Inhibition of epinephrine-induced lipolysis in isolated white adipocytes of aging rabbits by increased alpha-adrenergic responsiveness[1]

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Abstract The aim of this study was to explain the unresponsiveness of rabbit perirenal adipose tissue to epinephrine. The in vitro lipolytic response to isoproterenol and to epinephrine alone or associated with alpha- or beta-adrenergic blocking agents, was studied in the adipocytes of rabbits of various ages. Epinephrine induces a large glycerol release in young rabbit adipocytes whereas an increase in the rate of lipolysis cannot be shown with adult rabbit fat cells. Moreover, an antilipolytic effect can be shown for low concentrations of epinephrine when the basal rate of lipolysis is high in older rabbit adipocytes. Isoproterenol (beta-adrenomimetic) always exerts a strong adipokinetic effect, thus revealing functional beta-receptor sites. The blockade of alpha-adrenoceptor sites by phentolamine, which has no effect on young rabbits, abolishes the antilipolytic effect and unmasks a strong lipolytic effect of epinephrine on aged and normal rabbit adipocytes. The loss of beta-adrenergic responsiveness towards epinephrine in the aging rabbit is linked to the involvement of an increased alpha-adrenergic responsiveness. The stimulation of alpha receptor sites by epinephrine leads to a depressive effect on lipolysis (lack of adipokinetic effect or antilipolytic action).—**Lafontan, M.** Inhibition of epinephrine-induced lipolysis in isolated white adipocytes of aging rabbits by increased alpha-adrenergic responsiveness. *J. Lipid Res.* 1979. **20:** 208–216.

Supplementary key words perirenal adipocytes · glycerol · isoproterenol · beta-receptor sites · alpha-receptor sites · fat cell size.

Lipid mobilization and in vitro hormone-stimulated lipolysis remain puzzling questions in the rabbit. Rabbit adipose tissue is known to be relatively unresponsive to the adipokinetic effect of catecholamines while corticotropin exerts strong lipolytic effects. There are, however, contradictory reports about catecholamine-induced lipolysis in the rabbit. Most in vivo experiments have shown that epinephrine and norepinephrine both induce an increase of free fatty acids and glycerol levels in anesthetized and nonanesthetized rabbits (1–6). Several authors however have shown that isolated white fat cells cannot respond to epinephrine or norepinephrine by an increase in FFA output or in glycerol release (7–11). Kumon, Hara, and Takahashi (5) have recently shown that catecholamine infusions were able to induce an increase of plasma glycerol concentration in the adult rabbit and to promote a striking in vitro lipolysis in interscapular adipose tissue; perirenal, omental, and epididymal adipose tissue were unresponsive to catecholamines as described previously by other investigators. Kumon et al. (5) speculated, from their results, that fat mobilization could be explained by the action of endogenously liberated catecholamines on part of the whole systemic adipose tissue, but the unresponsiveness of the remainder was still unexplained. In a previous paper (11) we clearly demonstrated the presence of alpha- and beta-adrenergic receptors in the white perirenal adipose tissue of the adult rabbit. We proposed an interpretation of the absence of a noticeable lipolytic effect of epinephrine and focused on the possible role of inhibitory alpha-adrenoceptors, their stimulation by adrenaline leading to a depressive effect on lipolysis.

Recent investigations on different species have shown that adipose cell size is an important parameter for the basal rate of lipolysis and hormone-stimulated lipolysis. In fat cells, there is a probable link between the lipolytic response to hormonal stimuli and the cell size (12–17). Most of the numerous investigations have been conducted on rat, mouse, or human adipose tissue, well known for their strong responsiveness towards the lipolytic action of catecholamines. The ontogenic development of catecholamine resistance in rabbit adipose tissue has never been considered, nor has lipolytic activity or changes in adipocyte size. Some discrepancies mentioned previously by several

Abbreviations: FFA, free fatty acids; ACTH, adrenocorticotropic hormone.

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authors might be linked to a lack of definition of several parameters (age, nutrition, fat cell size).

The purpose of the present study, carried out with rabbit isolated perirenal white fat cells, was to determine if aging, associated with increased body weight and adiposity, affects the epinephrine responsiveness with regard to adrenergic receptor site activity.

MATERIALS AND METHODS

Animals

Male Fauve de Bourgogne rabbits were maintained at 20°C under a natural light–dark cycle and fed ad libitum on a standard pellet diet (UAR Paris). They were kept in a steady nutritional state in order to avoid any of the lipid metabolism disorders previously mentioned (18, 19). The laboratory chow was composed of protein (13%), fat (2.7%), carbohydrate (49.3%), cellulose (17%), water (10%), and trace amounts of minerals and vitamins. The rabbits varied in age from 50 days to 16 months and the body weight from 980 g to 4 kg. Animals were placed in one of three groups according to body weight (see Results).

Experimental procedures

Preparation of isolated adipocytes. The experiments took place from September to March for two years. All the studies were performed in the morning. The animals were killed by cervical dislocation after an 18-hr fast. Isolated adipocytes were obtained using Rodbell's method (20), slightly modified. The perirenal fat pads were removed, quickly cut into several small pieces, and digested at 37°C with gentle shaking in Krebs–Ringer bicarbonate buffer containing 2 mg/ml of crude collagenase. The collagenase method allowed the study of large rabbit fat cells, but some modifications were necessary. Large fat cells of aged rabbits were more fragile than those of younger ones. We noticed an increased cell breakage of isolated adipocytes in some preparations and rejected them. Some collagenase batches were unsuitable for the preparation of isolated adipocytes because they brought about cell lysis. Recently the same problem was reported for human fat cells (21). In the experiments reported here, the same suitable batch of collagenase was used throughout. The use of freshly siliconized glassware and the omission of the centrifugation procedure originally suggested by Rodbell (20) gave good preparations of large fat cells, with a reduced cell breakage yielding sufficient quantities for the study. The incubation medium used in all experiments was Krebs–Ringer bicarbonate buffer containing 6 μmol/ml of glucose and 3.5 g/100 ml of defatted albumin (lyophilized bovine fraction V). The pH was adjusted to 7.4 with NaOH after the buffer had been equilibrated with 95% O_2–5% CO_2. After 30 min for the older animals and 60–80 min for the younger ones, the isolated adipocytes were separated from the remaining tissue fragments by filtration through a silk screen. Cells were washed three times with 10 ml of fresh buffer. As previously reported by Schwabe, Ebert, and Erbler (22), the effect of lipolytic agents on cyclic AMP levels and glycerol release is dependent on the concentration of adipocytes in the incubation medium. However, norepinephrine-stimulated glycerol production was less affected by a 8- to 10-fold increase in the adipocyte concentration than was the cyclic AMP increase. The possibility of such an effect with rabbit adipocytes was considered. So, throughout this study, by appropriate dilutions of the concentrated adipocyte preparation we tried to limit the possible modifications of cell concentration. Samples of 500 μl of cell suspension (or the equivalent of 40–50 mg of total lipids for group II experiments, 20–30 mg for group I, and 70–80 mg for group III experiments) were incubated for 90 min at 37°C in 1.5 ml of incubation medium under a 95% O_2–5% CO_2 atmosphere with gentle shaking. A strict control of the cell count and dilution procedures was not done; thus, underestimations of the lipolytic rates in younger rabbits (higher adipocyte concentration per incubation flask) could have occurred. Adipokinetic substances and other agents were added (10 μl) just before starting the incubation. Incubations were done in duplicate or triplicate. At the end of the incubation procedure, the incubation vials were quickly packed in an ice bath to stop the cell metabolic activity. The lipolytic response of each incubation set was checked with adrenocorticotropic hormone (0.1 μg/ml incubation medium), a strong lipolytic agent on rabbit adipocytes.

Analytical techniques. In all experiments, at the end of the incubation, one aliquot of the isolated fat cell suspension was taken and stained with Giemsa colorant to determine cell size. The diameters of 400 adipocytes were measured with a microscope fitted with a micrometer. The mean fat cell diameter and volume were calculated according to the method of Hirsch and Gallian (23). To express metabolic activities, total lipids of cell suspensions were extracted according to the method of Dole and Meinertz (24) and the mass was found by gravimetric determination (after complete evaporation of the solvent). The glycerol released into the incubation medium was taken as a measure of lipolysis because of the very low rate of its reutilization by fat cells as compared with the rate of fatty acid esterification. Glycerol was analyzed by the

enzymatic method of Wieland (25). The lipolysis results are expressed as the number of micromoles of glycerol released for 100 mg of total lipids after 90 min, according to Jeanrenaud (26). Each assay was carried out in duplicate and the titration values were averaged. The lipolytic activity of the cells towards the pharmacological agents was expressed on the basis of lipid content inside the same experimental group. However, when necessary, because of interexperimental variation in adipose cell responsiveness to hormone stimulation, the results of some experiments are expressed on a percentage basis according to the following formula: (stimulated lipolysis minus basal lipolysis/basal lipolysis) × 100.

The mean values are given with standard error. The significance of the differences among the sets within an experiment was estimated with Student's paired *t* test. The test of Mann–Whitney was used to estimate the significance between experiments.

The following pharmacological agents were used in incubations: epinephrine hydrochloride (Sté Organotechnie), phentolamine (Ciba-Geigy), propranolol (I.C.I.-Pharma), isoproterenol (Winthrop), phenylephrine (Sigma), and ACTH (Choay Labs). These products were dissolved in saline and added to certain vials to obtain the concentrations given in the text. Bovine serum albumin (fraction V) was obtained from N.B.C., crude collagenase from Worthington Biochemical corporation, NJ. Enzymes came from Boehringer Mannheim.

RESULTS

Adipocyte preparations from three groups of animals were studied.

Group I was composed of young rabbits, 50–70 days old; the mean body weight was 1.100 ± 0.020 kg. The perirenal adipocyte population ranged in fat cell diameter from 30 μm to 100 μm with a mean fat cell diameter of 55 μm. Mean fat cell volume was 108 ± 18 pl.

Group II was composed of normal adult rabbits, 4–5 months in age; the mean body weight was 2.850 ± 0.170 kg. Fat cell diameters range from 40 to 136 μm, average 75 μm; the mean fat cell volume 237 ± 30 pl.

Group III consisted of older rabbits, 14–16 months old; their mean body weight was 4.100 ± 0.140 kg. The range in cell diameters was 90–160 μm, average 120 μm; the mean fat cell volume 882 ± 99 pl. The mean lifespan of Fauve de Bourgogne rabbits is 3–4 years under our housing conditions (individual cages and limited physical activity associated with ad libitum feeding of a synthetic diet).

The average diameter of rabbit perirenal adipocytes increased with age. As expected, the number of cells per gram of tissue decreased with the increase in cell diameter. Our investigation was not focused on a clear determination of the development of the adipose tissue organ during growth. We chose the mean cell volume as a rough determination of adiposity. Several authors showed that an increase of the adipose tissue mass during growth was associated with an increase in both cell number and cell size. Recently, the growth of perirenal fat pads of male New Zealand rabbits was studied (27). Until 6 months the growth was due to an increase in both the number and size of adipocytes. Between 6 months and 10 months, the weight of this fat pad doubled through an increase in the number of fat cells. The cellularity of this fat pad was stable after 10 months.

Epinephrine responsiveness of perirenal adipocytes of rabbits at various ages

As shown in **Table 1,** epinephrine exerts a strong adipokinetic effect on the adipose tissue of young rabbits (group I). In adult, commonly used, rabbits (group II) we found the same results as those obtained by previous investigators, namely, the absence of any noticeable lipolytic stimulation except for the extra-physiological concentration of epinephrine (2×10^{-4} M). In the older rabbits (group III) we did not find any stimulation of lipolysis; on the contrary, a reduction of spontaneous basal lipolysis occured for epinephrine concentrations of 0.02, 0.2, and 2×10^{-6} M. On the basis of the results shown in Table 1 we tried to obtain more detailed data on the lipolytic potentials of these fat cells. We investigated the effect of a beta-adrenoceptor-stimulating agent (isoproterenol) as well as the effect of the adrenocorticotrophic hormone on the lipolytic activity of the different fat cell batches.

Lipolytic responses of various tissues to isoproterenol and ACTH

ACTH (0.1 μg/ml or 1 μg/ml in the incubation of medium) strongly stimulated lipolysis in each adipocyte set, thus showing the efficiency of the intracellular lipolytic system (**Table 2**). Isoproterenol, 0.2 or 2×10^{-5} M, was as lipolytic as epinephrine for group I (young rabbits). An adipokinetic effect was clearly shown in both groups II and III. However, in group III, there was an important reduction in the lipolytic response to ACTH as well as to isoproterenol. The lipolytic response of larger cells is reduced on expressing the adipocyte responsiveness on a percentage

TABLE 1. Epinephrine-stimulated lipolysis in perirenal isolated fat cells of rabbits of various ages

Epinephrine concentrations $\times 10^{-5}$ M	0.002	0.02	0.2	2	20
Group I young rabbits[a] (16)	—	2 ± 3[b]	94 ± 20	1019 ± 233	1304 ± 212
Group II adult rabbits (32)	1.2 ± 2.4	1.3 ± 3.1	0.5 ± 2.6	15 ± 7	124 ± 15
Group III old rabbits (15)	—	−34.8 ± 7.5	−48.3 ± 6.3	−21.4 ± 10.4	33.6 ± 13.8

Dose response studies using epinephrine were done on different rabbit perirenal white fat cell preparations. Adipocyte responsiveness to epinephrine stimulation is expressed according to the formula: (stimulated lipolysis − basal lipolysis/basal lipolysis) × 100.

[a] Number of animals in each group of rabbits in parentheses.

[b] Positive results indicate a stimulation of lipolysis and negative results an inhibition of basal lipolysis.

Results are expressed as mean ± SEM.

basis when the basal lipolytic rate is increased. The glycerol production induced by these agents does in fact reach those obtained in group II rabbits. Nevertheless, it could be envisaged that the larger adipose cells are less sensitive than the smaller cells to the lipolytic agents, as previously reported for rat fat cells (13, 16, 18).

On the different batches of fat cells, isoproterenol was several times more effective than epinephrine. This result indicates the existence of efficient catecholamine beta-receptor sites in all the fat cell preparations investigated. The epinephrine unresponsiveness already mentioned (Table 1) seemed to be linked to membrane receptor modifications and not to a defect of the lipolytic system. It was obvious that the different lipolytic responses might be due to the involvement of alpha-adrenergic receptor sites by epinephrine which is a dual alpha and beta agonist. Investigations were carried out using an alpha-adrenoceptor blocking agent (phentolamine) and the almost pure alpha-adrenomimetic (phenylephrine).

Lipolytic responses of adult rabbit adipocytes (group II)

The basal rate of lipolysis (Table 2) was 0.261 ± 0.024 μmol/100 mg of lipid. The dose response of fat cells, to a beta-adrenomimetic (isoproterenol) or to epinephrine alone and in combination with phentolamine, is shown in **Fig. 1.** Epinephrine did not induce a clear lipolytic effect except for the pharmacological dose of 2×10^{-4} M. Isoproterenol elicited a highly lipolytic response: a significant ($P < 0.02$) lipolytic response appeared at 2×10^{-6} M isoproterenol. Higher doses induced a 3-5-fold stimulation of the basal lipolytic rate. Adrenaline in combination with phentolamine (5×10^{-5} M) enhanced glycerol release. There was no significant difference between the two responses at 20×10^{-5} M catecholamine. A more complete study of phentolamine effect was published previously (11). We expanded these results and explored the effects of alpha-adrenoceptor stimulation by phenylephrine, considered as an almost pure alpha-adrenomimetic (28). We investigated its action on isoproterenol-induced lipolysis (**Fig. 2**). Phenylephrine had no significant lipolytic effect on rabbit white fat cells and reduced significantly the lipid mobilizing effect of the standard concentrations of isoproterenol (0.2 and 2×10^{-5} M).

Lipolytic responses of young rabbit adipocytes (group I)

The spontaneous glycerol release was 0.269 ± 0.045 μmol/100 mg of lipid (Table 2). In adipocytes from young rabbits epinephrine and isoproterenol

TABLE 2. Basal rates of lipolysis and lipolytic responses of isolated perirenal adipocytes of rabbits at various ages to different concentrations of isoproterenol and ACTH

		Isoproterenol		ACTH	
	Basal lipolysis[b]	0.2×10^{-5} M	2×10^{-5} M	0.1 μg/ml	1 μg/ml
Group I young rabbits (10)[a]	0.269 ± 0.045	75 ± 15[c]	1018 ± 192	1826 ± 308	
Group II adult rabbits (15)	0.261 ± 0.024	62 ± 17	235 ± 38	895 ± 120	1130 ± 230
Group III old rabbits (11)	0.865 ± 0.159	60 ± 15	160 ± 53	184 ± 43	250 ± 62

Adipocyte responsiveness to lipolytic agents is expressed according to the formula: (stimulated lipolysis − basal lipolysis/basal lipolysis) × 100.

[a] Number of animals in each group of rabbits in parentheses.

[b] Basal lipolysis is expressed as μmol of glycerol released/100 mg total lipids. Incubation time was 90 min.

[c] Positive results indicate a stimulation of lipolysis.

Results are expressed as mean ± SEM.

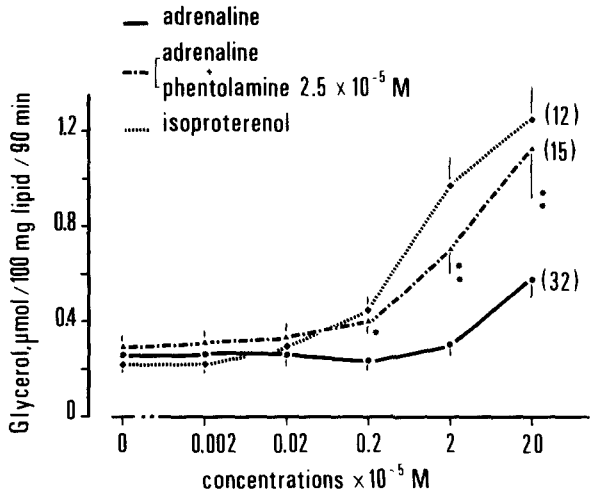

Fig. 1. Dose response curves for isoproterenol and epinephrine alone and in the presence of phentolamine in adult rabbit adipocytes (group II). Drugs were added at zero time. Isoproterenol response is not significantly affected by phentolamine. Vertical lines represent standard errors. The number of animals is in parentheses. *, $P < 0.02$; **, $P < 0.01$: result significantly different from epinephrine responses (by Student's paired t test).

elicited a strong adipokinetic effect (**Fig. 3**). No significant difference was found between the effect of epinephrine and that of isoproterenol. Phentolamine did not enhance the lipolytic effect of epinephrine. Phenylephrine did not reduce the adipokinetic action of isoproterenol.

Lipolytic responses of older rabbit adipocytes (group III)

Large adipocytes of the older rabbits studied in our investigations showed a significant increased basal rate of lipolysis compared with group II (0.865 ± 0.169 vs. 0.261 ± 0.024 µmol/100 mg of lipid; $P < 0.01$), as shown in Table 2, although the difference was underestimated by the expression of the results on a total lipids basis. An expression according to fat cell number would show an even more striking difference. Isoproterenol (2×10^{-5} M) exerted a clear lipolytic action (2.036 ± 0.240 vs. 0.865 ± 0.159) of basal lipolysis and ACTH (1 µg/ml) was strongly lipolytic (3.027 ± 0.292 vs. 0.865 ± 0.159). Under these conditions a significant decrease of the basal rate of lipolysis could be seen at low concentrations of epinephrine (2×10^{-7} M and 2×10^{-6} M), **Fig. 4;** the others being ineffective on basal lipolysis. Phentolamine (5×10^{-5} M or 5×10^{-4} M) although slightly inhibitory alone, suppressed the inhibitory effect of epinephrine, and unmasked a significant stimulation of lipolysis with ineffective higher epinephrine concentrations. However the level of isoproterenol-induced lipolysis was not reached; an incomplete alpha-blocking action linked to an insufficient phentolamine concentration could explain this slight difference. Moreover, a beta-adrenoblocking agent (propranolol) at the concentration of 2×10^{-5} M, associated with epinephrine, induced an important reduction of glycerol release as shown in Fig. 4, even for the higher concentrations of the hormone. Lipolysis stayed under the spontaneous basal rate.

The weak cell breakage noticed in this set of experiments tended to reduce the activity but not to falsely magnify the observed phenomenon, as clearly discussed previously by Zucker (29).

DISCUSSION

The present study of isolated perirenal fat cells of the rabbit was carried out to investigate the relationship between aging and the epinephrine effect on the adipose tissue. The data presented here deal with isolated adipocytes of clearly different sizes obtained from the perirenal adipose tissue of rabbits different in age and body weight. Mean fat cell volume increased 5–6-fold (108 pl to 882 pl).

The results in Table 1 indicate a striking difference in the lipolytic action of epinephrine on the adipocytes of the three groups of rabbits. Aging is

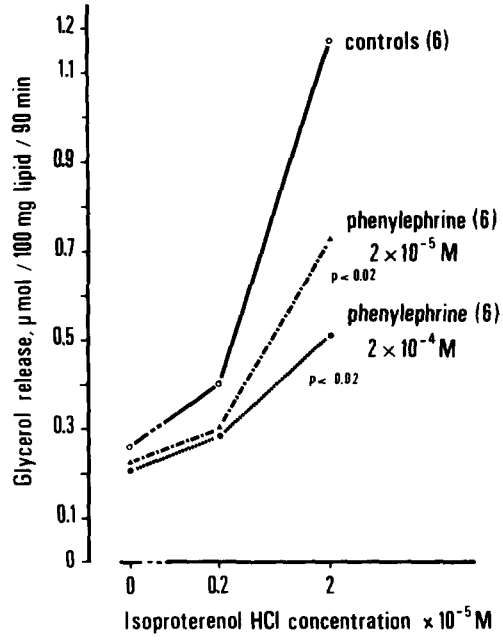

Fig. 2. Inhibition of isoproterenol-induced lipolysis in adult rabbit fat cells (group II) by an almost pure alpha-adrenomimetic (phenylephrine). Isoproterenol and phenylephrine added at zero time. Phenylephrine alone failed to modify the basal rate of lipolysis. The number of animals is in parentheses. $P < 0.02$, by Student's paired t test.

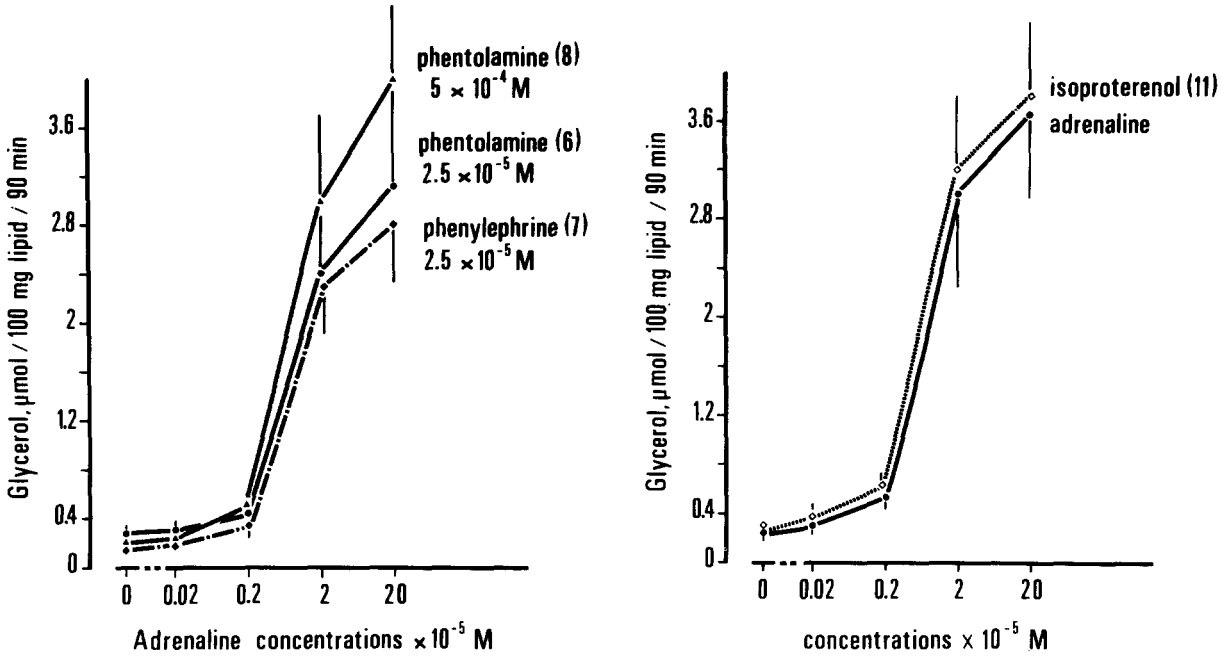

Fig. 3. Right, dose response curves for epinephrine and isoproterenol in young rabbit adipocytes (group I). Left, effects of phentolamine and phenylephrine on epinephrine-induced lipolysis in young rabbit fat cells (group I). The addition of phentolamine or phenylephrine failed to modify significantly the response to epinephrine at any concentration of that substance. Drugs were added at zero time. Vertical lines represent standard errors. The number of animals is in parentheses.

curiously marked by a quick loss of the adipokinetic effect of epinephrine. A strong lipolytic effect was observed in the younger animals, then, epinephrine became weakly active in adult rabbits, as mentioned by other investigators (7–11). At later ages, a fat-mobilizing capacity was not shown; an antilipolytic effect of epinephrine appeared in large fat cell preparations. On the other hand, spontaneous basal glycerol release was increased with aging; large fat cells of older animals had a 3–4-fold greater rate of baseline lipolysis than small fat cells of young rabbits (Table 2). These results are in agreement with previous reports on the rat or man (12, 13, 15, 17, 30, 31). Generally, basal lipolysis of fat cells from rats or humans in vitro increases with rat weight or human fat cell enlargement, respectively. The expression of lipolytic rates for rats on a per cell basis showed higher rates with increased cell size or animal age (17, 30). Catecholamine-induced lipolysis, commonly studied on epinephrine-responsive adipose tissues of different species led to conflicting results. In rat adipose tissue, Molgaard Hansen, Hoiriis Nielsen, and Gliemann (17) reported that norepinephrine-dependent lipolysis is positively correlated to rat weight; a similar result was reported for human fat cells (15). Hartmann et al. (31), however, working with rat adipose cells and Goldrick and McLoughlin (12), with human fat cells, could not find a correlation between norepinephrine-stimulated lipolysis and cell size. On the other hand, a decreased lipolytic activity (maximal response and sensitivity to epinephrine) was reported for old swine adipocytes (32) and rat adipocytes (14).

This age-dependent modification on epinephrine-induced lipolysis in the rabbit was studied in conjunc-

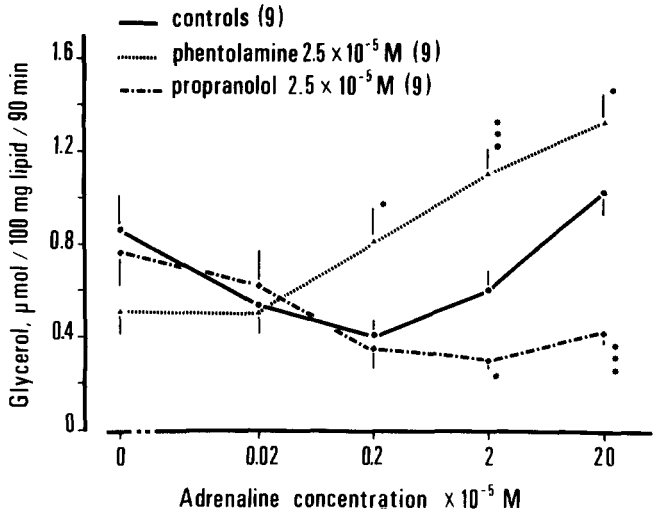

Fig. 4. Effect of various concentrations of epinephrine alone and in the presence of adrenergic blocking agents on lipolysis in aged rabbit fat cells (group III). Vertical lines represent standard errors. The number of animals is in parentheses. *, $P < 0.02$; ***, $P < 0.001$: results significantly different from control values (by Student's paired t test).

tion with our previous investigations in which we postulated the existence of beta- and alpha-adrenergic receptors in rabbit white fat cells (11). Thus, we tried to characterize, in terms of responsiveness to lipolytic substances (isoproterenol and ACTH) and in terms of adrenergic receptors sites activities, adipocytes from the different experimental sets. These results (Table 2) bring to light the existence of functional beta-receptor sites on small and large rabbit fat cells; they are able to induce an increase of lipolysis when stimulated by a pure beta-adrenomimetic drug. Expression of the lipolytic rates on a per cell lipids basis did not allow us to take into account the decreased cell number per unit of tissue weight as adipocytes increased in size. Under such circumstances an underevaluation of the true lipolytic activity is made in older rabbits. The investigation of possible modifications of the adipose tissue alpha-adrenergic responsiveness was prompted by the comparison between isoproterenol responses and the modifications of the reactivity of rabbit fat cells to a mixed agonist such as epinephrine. The unresponsiveness of the adipose tissue of normal and older rabbits to epinephrine could be related to an increase of fat cell alpha-adrenergic stimulation. So, alpha-adrenergic responsiveness was explored in our experiments with different drug combinations. *1*) Alpha-receptor site blockade suppressed the antilipolytic effect of epinephrine on older adipocytes (Fig. 4) and unmasked epinephrine-stimulated lipolysis on weakly responsive cells (Fig. 1). The response nearly reached those elicited by isoproterenol. Phentolamine had no action on the adipokinetic effect of epinephrine on small cells of young rabbits (Fig. 3). *2*) Phenylephrine reduced the lipolytic effect of isoproterenol on adult rabbit adipocytes (Fig. 2) but did not modify the adipokinetic action of isoproterenol on small fat cells of young rabbits (Fig. 3). *3*) A complete beta-receptor inhibition by propranolol combined with the epinephrine action increased the antilipolytic effect mentioned with epinephrine alone on large fat cells (Fig. 4). We think that such a combination allows the alpha agonist action to take place unopposed.

These investigations confirm an earlier report (11) and clearly indicate that alpha-, as well as beta-adrenergic receptors are present in rabbit adipose tissue. They allow a dynamic interpretation of the role of alpha-adrenoceptor activity on the fat cells of aging rabbits. Beta-receptor stimulation promotes lipolysis and alpha-adrenergic receptor stimulation is able to inhibit lipolysis. Epinephrine, a mixed agonist, can stimulate both beta- and alpha-adrenergic receptors of isolated adipocytes, the net result of these opposed effects on lipolysis will be under the control of the activity of the receptor sites. In young rabbits, with small fat cells (Fig. 3), beta-adrenergic receptors seem strongly effective either with the mixed agonist, epinephrine, or the pure beta-agonist, isoproterenol. Alpha-adrenergic responsiveness is neither clearly shown by the alpha-adrenolytic nor by the pure alpha-agonist. The loss of epinephrine efficiency, except for very large doses, on adult rabbit adipocytes (Fig. 1) may be interpreted as an increased alpha-adrenergic responsiveness as shown by the alpha-adrenolytically enhanced lipolytic effect and the alpha-agonist antagonization of the adipokinetic effect of isoproterenol. Although masked, the beta-adrenergic receptivity and the lipolytic efficiency is maintained as shown by the adipokinetic effects of isoproterenol and the remaining weak lipolytic effect noticed for pharmacological doses of epinephrine. On older rabbits with larger fat cells, the inhibitory influence of alpha-adrenoceptor stimulation can be clearly shown either with epinephrine alone or in combination with propranolol.

A clear antilipolytic effect of epinephrine is shown without any lipolytic action at higher doses. On the other hand, similar doses of alpha-adrenolytics used with other fat cell preparations prevent the antilipolytic effect and elicit a lipolytic action. However the isoproterenol-induced level of lipolysis cannot be reached and can be associated with an increased alpha-adrenergic efficiency; a higher dose of phentolamine would probably have been necessary. It is difficult to confirm that the antilipolytic action represents a stronger alpha-adrenergic effect than in adult rabbits, because an increased basal rate of lipolysis clearly brings to light the phenomenon of inhibition. We found the same result on fat cells with an increased basal rate of lipolysis in winter on 9-month-old rabbits (11). Schimmel (33) reported that the inhibitory influences of alpha-agonists towards lipolysis and glucose oxidation are only evident when these metabolic processes are both increased in the hamster fat cells.

Several investigators have reported the existence of alpha-adrenoceptors in the adipose tissue of man (34–36) and hamster (33, 37, 38). Their existence in rat fat cells is controversial. Some investigators explored the mechanism by which alpha-adrenergic agonists inhibit lipolysis and they reported a fall in the intracellular level of cyclic AMP (35–38) and adenylate cyclase inhibition (39, 40). The mechanism by which alpha-receptors mediate a reduction of cyclic AMP levels is quite unknown, and inhibition of adenylate cyclase should be considered as only one of nu-

merous possibilities. Even so, presently, the mechanism is not clearly understood and the significance of alpha-adrenergic effects on adipose tissue at physiological concentrations is still uncertain. Human fat cells or the animal fat cells generally studied (rat, hamster) have a clear catecholamine lipolytic responsiveness and a strong beta-adrenergic effect can be shown. A pathologically increased alpha-adrenergic responsiveness on adipose tissue was reported in hypothyroid man, explaining the failure of norepinephrine to promote an adipokinetic effect on this tissue (36). Recently we described a human subcutaneous adipose tissue that was unresponsive to the lipolytic effect of catecholamines and we also showed a strong alpha-adrenergic effect on the isolated adipocytes (41).

In conclusion, the data presented here confirm the presence of inhibitory alpha-receptors in rabbit perirenal fat cells and focus on the interest of this species as a model for the study of catecholamines and receptor site interactions. A development of the alpha-adrenergic responsiveness with aging is demonstrated and a dynamic interpretation of the physiological role of alpha-sites is proposed. Loss of epinephrine-induced lipolysis with aging in rabbit fat cells can be explained by an increased alpha-adrenergic responsiveness, suggesting that old adipocytes show an increased number of alpha-receptor sites or that the alpha-sites present are more active. These data also permit a good interpretation of several contradictory results previously reported for rabbit adipocytes. Fain (42) showed a clear lipolytic action of epinephrine on fat cells from very young rabbits; his data fit our present findings. On the other hand, some investigators observed an extremely heterogeneous response using the same strain of rabbit; some of them hardly reacted to catecholamines while adipokinetic reactions can be shown on the others (5, 10). These results can be linked to differences in fat cell size or age, leading to a different lipolytic effect as shown in this report.

The results reported here cannot clearly dissociate aging, increased body weight, or fat cell size. Aging is a complex phenomenon; in our experimental conditions, this problem is complicated by the development of large fat stores. The rabbits, with free access to food and limited physical activity become obese; differentiation between an obese rabbit and an old rabbit cannot be done in the present studies. Miller and Allen (43) mentioned such a problem for the rat. This study raises a hypothesis that needs more research to clarify and clearly dissociate obesity and aging and to provide grounds for studies of the physiological role of alpha-sites in other species.

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