Selective loss of adipose cell responsiveness to glucagon with growth in the rat

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Abstract In isolated fat cells, the same maximal rate of glycerol production can be induced by epinephrine or ACTH, alone or in combination with each other or with glucagon. With fat cells from rats weighing 150–175 g, the maximal rate of lipolysis attained with glucagon was 75–80% of that produced by epinephrine or ACTH, and with increasing size of the donor rat, the magnitude of the effect of glucagon relative to that of the other hormones declined markedly. In particulate preparations from fat cells of rats weighing 100–125 g, the maximal effect of glucagon on adenyl cyclase activity was about 60% of that of epinephrine, and was significantly less (30%) in preparations from 350–400 g rats. These data are consistent with the hypothesis that with growth of the rat there is a selective decline in the number of glucagon receptors relative to those for epinephrine or ACTH in the fat cell membrane.

Supplementary key words hormone-stimulated lipolysis · ACTH · epinephrine · cyclic 3′5′-AMP · adenyl cyclase · hormone receptors

Although the several hormones that stimulate lipolysis in rat adipose cells apparently do so through a single adenyl cyclase–triglyceride lipase system, it seems clearly established that epinephrine, ACTH, and glucagon stimulate cyclic AMP formation as a result of their interactions with different hormone-specific receptor sites on the fat cell membrane (1–4). Because of the independence and specificity of these receptors, it is possible to modify in vitro the responsiveness of intact fat cells or of adenyl cyclase preparations derived from them to one of the hormones without altering the action of the others (1–6).

We have found that, as rats grow, the fat cells exhibit a selective change in their responsiveness to glucagon, i.e., the maximal stimulation of lipolysis inducible with glucagon relative to that produced by epinephrine or ACTH declines with increasing age and size of the donor rat. A parallel change in the effect of glucagon on fat cell adenyl cyclase activity has been demonstrated.

METHODS AND MATERIALS

Epididymal fat pads were obtained from rats of the NIH Osborn-Mendel strain. Except where noted, all animals were allowed free access to food and water until killed by decapitation. Fat cells were isolated essentially as described by Rodbell (7). Krebs-Ringer phosphate medium containing bovine serum albumin, 30 mg/ml, and no glucose was used throughout. All incubations were carried out at 37°C in air. Minced fat pads, ca. 0.8 g/ml, in medium were incubated with collagenase for 1 hr. Fat cells were washed three times before being distributed to individual vials for experimental incubation. There were no obvious differences in the yield of fat cells from tissues from rats of different sizes. Fat cell weight was estimated from the amount of hexane-extractable, hydroxamate-reactive ester (8), assuming that 3 μmoles of ester are equivalent to 1 mg of cells. Cells, 25–50 mg, plus 2.5 ml of medium were incubated for 30 min before addition of hormone (in a volume of 25 μl). Samples (0.5 ml) of cells plus medium were taken at the beginning of incubation period and at several times after the addition of hormone for determination of glycerol by the method of Chernick (9), except that NADH was measured spectrophotometrically. All calculations are based on verified initial rates of glycerol production. With each preparation of cells, two or more concentrations of glucagon and of epinephrine or ACTH, or both, were used to establish that maximal rates were in fact attained. In the absence of hormone, the rate of glycerol production was usually less than 2 μmoles/g/hr, often not measurable. When measurable, basal values have been subtracted from values presented.

Adenyl cyclase activity in particulate fractions from frozen–thawed fat cells was measured as described previously (2). Cyclic 3′,5′-adenosine monophosphate (cyclic AMP) was isolated and purified from fat cell incubations (10, 11) and quantified by the method of Gilman (12). Crystalline glucagon (lot 258-234B-167-1) was a gift from the Eli Lilly Co. Porcine ACTH, 120 U/mg, was purchased from Calbiochem, fraction V from bovine serum from the Armour Pharmaceutical Co., and ^{3}H-labeled ATP (20.7 Ci/mmole) from Schwarz Biochemicals. Solutions of L-epinephrine were prepared from the bitartrate, and concentrations are expressed in terms of the free base.

RESULTS

We have previously reported that the same maximal rate of glycerol production could be induced by either epinephrine or ACTH (11). Addition of both together at maximally effective concentrations or in combinations with glucagon did not further stimulate lipolysis. In those studies, with fat cells from rats weighing 150–170 g, the maximal rate attained with glucagon alone was only 75–80% of that produced by the other hormones. In the experiment shown in Fig. 1, two preparations of fat cells, one from rats weighing about 175 g and the other from 500-g rats, were incubated with concentrations of epinephrine, ACTH, or glucagon that had been previously shown to produce maximal effects on lipolysis. As expected, the rate of hormone-stimulated lipolysis expressed per gram of fat cell was considerably lower with cells from the larger rats. In addition, however, the magnitude of the glucagon effect relative to that of epinephrine or ACTH was markedly decreased. In order to compare fat cells from rats of different sizes in terms of their responsiveness to glucagon without reference to number or weight of cells, rates of glycerol production in Figs. 2 and 3 are presented relative to the maximal rate induced by epinephrine or ACTH, or both, for each pool of cells. Rates of glycerol production induced by maximally effective concentrations of the latter two hormones were not increased by 0.1 mM theophylline. In the presence of the methyl xanthine, glucagon stimulated lipolysis to the same level as did epinephrine or ACTH.

As shown in Fig. 2, the concentration of glucagon required for maximal stimulation of glycerol production was apparently not very different for cells from 250–300-g rats and those from 125–155-g rats, but the rate attained with the latter cells was much greater. Fig. 3 summarizes data from experiments done over a period of more than a year, showing that although there is no precise quantitative relationship between rat size and the relative effect of glucagon on lipolysis, responsiveness to glucagon declines strikingly with increases in rat weight above 100 g.

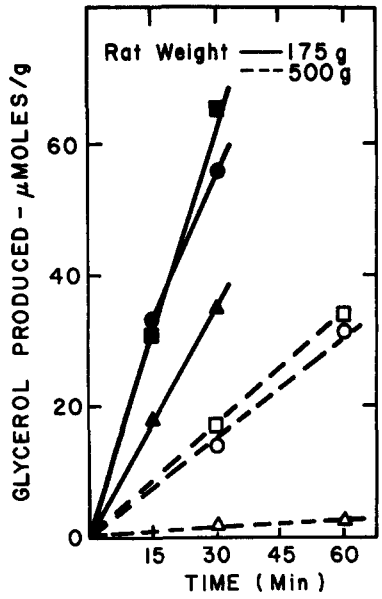

FIG. 1. Glycerol production by fat cells from large and small rats. Cells were incubated for 30 min before addition of 0.2 μg of epinephrine/ml (■,□), 1250 mU of ACTH/ml (●,○), or 1 μg of glucagon/ml (▲,△). Values represent the means of duplicate incubations.

The diminished glucagon response of the cells from larger rats was apparently not due to destruction of glucagon in the medium, since when cells from small rats (150 g) were incubated in medium in which cells from 380-g rats had previously been incubated for 30 min, stimulation of lipolysis by the "used" medium (originally containing 1 μg of glucagon/ml) was as great as that produced by fresh medium with 1 μg of glucagon/ml.

It has previously been found that the responsiveness of fat cells to epinephrine and ACTH is influenced by the ionic composition of the incubation medium (13–16). Ouabain, 0.5 mM, in the complete medium decreased the sensitivity to epinephrine more than that to ACTH and did not alter sensitivity to glucagon. Omission of Ca^{2+} from the Krebs-Ringer phosphate medium markedly decreased the sensitivity of fat cells to ACTH and had a considerably smaller effect on that to epinephrine. The absence of Ca^{2+} sometimes slightly decreased the sensitivity to glucagon, and omission of Mg^{2+} slightly increased it, but these effects were inconsistent and not demonstrably correlated with the size of the donor rats (data not shown).

By starving large donor rats for 48–90 hr, fat pad weight, and presumably cell size, was reduced (17). The relative stimulatory effect of glucagon in cell preparations from large donor rats was not, however, increased by this maneuver. In several preliminary experiments the lipolytic effect of glucagon relative to that of epinephrine was doubled in cells from rats that had been treated with

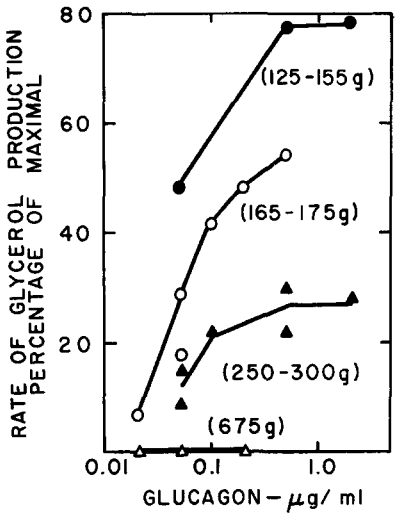

FIG. 2. Effect of glucagon concentration on the rate of glycerol production expressed relative to the maximal rate produced by epinephrine or ACTH. Rates of glycerol production are presented as a percentage of the maximal rate induced by epinephrine or ACTH, or both. Values for cells from rats weighing 125–155 g (●) and 675 g (Δ) represent single experiments; from rats weighing 165–175 g (○) and 250–300 g (▲), two experiments.

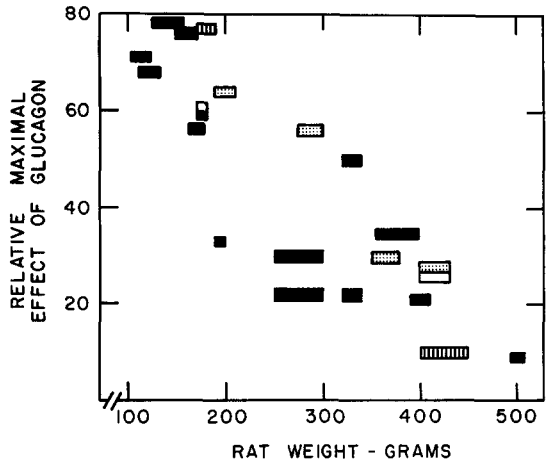

FIG. 3. Relative maximal effect of glucagon on the rate of glycerol production as a function of rat weight. Maximal rates induced by glucagon are presented as a percentage of the maximal rate induced by epinephrine or ACTH or both, for each preparation of cells from rats of the size range indicated. Some of the data have been presented previously (9), and some are taken from Figs. 1 and 2. In some of these experiments (■) and in two others (□,▥), cells from large and small animals were tested on the same day; ▩ represents studies carried out over a period of 1 month using tissues from a single litter of rats.

dexamethasone for 48–72 hr (which caused significant weight loss) compared with untreated littermates.

As shown in Table 1, the maximal effect of glucagon on adenyl cyclase activity in particulate fractions from fat cells of 100–125-g rats was about 60% of that of epinephrine, whereas with preparations from rats weighing 350–400 g, it was significantly less. It has usually been

TABLE 1: Effect of epinephrine and glucagon on adenyl cyclase activity

Rat Weight	Additions	Cyclic AMP Produced Expt. 1	Expt. 2	Expt. 3	Effect of Glucagon
		nmoles/mg protein			*%*[a]
100–125 g	None	0.7	0.4	0.7	
	NaF, 10 mM	3.7	1.5	2.7	
	Epinephrine, 62 μg/ml	2.6	1.5	3.1	
	Glucagon, 24 μg/ml	2.1	1.2	2.0	66.8 ± 6.4
	Glucagon, 48 μg/ml	1.8	1.2	1.9	60.2 ± 6.6
350–400 g	None	0.5	0.5	0.9	
	NaF, 10 mM	2.5	2.7	5.5	
	Epinephrine, 62 μg/ml	1.9	2.2	4.7	
	Glucagon, 24 μg/ml	0.9	0.8	2.3	27.7 ± 5.5[b]
	Glucagon, 48 μg/ml	0.9	0.9	2.6	32.3 ± 6.4[c]

Particulate fractions from fat cells were prepared as previously described (2), except that both centrifugations were carried out at 15,000 *g* for 15 min and the washed particles were suspended in 25 mM Tris buffer, pH 8.0, containing 1 mM EGTA (ethyleneglycol-bis[β-aminoethyl ether]-*N,N'*-tetraacetic acid). Adenyl cyclase activity was assayed using 3.1 mM ^{3}H-labeled ATP with incubation for 10 min at 30°C (2).

[a] (Δ due to glucagon)/(Δ due to epinephrine) × 100; mean ± SEM, n = 3.

[b] $P < 0.02$, large vs. small rats.

[c] $P < 0.05$, large vs. small rats.

found that hormonal stimulation of adenyl cyclase activity in fat cell ghosts or particles is greatest with epinephrine, and relative to the effect of epinephrine, the maximal levels attained with ACTH and glucagon have tended to be variable (2–4). In the present experiments, procedures and conditions for preparation of fat cell particulate fractions were carefully controlled and replicated. Adenyl cyclase activity was assayed in the presence of EGTA. Perhaps for these reasons, variability was minimized sufficiently to make possible the demonstration of a significant difference between preparations from large and small rats in terms of their relative stimulation by glucagon. There was no consistent difference between the two groups of preparations in terms of basal, fluoride-, or epinephrine-stimulated cyclase activity per milligram of protein. (Forn et al. [18] reported that in homogenates of fat cells assayed in the presence of sodium fluoride, adenyl cyclase activity per fat cell decreased with increasing size of the donor rat.)

In fat cells from rats weighing 150–200 g, levels of cyclic AMP 5 min after the addition of glucagon were lower than those produced by exposure to epinephrine for the same period (11). With cells from rats weighing 370–400 g, hormone-induced changes in cyclic AMP were difficult to detect in the absence of a methyl xan-

thine to inhibit partially cyclic nucleotide phosphodiesterase. In the presence of theophylline, glucagon produced in 5 min a somewhat smaller rise in cyclic AMP than did epinephrine.[1] Since cyclic AMP levels are not a good index of adenyl cyclase activity in intact cells and do not correlate well with rates of lipolysis (11), no attempt was made to compare fat cells from rats of different sizes in studies of this type.

DISCUSSION

The mean size of fat cells in the rat epididymal fat pad increases with increasing age and weight of the rat (19). Using fat cell preparations of different mean sizes obtained from a single donor rat or from rats of varying weights (100–300 g), Zinder and Shapiro (19) found that with increasing cell size the maximal rate of lipolysis induced by epinephrine or ACTH declined when expressed per weight of cell triglyceride, increased when calculated per cell, and changed very little in relation to the total surface area of the cells. They concluded that the maximal rates of ACTH- or epinephrine-stimulated lipolysis were independent of age or weight of the donor rat, but directly related to total cell surface area. We have found that independent of any reference to cell weight or number, the maximal rate of glycerol production induced by glucagon and expressed relative to the maximal rates produced by epinephrine or ACTH (which were similar with each cell preparation) declines with increasing size (age) of the donor rats. In order to determine whether this change is directly related to fat cell size, it would be necessary to compare the behavior of fat cells of different sizes obtained from a single rat (19). We did not observe any change in the magnitude of the glucagon effect when fat cell volume (although perhaps not surface area) was decreased by starvation of large donor rats for 48–90 hr.

It is probable that the observed age-related decrease in the lipolytic response to glucagon results from a decline in responsiveness of the fat cell adenyl cyclase to this hormone, although it is necessary to be cautious in drawing conclusions concerning enzyme activity in the intact cell on the basis of assays in fat cell ghosts or particulate preparations. Only a fraction of the total cell adenyl cyclase is contained in these preparations, and the concentrations of hormones required for stimulation of cyclase activity are much higher than those needed to produce maximal effects on cyclic AMP levels or on glycerol production in intact cells. In addition, it is conceivable that some damage to the glucagon receptors, similar to that produced by trypsin (5, 6), may occur during incubation of the cells with crude bacterial collagenase in the course of their preparation. The findings reported above must, nevertheless, reflect some age- or size-related modification of the portion of the glucagon response system that is hormone-specific, i.e., the glucagon receptors, since the change is evident when effects of glucagon are expressed relative to the maximal effects produced by epinephrine or ACTH. This could be an alteration in the extent or efficiency of the mechanism for coupling the primary reaction of glucagon with its receptor to the catalytic adenyl cyclase, assuming that this "transducer" (20, 21) is hormone-specific, or a change in the rate of inactivation of glucagon at or near its receptor site if such in fact occurs. Taking into account the observations of Zinder and Shapiro (19) that the maximal rate of epinephrine- or ACTH-induced lipolysis was related to cell surface area, it may be suggested that as the fat cells increase in size during growth of the rat, the composition of the new plasma membrane that is formed remains constant in terms of its content of receptors for epinephrine and ACTH, while the capacity of the cell to add and/or replace glucagon receptors declines, leading to an apparent loss of receptors for glucagon relative to those for the other two hormones.

It is possible that this phenomenon is the result of a primary change in the fat cell itself, but it seems to us more likely secondary to alterations of some other factors, perhaps one or more of the hormones (e.g., thyroid hormone, corticosteroids, growth hormone) that are known to modify the responsiveness of the fat cell to the so-called lipolytic hormones. In most studies of the effects of these "permissive" hormones, the possibility that they might specifically or differentially influence responsiveness to epinephrine, ACTH, or glucagon has not been directly investigated. Braun and Hechter (20) reported that treatment of intact or adrenalectomized rats with dexamethasone selectively increased the response of fat cell adenyl cyclase to ACTH without altering the response to the other two hormones or to fluoride. Our preliminary finding that treatment of large rats with dexamethasone increased somewhat the relative lipolytic effect of glucagon appears to be at variance with their work and will require further investigation. Nevertheless, the selective loss of glucagon responsiveness with age reported here and the specific effect of dexamethasone treatment on adenyl cyclase responsiveness to ACTH are two examples of perhaps multiple ways in which the responsiveness of the fat cell to individual hormones can be physiologically controlled or modified. It seems likely that the mechanisms involved here are similar or analogous to those underlying the regulation of hormone receptors in other types of cells, their specificity, and time of appearance or disappearance in the course of development.

Gold et al. (22) have recently reported that in the chronically failing heart the adenyl cyclase exhibits a

[1] Manganiello, V., F. Murad, and M. Vaughan. Unpublished data.

selective loss of responsiveness to glucagon (relative to norepinephrine). In a few experiments, we have compared adenyl cyclase activity in particulate fractions of heart muscle from 150- and 450-g rats. The effect of glucagon relative to that of epinephrine was not significantly different in the two groups. It will be of interest to learn whether there are age-related changes in the responsiveness of other cells, e.g., the beta cells of the pancreatic islets, to glucagon.

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