chosen at ages that would allow distinction to be made between changes due to maturation and those related to senescence. Finally, several components of the β-adrenergic receptor–adenylate cyclase system, viz., β-receptors, adenylate cyclase, cyclic AMP accumulation, and phosphodiesterase activity, have been examined in order to investigate whether these membrane-associated parameters mediate changes in β-adrenergic lipolytic function with age.

MATERIALS AND METHODS

Male Wistar rats bred and maintained at the Gerontology Research Center were used in this study. The rats have a median life span of 24 months (50% mortality at 24 months) (11). The mean weights of a representative group of rats over the period of these experiments at 2, 6, 12, and 24 months were 301 ± 15,571 ± 13,614 ± 8, and 595 ± 15 g, (mean ± S.E.M.), respectively. (N = 10 in each group). The rats were fed standard laboratory chow with a 5% (by weight) crude fat content.

The rats were killed by decapitation or CO_2 narcosis between 9:30 and 10:00 AM. There was no difference in results from rats killed by either method.

Chemicals used were reagent grade or better. Isotopes were obtained from New England Nuclear. Collagenase (*Clostridium histolyticum*, Type I) was obtained from Worthington.

Preparation and sizing of isolated fat cells

Epididymal fat pads from 2–6 animals in each age group (2, 6, 12, and 24 months) were immediately dissected and rinsed in normal saline at 37°C. After weighing the fat pads from each age group, isolated fat cells were prepared according to the method of Rodbell (12) but with the following minor modifications. The fat pads were minced with scissors and placed in plastic conical flasks in Krebs-bicarbonate buffer (3 ml per g wet weight of fat) with 4% bovine serum albumin and 5 mg collagenase per g wet weight of fat. A single batch of collagenase was used throughout these experiments. Collagenase digestion was carried out at 37°C in a metabolic shaker at 100 cycles per minute. Cells were washed twice in fresh Krebs-albumin buffer and allowed to separate from the infranatant by flotation. A 500-μl aliquot of appropriately diluted cells was fixed in osmium tetroxide for counting and sizing in a Coulter counter (13) as previously reported from this laboratory (11). The packed cell volume for each preparation was noted for use in calculations to establish the number of adipocytes in each assay.

Incubation of isolated fat cells for determination of lipolysis rate and cyclic AMP

Packed fat cells were diluted 1 to 4 in Krebs-albumin buffer and 350 μl was added to polystyrene tubes containing Krebs-albumin buffer and epinephrine to give the desired final concentration of epinephrine in a total volume of 1 ml. After incubation at 37°C with gentle rocking (~60 cycles per min) in a metabolic bath for 1 hr, the tubes were placed on ice. Two hundred μl of cells and infranatant was transferred to tubes containing 20 μl of 2 M HCl for cAMP determinations and 200 μl of infranatant was reserved for determination of glycerol release. All incubations were carried out in duplicate. The mean numbers of cells in the incubates were $1.4 \pm 0.4 \times 10^4$, $6.7 \pm 0.6 \times 10^3$, $6.4 \pm 0.7 \times 10^3$, and $1.4 \pm 0.3 \times 10^4$ adipocytes per 200 μl for the 2, 6, 12, and 24-month adipocytes, respectively. The number of experiments is indicated in Table 1.

Measurement of lipolysis

The rate of lipolysis was measured as glycerol release. Glycerol was measured by the enzymatic method described by Weiland (14).

Cyclic AMP

Cyclic AMP was measured by radioimmunoassay of acetylated cAMP (New England Nuclear Kit). The tubes containing the acidified incubation mixture (20 μl of 2 M HCl and 200 μl of incubation mixture) were placed in a boiling water bath for 1 min and after cooling were neutralized with 10 μl of 4 M NaOH. Samples were frozen for storage. After being thawed and centrifuged (3000 *g*, 20 min, 4°C) 3.3 μl was taken for radioimmunoassay. Measurements of cAMP reported in this paper are expressed as the net accumulation of cAMP in the combined cells and medium. Phosphodiesterase activity was not inhibited during the fat cell incubations.

[^{3}H]dihydroalprenolol ([^{3}H] DHA) binding

A measured volume of isolated fat cells was homogenized in 10 mM Tris-HCl–0.25 M sucrose containing 1 mM EDTA at pH 7.4 in a chilled Dounce homogenizer with a glass to glass pestle. The homogenate was centrifuged at 16,000 *g* for 15 min at 4°C and the fat cake and supernatant were discarded. The pellet was washed in 50 mM Tris-HCl with 10 mM $MgCl_2$ at pH 7.4 (assay buffer), rehomogenized, and used directly in binding assays.

The binding assays were performed essentially

according to the method of Williams, Jarret, and Lefkowitz (15). Approximately 1 mg/ml of protein was incubated with 1 to 100 nM [^{3}H]DHA at 37°C for 10 min in a total volume of 250 μl. Specific binding was defined as the difference between counts bound to fat cell membranes in the absence (total binding) and presence (non-specific binding) of 5×10^{-6} M (−) propranolol. The specific binding represented 60–80% of the total binding at 5nM [^{3}H]DHA. Twelve concentrations of [^{3}H]DHA were used per experiment. Membrane bound [^{3}H]DHA was separated from "free" [^{3}H]DHA by washing the tube contents over a GF/C glass filter (Whatman) with 20 ml of ice-cold assay buffer. Membrane bound [^{3}H]DHA was retained on the filters that were counted in 10 ml of ACS solution (Amersham) at 34% efficiency. Analysis of the resulting binding data was by the method of Scatchard (16) and resulted in curvilinear plots with upward concavity (Fig. 5). Curve fitting was therefore achieved by least squares analysis using a computer program of Rodbard (17). The program was used to differentiate high and low affinity binding sites mathematically.

Adenylate cyclase

Adenylate cyclase activity was measured in fat cell ghosts by the method of Salomon, Londos, and Rodbell (18). This technique involves the conversion of [α-^{32}P]ATP to [^{32}P]cyclic AMP and isolation of the product by ion-exchange columns. Corrections for losses were made as described (11).

Phosphodieserase activity

Phosphodiesterase activity was measured in cell membrane particles and cell sap. The adipocytes were diluted 1 to 5 in Krebs-albumin (4%) buffer and the volume of incubation was 5 ml. Measurements were made in the presence and in the absence of 5×10^{-5} M isoproterenol, the dose at which maximum activity was achieved. The method of Filburn, Colpo, and Sacktor (19) was used with 1 μM cyclic AMP as substrate. This concentration of cAMP was saturating for low K_m phosphodiesterase. Incubation time was 20 min at 37°C. The time course of phosphodiesterase activity was linear up to 40 min.

Protein

Protein was measured by the method of Lowry et al. (20) using bovine serum albumin as the standard.

Data presentation

Data are presented as mean ± S.E.M. The unpaired t test and analysis of variance and Duncan's multiple range test were used as the tests of statistical significance. The ED_{50} refers to the dose of epinephrine producing a half maximal response. For the purposes of statistical analysis, mean values were calculated as follows. Within a given age group, all maximum levels were not achieved at the same dose of epinephrine. Data in Table 1 are calculated from the maximum levels achieved in individual experiments. When the glycerol release for each dose of epinephrine was averaged to obtain the mean dose–response curves, the plateaus seen in individual experiments were not evident. Similarly, the mean ED_{50} values were calculated by taking the mean of the ED_{50} values achieved in individual experiments. The mean dose–response curves (Fig. 3) were calculated by assessing the mean values of release at each dose of epinephrine.

RESULTS

Cell size and age

The mean diameters of the isolated fat cells increased from 71 ± 2 μm (N = 19 preparations) in the 2-month animals to 101.3 ± 2 μm (N = 20) in the 6-month animals. The mean diameter of the 12-month adipocytes was similar to those at 6 months (100.8 ± 2 μm, N = 22) and larger than those from the 24-month old rats (91 ± 2 μm, $P < 0.005$, df = 38). The recovery ratio (assessed as fat cell volume/wet weight of the fat pads) was 0.85 ± 0.03 in the old cells compared with 0.97 ± 0.01, 1.02 ± 0.03, and 0.98 ± 0.03 from cells of the 2-, 6-, and 12-month rats, respectively ($P < 0.005$). The cause of the lower recovery ratio for the cells from 24-month animals was not determined, but appeared to be due in part to greater cell fragility. Occasionally, preparations were encountered with more than 5% of free fat after collagenase digestion; these were discarded. After modification of the cell preparation procedure such that the cells were separated from the medium by flotation, the fragility problem in the adipocytes from senescent rats was markedly less pronounced.

Rates of lipolysis

Glycerol release (μmol/10^6 cells) was measured over 60 min. Basal rates of lipolysis were defined as the glycerol release in the absence of epinephrine. Lipolysis rates were linear over this time interval for rat adipocytes of all age groups.

Individual dose–response curves for epinephrine-stimulated lipolysis showed plateau formation in most cases (**Fig. 1**). In the experiments reported in this paper, eight of nine, eight of eleven, eight of thirteen, and eight of eight experiments showed definitive

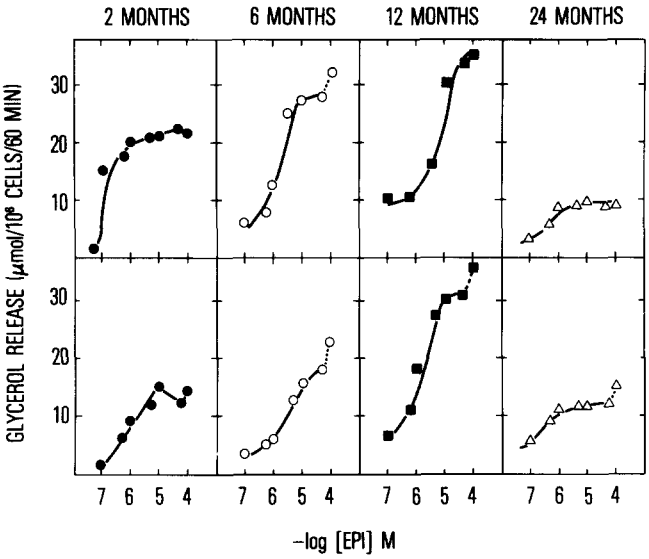

Fig. 1. Glycerol release in response to increasing doses of epinephrine from epididymal adipocytes of 2-, 6-, 12-, and 24-month rats. Adipocytes ($7-14 \times 10^3$ cells/ml) were incubated in Krebs-albumin (4%) buffer at 37°C for 60 min. Glycerol concentrations were measured in 200 μl of the infranatant by the enzymatic method of Weiland (14). Results from two separate experiments are shown. The dotted lines above epinephrine concentrations of 5×10^{-5} M epinephrine indicate nonspecific release (see Fig. 2).

plateau formation for the 2-, 6-, 12-, and 24-month groups. However, above an epinephrine dose of 5×10^{-5} M, there was a nonstereospecific stimulation of glycerol release (**Fig. 2**). The nonstereospecific stimulation was not blocked by 10^{-5} M propranolol (4). Thus, the dose of epinephrine producing half maximal response (ED_{50}) and maximum levels were calculated from the *individual* dose–response curves of each experiment. The *mean* dose–response curves did not necessarily show plateau formation, since the maximum responses were not achieved at exactly the same dose of epinephrine, and the non-specific release obliterated the plateau (**Fig. 3A**) in a few experiments. (The last paragraph of the Materials and Methods section explains how the mean dose response curves were achieved.)

The glycerol release in response to a maximum dose of epinephrine for each age group is shown in **Table 1.** The adipocytes from old rats released less glycerol per cell with a maximally stimulating dose of epinephrine than the 6- or 12-month groups ($P < 0.01$). The larger 6- and 12-month adipocytes were capable of releasing greater amounts of glycerol per cell than the smaller 2-month adipocytes ($P < 0.05$).

When the ED_{50} for glycerol release of the different age groups was compared, the sensitivity of the 24-month group to epinephrine was about one-half log unit less than in the 6-month and 12-month groups (Table 1). This apparent increase in sensitivity was in some measure difficult to interpret, since the maximal rate of lipolysis was also less and was achieved at lower doses of epinephrine in the old cells (see Discussion). Cells from young rats were also more sensitive (lower ED_{50}) than those from the mature animals.

The basal levels of glycerol release in the 6- and 12-month groups (6.7 ± 1.1 and 5.5 ± 1.1 μmol/10^6 cells/60 min, respectively) were greater than in the 2- and 24-month groups (3.0 ± 0.5 and 2.8 ± 0.9 μmol/10^6 cells/60 min, respectively). Thus, when glycerol release was expressed as a percentage of basal, there was an apparent decrease in lipolysis in the mature compared with either young or senescent groups. The changes in ED_{50} described above were, however, present regardless of the manner in which the data were expressed (Fig. 3).

Since the mean size of the cells from the old animals was less than that from the mature groups, and since smaller cells (i.e., from young animals) had lower lipolytic rates, we attempted to distinguish between differences in glycerol release due to age and to cell

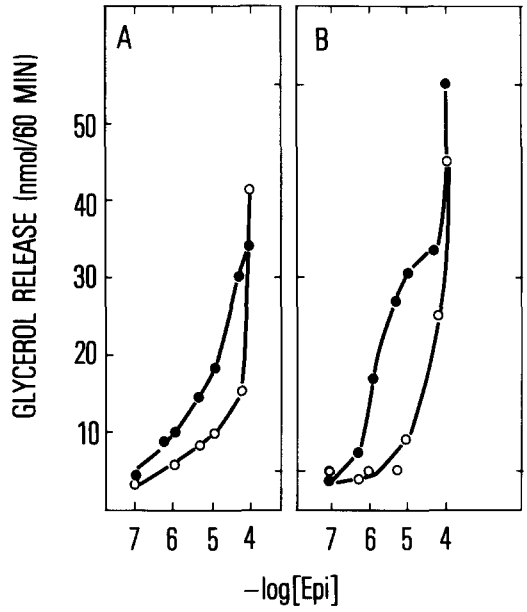

Fig. 2. The stereospecific stimulation of glycerol release by high doses of (−) and (+) epinephrine in 12-month (panel A) and 24-month (panel B) adipocytes in a single representative experiment. Adipocytes (87.5 μl of packed cells) were incubated with increasing doses of the (−) isomer (●) or (+) isomer (○) of epinephrine in a total volume of 1 ml Krebs-Ringer bicarbonate buffer, pH 7.4, with 4% bovine serum albumin for 1 hr at 37°C. Glycerol release was measured by the method of Weiland (14) in 200 μl of the infranatant. The nonstereospecific binding observed above doses of 5×10^{-5} M epinephrine was more pronounced in rats after maturation (12 and 24 months shown) and was not suppressed by 10^{-5} M propranolol (data not shown). The experiment was repeated twice.

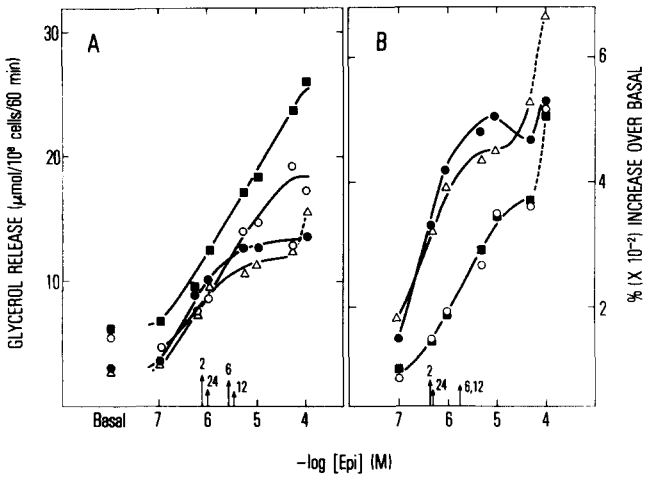

Fig. 3. Glycerol release in response to increasing doses of epinephrine in rat adipocytes of 2-(●), 6-(○), 12-(■), and 24-(△) month rats. Panel A shows the mean glycerol release (measured as in Fig. 2) expressed as μmol glycerol released per 10^6 cells; whereas panel B shows the same data expressed as percentage increase over basal. The arrows indicate the ED_{50} for each age group. Standard errors are shown in the text and Table 1. The number of experiments for each age group is as indicated in parentheses for Table 1.

size. Small subgroups (N = 5 in each case) of adipocytes with the same mean cell diameter were selected and analyzed separately. In the 6- and 12-month subgroups (cell diameters 104.6 ± 2.1 and 105.2 ± 2.7 μm, respectively,) the differences in glycerol release rates were not significant. The ED_{50} of epinephrine-stimulated lipolysis was also the same in both subgroups (**Fig. 4A**). In the 12- and 24-month subgroups (cell diameters 90.8 ± 3.8 and 91.4 ± 3.0 μm, respectively) the glycerol release rates were not statistically different, unlike results when the entire groups were analyzed. Although the ED_{50} of the 24-month subgroup was less (1.2 ± 0.5 μM) than that of the 12-month subgroup (4.9 ± 2.2 μM), the differences did not reach statistical significance, perhaps because the numbers were too small. The same trend, however, was evident in comparing the ED_{50} values for the subgroups as in comparing the large groups.

[^{3}H]DHA binding studies

The binding characteristics of the β-adrenergic antagonist in young, mature, and senescent adipocyte membranes were shown to be identical. Thus, binding was stereospecifically inhibited by the (−) isomers of β-adrenergic agonists 300 to 500 times more effectively than the (+) isomers. Scatchard analysis of [^{3}H]DHA saturation data showed curvilinear plots (**Fig. 5**) with upward concavity as had been previously described by Malbon and Fain (21). The data can be interpreted as showing two classes of binding sites, one of high affinity (K_D 2–6 nM) and low capacity, and the other of low affinity (K_D 15–30 nM) and with a capacity two to three times that of the higher affinity site. We assume that only the high affinity site

TABLE 1. Epinephrine-stimulated lipolysis and associated adenylate cyclase activity, cyclic AMP accumulation, and β-adrenergic binding parameters in young, mature, and senescent rat adipocytes[a]

	Age in Months			
	2	6	12	24
Maximum glycerol release (μmol/10^6 cells)	11.8 ± 2.1[c] (9)	21.2 ± 2.5 (11)	22.9 ± 2.6 (13)	12.5 ± 2.5[c] (8)
ED_{50} glycerol release (×10^{-6} M)	0.8 ± 0.2[c] (9)	5.2 ± 1.5 (11)	6.2 ± 1.5 (13)	1.1 ± 0.2[c] (8)
β receptors/cell (×10^{-4})	5.8 ± 1.4 (11)	9.2 ± 2.0 (13)	7.4 ± 1.8 (12)	5.1 ± 1.5 (16)
K_D [^{3}H]DHA binding (×10^{-9} M)	4.7 ± 1.5 (12)	5.7 ± 1.3 (15)	4.8 ± 0.9 (14)	7.6 ± 1.3 (17)
Adenylate cyclase activity at 10^{-4} M epinephrine (nmol/10^6 cells/10 min)	1.8 ± 0.4 (5)	1.2 ± 0.2 (8)	1.0 ± 0.2 (9)	0.9 ± 0.3 (7)
ED_{50} adenylate cyclase activity[b] (×10^{-5} M)	2.4 ± 0.8 (5)	1.8 ± 0.2 (8)	2.5 ± 1.0 (9)	2.2 ± 0.4 (7)
Maximum cAMP accumulation (pmol/10^6 cells/60 min)	152 ± 52 (6)	295 ± 79 (8)	296 ± 38 (8)	234 ± 73 (5)
ED_{50} cAMP accumulation (×10^{-6} M)	2.8 ± 1.5[c] (5)	24.4 ± 4.4 (9)	25.3 ± 4.7 (11)	1.9 ± 1.2[c] (6)

[a] Methodology as discussed in Materials and Methods.
[b] Taken as dose of one-half response at 10^{-4} M epinephrine.
[c] Value differs significantly from 6- and 12-month groups by analysis of variance ($P < 0.05$).

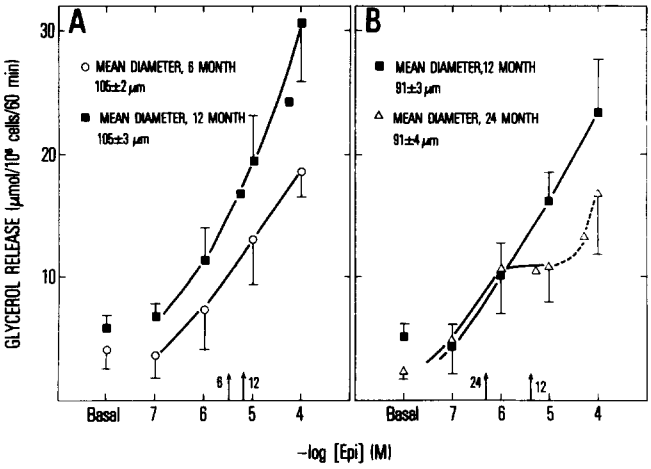

Fig. 4. The glycerol release in 6- and 12-month (panel A) and in 12- and 24-month (panel B) rat adipocytes with matched mean diameters in response to epinephrine. The groups of rats were selected from the experimental groups shown in Fig. 3, but had the same mean adipocyte diameter. Five experiments were selected for each of the age groups shown. The mean glycerol release in the 6-month (○) adipocytes was not significantly different from the 12-month (■) adipocytes nor was the glycerol release between the 12- and 24-month (△) adipocytes in panel B. The dotted line indicates that the glycerol release above 5×10^{-5} M epinephrine was nonstereospecific.

is of physiologic significance. This issue will be presented in greater detail elsewhere (manuscript in preparation).

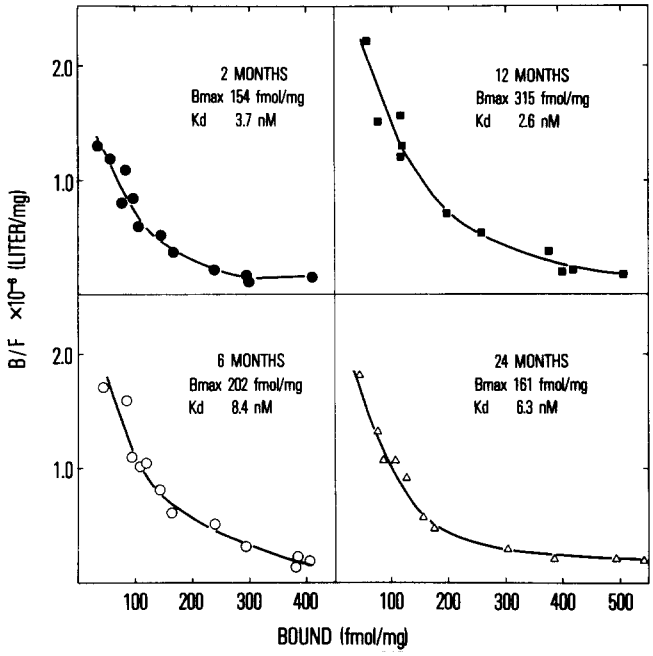

Fig. 5. Scatchard analysis of the steady state binding of varying concentrations of [^{3}H]dihydroalprenolol to adipocyte membranes of 2-, 6-, 12-, and 24-month rats. Adipocyte membranes were incubated with 1-100 nM [^{3}H]DHA at 30°C for 12 min. Curvilinear plots achieved were analyzed by the method of Rodbard et al. (17). Results are from a single representative experiment.

The results are presented in Table 1. Mean receptor number per cell varied between 60,000 and 90,000 and no significant differences were apparent. When receptor capacities (fmol/mg protein) were analyzed, there was still no difference between the age groups. Receptor affinity was the same in all groups.

Adenylate cyclase activity

Adenylate cyclase activity was not found to differ among the four age groups tested. At all doses of epinephrine, adipocytes from each of the age groups generated similar activity. The levels of activity in each group at 10^{-4} M epinephrine is shown in Table 1. The adenylate cyclase activity per 10^6 cells generated by 5 mM sodium fluoride was not different between the different age groups. The ED_{50} in each age group was also similar (Table 1).

Net cAMP accumulation

In cells and medium, the net cAMP accumulation in response to epinephrine produced a sigmoidal dose–response relationship. The maximum net accumulation by adipocytes from mature and old rats was not significantly different. The adipocytes from young rats accumulated less cAMP in response to maximum doses of epinephrine. However, the dose of epinephrine producing a half maximal response was less in the 24-month adipocytes than in adipocytes for the mature groups (Table 1).

Phosphodiesterase activity

Phosphodiesterase activity was measured in adipocyte membrane and supernatant after homogenization of adipocytes. In a limited number of experiments (N = 4) no statistical difference was found in the membrane particle nor supernatant phosphodiesterase activity of 3-, 12-, or 24-month rats, either at basal levels or after stimulation with 5×10^{-5} M isoproterenol. In each group the stimulation with isoproterenol was 10–12% above basal and the extent of stimulation by a single maximal concentration was not different between groups.

DISCUSSION

In inducing lipolysis, catecholamines act through the adipocyte β-adrenergic receptors via the enzyme adenylate cyclase which catalyzes the conversion of ATP to cyclic AMP, the phosphorylation of a protein kinase by cyclic AMP, and finally phosphorylation and activation of triglyceride lipase. Whether changes in the lipolytic response under various physiologic states,

including aging, are regulated via changes in the membrane receptor–adenylate cyclase–cyclic AMP system is not known, although some evidence suggests that this may be the case. Several studies have reported age-related changes in the catecholamine-stimulated lipolytic response (1–4), but detailed information on receptor number and affinity, adenylate cyclase, or accompanying changes in cyclic AMP accumulation that may mediate the changed response have not been previously available. The studies reported here revealed that adipocytes became less sensitive to epinephrine as they matured, but during senescence the adipocytes became more sensitive to epinephrine. The maximum glycerol release was found to be higher from mature adipocytes than from either young or senescent adipocytes, but these changes were apparently not mediated by cell size changes per se (see below). Beta-adrenergic binding parameters and adenylate cyclase activity did not change and could not explain the observed changes in lipolytic responses with age.

The present experiments on catecholamine-induced lipolysis studied the epinephrine dose–response relationship in young, growing animals (2 months), mature animals in which weight gain had ceased (6 and 12 months) and in old or senescent animals (24 months) that had reached their median life span. It was found that mature adipocytes were capable of releasing more glycerol in response to a maximum dose of epinephrine than either adipocytes from young or senescent rats. However, in contrast to many earlier studies, we have measured glycerol release through the entire dose response so that sensitivity to epinephrine could be estimated. It was found that young adipocytes or senescent adipocytes had greater sensitivity (lower ED_{50}) than the adipocytes from the mature rats. These results do not, of course, imply that the mechanism of the differences seen between young and mature and between the mature and senescent adipocytes is necessarily similar. However, they are of importance in studies such as these and may in part explain some confusion in the literature. If single submaximal doses are used to define lipolytic changes where there are differences in sensitivity, a change will be observed. However, this may not represent a change in the adipocyte's ability to achieve a similar maximum level of lipolytic activity. This would be particularly true if results were expressed as a percentage of basal activity (Fig. 3B).

The meaning of the finding that adipocytes of different age groups showed different sensitivities to epinephrine is difficult to define. If the data were expressed in terms of percentage increase over basal levels (Fig. 3B), it may be seen that there is a clear-cut shift to the right (and therefore decreased sensitivity to epinephrine) in responses of adipocytes from the 6- and 12-month groups. However, if the data were expressed in absolute terms (lipolytic rate/10^6 cells) (Fig. 3A), the response at lower doses of epinephrine is similar in all age groups, but the maximum rates of release are achieved at lower doses of epinephrine in the 2- and 24-month groups than in the mature groups. Thus, the change in sensitivity is not achieved by an obvious shift to the right of the epinephrine dose–response curve. We are unable to advance a physiological explanation for these results at present.

Hartman and Krause (22) have recently reported differing rates of reesterification in rats of different ages which might be one explanation of the changes in lipolytic sensitivity to epinephrine we have seen. Although the studies of Hartman and Krause have not included rats at all ages throughout their life span, they do indicate that future studies of lipolytic changes with age should examine both free fatty acid release and glycerol release. At present, the possibility that reesterification rates may change within a given depot as a function of age throughout the life span has not been tested.

The work of Hartman and co-workers emphasizes that the epididymal depot is only part of the adipose "organ". Hartman and Krause (22) found adipocyte responses in the mesenteric, perirenal, epididymal, and subcutaneous depots change as a function of weight (age) of the rat (up to 700 g). On the other hand, Yu et al. (1) found essentially parallel decreases in lipolysis in perirenal and epididymal sites. Although future studies should attempt to keep some of the regional differences in mind, we were able, for technical reasons, to examine only a single depot.

The role of changing cell size in control of the response of the aging adipocyte to catecholamines has been extensively discussed, but it still remains unclear whether the aging effect is separate from the effect of changing cell size per se on lipid mobilization (3, 23). The problem has been addressed in several different ways in the literature and we have attempted to compare our results with each of these workers' approaches. First, Gonzalez and deMartinez (3) examined adipocytes from very small subgroups of rats (N = 5 in each group) of different ages but with matched mean diameters. They found an increase in lipolytic response between maturity and senescence but a decline in lipolytic response between young and senescent adipocytes in the subgroups. The lipolytic response in the small subgroups with matched cell size was different from the entire group. In the

entire group, the lipolytic response was greater in the senescent adipocytes than in the mature. These workers concluded therefore that both cell size and age modified the lipolytic response and that cell diameters had to be taken into account in aging studies. In our study we examined a selected portion of our data in a similar way, but found that the lipolytic dose responses seen in the subgroups with matched mean cell diameters followed similar dose–response relationships to the entire groups. That is, the change in sensitivity between the senescent and mature groups was still observed when the cell size variable was controlled. Second, it had been demonstrated that there is a positive correlation between increasing mean cell size and lipolytic response (6, 7, 9), at least in growing and maturing rats. Our results were similar in that a greater maximum response of the larger mature adipocytes was exhibited compared with the lesser maximum response of the young adipocytes. However, our results differed in that the senescent and young adipocytes showed similar maximum lipolytic responses while there was a marked and significant difference between their mean cell sizes. This was strong evidence that age-related change in lipolysis was independent of mean cell size per se. There is evidence that adipocytes of differing cell size taken from a single pool positively correlate with the lipolytic response (7). When we made a correlation between mean cell diameter and maximum lipolytic response in the adipocytes from all experiments in this report, no linear correlation was seen ($r = 0.09$, N = 38). Thus changing cell size did not appear to be the principle mechanism responsible for the age-related responses seen by us. Finally, in the mouse, Jolly et al. (23) suggested that the glycerol output of adipocytes from animals of differing ages was equalized by factors influencing the adipocyte size. The changes that were observed with age were attributable to changes that occurred in response to changing cell size alone. When our results were expressed per unit of cell surface area, differences in the sensitivity to epinephrine between young and mature and between mature and senescent rats were maintained (data not shown). Thus, it is unlikely that cell size alone relates to the mechanism of age-changes in the Wistar rat.

Beta-adrenergic binding parameters and adenylate cyclase activities were shown not to change with age. Thus, our studies indicated that these parameters did not mediate the changes in lipolytic response. The results achieved here were in direct contrast to the only other study of β-adrenergic binding mechanisms as reported by Guidicelli and Pecquery (2). In their study, β-adrenergic receptor number was reported to decrease along with decreasing lipolysis in fat cells of the aging rat. Several deficiencies can be identified in their study, problems that we have attempted to circumvent here and that may explain the differing results. First, Guidicelli and Pecquery (2) used only a minimal number of adipocyte preparations and their results must be considered statistically inadequate. Furthermore, during our studies we found that adipocytes from senescent animals were particularly susceptible to lysis and that if the cells were centrifuged during preparation that lysis was exaggerated. Fragility of aged adipocytes and the necessity to modify isolation procedures of adipocytes from aging rats have also been reported by other workers (24). In the study cited (2), the centrifugation step was not omitted. The method used, therefore, may have produced membranes from a biased (less fragile) population of adipocytes. Each of these factors may have biased the capacity of β-adrenergic receptors on the resultant membrane preparations. Since no statistical analysis was presented and since we have found considerable variation from preparation to preparation (Table 1), we must question the validity of their conclusions. Yet another major difference between this work and ours concerns the affinity of the β-adrenergic binding sites. Since the Scatchard plots of [^{3}H]DHA binding in adipocyte membranes are curvilinear, it is essential to obtain raw data using several concentrations of [^{3}H]DHA less than 10 nM in order to describe the high affinity site we are describing. If minimum concentrations of [^{3}H]DHA around 10 nM are used, the Scatchard plots will still appear curvilinear but the affinities will not be as high as we have found. For these reasons it is necessary to compute lines of best fit. Guidicelli and Pecquery reported sites with an affinity for [^{3}H]DHA that was two to five times less than the affinity of the receptors we are reporting (K_D = 15–20 nM versus 4–7 nM, respectively).

In our present study, the adenylate cyclase activity was found not to differ between the different age groups. This finding is in accordance with other work we have published (11) and also with the finding that β-adrenergic receptor number does not change between the age groups.

The ED_{50} of net cAMP accumulation in young rats was considerably less (half a log unit) than in the mature (6- and 12-month) rats. In the old rats the ED_{50} for net cAMP decreased considerably from that in the mature rats. Since adenylate cyclase was unchanged with age, these results suggest that phosphodiesterase activity might differ in young and senescent rats. Forn et al. (5) showed an increase in phosphodiesterase activity in maturing rats (18–24 weeks) compared with very young (5–6 week) animals.

However, in contrast to the results we have seen, these workers found a decrease in adenylate cyclase between young and mature groups. We have no easy explanation for this difference. These workers used another strain of rat (Sprague-Dawley) and methodology different from that which we describe. Finally, it was not stated in the paper by Forn et al. (5) what diet the rats were fed. It has been shown that the fat content of rat chow may radically affect the response to epinephrine-stimulated lipolysis (25). This has been shown in rats of similar ages but the effect of aging has not been tested except with respect to dietary restriction (1).

We did not observe age-related differences of phosphodiesterase. Our observations in this regard were, however, limited in the numbers of experiments and in the use of only basal levels and maximal stimulation. The data reflected activity of low K_M phosphodiesterase. Although the possible importance of changing phosphodiesterase levels during altered age-related response to epinephrine remains less than fully examined, decreased lipolysis was seen even at comparable levels of cyclic AMP, indicating that phosphodiesterase activity per se is not the critical issue. Recent evidence in human adipose tissue suggests that, in collagenase digestion of fat during preparation of adipocytes, phosphodiesterase activity may be decreased and cyclic AMP levels may be altered (26). If this proves to be the case in the rat, the interpretation of these results is further complicated.

In the rabbit, an increase in the number of α-adrenergic receptors with increasing age has been observed (27). The α-adrenergic response is inhibitory to the β-adrenergic response in rabbit fat, so that the increasing α effect accounted for the decreasing β-adrenergic response. This is not the mechanism of the changing β-adrenergic response in the rat, since no modification of the β-adrenergic lipolytic response was observed with α-adrenergic antagonist. Furthermore, we have demonstrated no α-sites in the rat through use of the α-adrenergic antagonists [^{3}H]dihydroergocryptine or ^{3}H-labeled WB 4101 (data not shown).

The lipolytic rate in adipocytes of old rats was shown to decrease by about 50% when compared with mature rats. The sensitivity of the dose responsiveness was increased in old rats. No changes were observed in the membrane-receptor-adenylate cyclase systems suggesting that changes in these parameters were not the mechanisms responsible for the lipolytic changes. There were changes in the dose-responsiveness of cAMP accumulation but we cannot propose that these changes were directly responsible for the lipolytic changes. As cited, it has been reported that collagenase digestion distorts cAMP levels and phosphodiesterase activity in human fat cells (26). This warrants investigation in rat adipocytes. Also, lipolytic activity has been shown to correlate more closely with protein kinase activity in hamster adipose tissue than with cAMP accumulation (28), while in the rat it has been reported that the activated triglyceride lipase activity correlates with lipolysis better than cAMP accumulation. Although cAMP levels may be important they do not parallel lipolytic rates. Therefore, future studies will incorporate measurements of the intracellular enzyme activity of adipocytes.

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