Influence of development and reduction of fat stores on the antilipolytic α_2-adrenoceptor in hamster adipocytes: comparison with adenosine and β-adrenergic lipolytic responses[1]

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Abstract The response of the hamster adipocyte to various lipolytic (β-adrenergic) and antilipolytic (α_2-adrenergic and adenosine-dependent) stimuli was studied during the development and after cold-induced regression of fat stores. Alpha$_2$-adrenergic binding ([^{3}H]clonidine binding sites) was also investigated. Adipocytes came from young animals (4–5 weeks), adults (20–25 weeks), and adults submitted to a 6-week cold exposure (6°C) that promoted a large decrease in fat stores and in fat cell size. The lipolytic response induced by isoproterenol (β-agonist) was equivalent in the different groups. Adenosine and α_2-adrenergic antilipolytic effects were estimated through the inhibition of theophylline-induced lipolysis by phenylisopropyladenosine and clonidine, respectively. The adenosine effect was unchanged in all the groups. In contrast, the α_2-adrenergic effect, which was not present in young hamsters, increased simultaneously with fat cell size, was fully effective in adult hamsters, and had completely disappeared in small adipocytes from cold-exposed hamsters. In fat cell ghosts, α_2-adrenoceptors ([^{3}H]clonidine binding sites), followed similar modifications: they increased with fat cell enlargement and disappeared after cell size reduction following cold exposure. These results suggest that: *1*) the increased α_2-adrenergic antilipolytic response which is concomitant with fat cell enlargement could partly explain the growth-related decrease in the previously reported lipolytic effect of epinephrine; *2*) the α_2-receptivity of the adipocyte seems to be strictly fat cell size-dependent while the β-adrenergic and adenosine responses are unaffected; and *3*) the regulation in the adipocytes of the adenosine, α_2- and β-receptors seems to be unrelated.—**Carpene, C., M. Berlan, and M. Lafontan.** Influence of development and reduction of fat stores on the antilipolytic α_2-adrenoceptor in hamster adipocytes: comparison with adenosine and β-adrenergic lipolytic responses. *J. Lipid Res.* 1983. **24:** 766–774.

Supplementary key words fat cell size • α_2-receptor sites • β-receptor sites • adenosine receptor sites • [^{3}H]clonidine • isoproterenol • phenylisopropyladenosine • clonidine

A great number of studies have shown that both α- and β-adrenoceptors are present in adipocyte membranes of various species (1–6). The stimulation of lipolysis is mediated by the β-adrenoceptor while the α_2-adrenoceptor subtype is involved in the inhibition of lipolysis. So, it is now well accepted that the lipolytic differences in the responsiveness of the adipose tissue between various species are mainly dependent on the balance between α- and β-adrenergic receptors (6).

Most of the studies on the effect of aging on hormone responsiveness and sensitivity of adipose tissue have been carried out in the rat. Concerning the adrenergic agents, the most striking result is that aging is associated with a decrease in the lipolytic response to catecholamines (7–15) and that this effect is related to a decrease in β-adrenoceptor number (14) and adenylate cyclase activity (16, 17). Although the rat has been the most commonly used species for investigations into the age-dependent effect of catecholamines on adipose tissue, its adipocytes lack the α_2-adrenergic receptors controlling lipolytic processes (18, 19).

Little is known concerning changes of the α_2-antilipolytic adrenoceptor during aging. We have shown that in rabbit (20) and in dog adipocytes (21), the absence (22) or the loss (23) of the lipolytic response to epinephrine in the aging animal is linked to the involvement of an increased α-adrenergic response. Moreover, Pecquery and Giudicelli (24) reported that the α-adrenergic responsiveness increases with age in hamster adipocytes concomitantly with [^{3}H]dihydroergocryptine

Abbreviations: KRBA, Krebs-Ringer bicarbonate buffer containing albumin; PIA, phenylisopropyladenosine.

[1] A preliminary report of a portion of these data has been published in abstract form at the International Conference on the Adipocyte and Obesity: Cellular and Molecular Mechanisms, held in Toronto, June 1982.

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binding site number. In the hamster, as in the dog and the rabbit, aging is associated with an extension of the adipose tissue as demonstrated by the development of large fat pads with bigger adipocytes. It is of interest to distinguish the relative importance of aging and obesity in the appearance and the increment of the α-adrenergic antilipolytic effect in fat cells.

In the present report, a description of the antilipolytic α-adrenergic response in adipocytes of young and mature hamsters is associated with a study of α_2-adrenergic receptor binding sites. Moreover, we also present evidence suggesting that the main factor involved in the genesis of α_2-adrenergic responsiveness is the increase of the fat cell size rather than aging as previously supposed. In this study we also compare the antilipolytic effect initiated by α_2-adrenoceptor stimulation to that of phenylisopropyladenosine, a well known antilipolytic agent acting on inhibitory adenosine receptors of the fat cell membrane (25).

MATERIALS AND METHODS

Animals

All the studies were performed within a period ranging from May to July. The male golden hamsters used were reared in our laboratory. Hamsters that were 4–5 weeks old were considered as young, immature animals in our experiment. They were weaned 1 week before use. At this time, the mean body weight was 40–50 g. The diameters of the perirenal adipocytes ranged from 30 to 40 μm. We also used adult hamsters, 20–25 weeks of age, housed in a temperature-controlled room (22°C) and exposed to light–dark intervals of 12 hr. Another group of adult hamsters of the same age was housed in a cold temperature-controlled room (6°C), 6 weeks before use, under the same light–dark cycle. These animals had reduced physical activity and spent most of their time in a cotton bed included in each cage. They were normothermic over the entire course of the experiment. The experimental conditions were sufficient to produce significant weight loss and increased food intake (10–20%). All the animals were fed *ad libitum* with a standard diet (UAR, Paris) composed of protein (22%), fat (5%), carbohydrate (51%), cellulose (4%), water (12%), minerals, and vitamins. Body weight and other characteristics of the groups of hamsters are given in **Table 1.**

Preparation of isolated adipocytes

The fat cells were prepared as follows. The hamsters were fasted overnight. They were killed by decapitation and the perirenal and epididymal adipose tissue was immediately removed. Adipose tissue from 3–5 adults or 25–30 young or cold-treated hamsters was pooled to obtain sufficient yield of fat cells for lipolysis and binding studies. The tissue was cut into small pieces and incubated for 20–30 min at 37°C in 10 ml of Krebs-Ringer bicarbonate buffer containing 35 mg/ml of defatted bovine serum albumin (KRBA) and 6 mg of crude collagenase. The isolated fat cells were then prepared as previously described (3, 21, 22).

Lipolysis measurements

The isolated adipocytes (20–30 mg of cell lipid) were dispersed in plastic vials in 2 ml of KRBA and 6 μmol/ml glucose. Theophylline and other agents were added in portions of 20 μl just before starting the incubation procedure. After 90 min, an aliquot of the incubation medium was taken to determine glycerol release by Wieland's method (26). The metabolic activity is expressed as μmol of glycerol produced per 100 mg total

TABLE 1. Characteristics of various hamster groups: weight, fat cell size, basal lipolysis, and theophylline-induced lipolysis

	n	Weight		Fat Cell Size Diameter	Basal Lipolysis	Theophylline-induced Lipolysis	Ratio Stimulated/ Basal Lipolysis
		Before	After Cold Exposure				
		grams		*μm*			
Adult hamsters (20–25 weeks)	25	128 ± 3		70–90	0.97 ± 0.25[a]	2.76 ± 0.46	2.8
					2.22 ± 0.53[b]	7.47 ± 1.95	3.4
Young hamsters (4–5 weeks)	70	60 ± 6		30–45	1.48 ± 0.26	5.82 ± 1.41	3.9
					0.32 ± 0.04	1.77 ± 0.07	5.3
Adult hamsters placed in temperature controlled room (6°C, 6 weeks)	65	136 ± 5	111 ± 3	30–45	1.14 ± 0.22	4.46 ± 1.20	3.9
					0.33 ± 0.03	1.21 ± 0.23	3.6

Results are means ± SEM. Weight and fat cell diameters were estimated from the whole population of hamsters (n) used for all the experiments. In each group basal and theophylline-stimulated lipolysis are estimated from five experiments and expressed as:
[a] μmol glycerol/100 mg lipid per 90 min, or
[b] μmol glycerol/10^6 cells per 90 min.

lipid determined gravimetrically after extraction according to Dole and Meinertz's method (27) or as μmol of glycerol produced by 10^6 cells.

The fat cells of two vials were stained with Giemsa stain and the diameters of 400 cells were determined by optical sizing. The mean fat cell diameter and number were determined as previously described (22).

Binding studies

Pooled perirenal and epididymal adipocytes were washed three times in a lysing medium ($MgCl_2$, 2 mM; $KHCO_3$, 1 mM: ATP, 1 mM; Tris-HCl, 2 mM, pH 7.6). Crude adipocyte membranes were pelleted by centrifugation (10,000 × *g*, 10 min, 4°C). They were washed twice in a buffer (Tris, 5 mM pH 7.6; 1 mM EDTA) and finally suspended (2–3 mg protein/ml) in an incubation medium (10 mM $MgCl_2$; 50 mM Tris-HCl, pH 7.5). They were used immediately in binding assays or stored, at most for 1 week, at −80°C. Protein was determined using the method of Lowry et al. (28) with bovine serum albumin as standard.

Binding assays were carried out as follows. One hundred μl of membrane suspension (200–300 μg of protein) was incubated for 15–20 min at 25°C with 100 μl aqueous [^{3}H]clonidine solution in 400 μl total volume of Tris-HCl 50 mM, pH 7.5, containing 10 mM $MgCl_2$. Nonspecific binding was determined by addition of 100 μl of phentolamine (10^{-4} M). At the end of the incubation, duplicate 150-μl aliquots were diluted with 4 ml of ice-cold buffer and filtered immediately under vacuum, using Whatman GF/C glass fiber filters. The filters were washed rapidly with two 10-ml portions of incubation buffer at 4°C, dried at 50°C for 30–40 min, and counted in minivials in 4 ml of ACS scintillation mixture (Amersham-Searle) with an efficiency of 40–45%. All values refer to specific binding which represented 75–80% (adult hamsters) of the radioactivity retained on the filters. Receptor binding assay results were analyzed according to the general principles found in Williams and Lefkowitz's monograph (29).

Chemicals

Chemicals were obtained from the following sources: [^{3}H]clonidine (sp act 22.2 Ci/mmol) from New England Nuclear; clonidine chlorhydrate from Boehringer Ingelheim; phentolamine base from Giba-Geigy (Basle); and theophylline from Bruneau Labs (France). Bovine serum albumin (fraction V) and yohimbine-HCl were obtained from Sigma and crude collagenase was from the Worthington Biochemical Corporation (Freehold, NJ). Enzymes and phenylisopropyladenosine came from Boehringer-Mannheim Corporation. All other chemicals were of analytical grade.

RESULTS

In order to measure the biological effects of α_2-adrenoceptor stimulation on fat cell lipolysis, the action of clonidine, an α_2-adrenergic agonist, was studied during theophylline-stimulated lipolysis. The same experimental design, based on fat cell lipolysis stimulated by theophylline, was also used to test the inhibitory effect of phenylisopropyladenosine (PIA). This sort of experiment is an accurate model for the exploration of the inhibitory effect linked to α_2-adrenoceptor or adenosine-receptor stimulation (3, 6). Similar results were obtained when adenosine-deaminase was used instead of theophylline to promote stimulation of lipolytic activity (data not shown). When expressed as μmol glycerol/100 mg lipid, the basal lipolytic activity was very similar in young and adult hamster adipocytes (Table 1). However, when the lipolytic activity was related to 10^6 fat cells, the glycerol release was 6- to 7-fold higher in adults than in young or cold-exposed hamster adipocytes. Since non-stimulated fat cells showed a low basal lipolytic rate that did not permit lipolysis inhibition to be evaluated, the inhibiting effects were studied after theophylline stimulation of the cells. Theophylline (0.33 mM) promoted an increment of lipolytic activity of the fat cells that was of the same order of magnitude in the three groups, whatever the expression of the lipolytic rate as indicated by the values of the ratio of stimulated:basal lipolysis (Table 1).

Comparison of clonidine and PIA effects on theophylline-induced lipolysis in fat cells from young and adult hamsters

The effect of the α_2-adrenoceptor agonist, clonidine, on theophylline-induced lipolysis in fat cells from young and adult hamsters is shown in **Fig. 1.** Clonidine caused dose-dependent inhibition of the lipolytic response induced by theophylline (0.33 mM) in adult hamster adipocytes. The maximal inhibitory effect (65% inhibition) was reached with 5.10^{-7} M clonidine (lipolysis fell from 2.80 μmol glycerol/100 mg lipid in theophylline-stimulated state to 0.98 μmol/100 mg lipid with 5.10^{-7} M clonidine). A significant antilipolytic effect was observed with 10^{-8} M clonidine. In contrast, in adipocytes from young hamsters, clonidine had no inhibitory effect until 10^{-6} M; a weak inhibitory response appeared with large amounts of clonidine (5.10^{-6} and 5.10^{-5} M); the maximal effect did not exceed 10%.

The results obtained with the antilipolytic agent PIA are shown on the right of the Fig. 1. In both cases, PIA provoked an antilipolytic effect. Inexplicably, adipocytes from young animals were more sensitive to this agent since a significant antilipolytic effect was observed

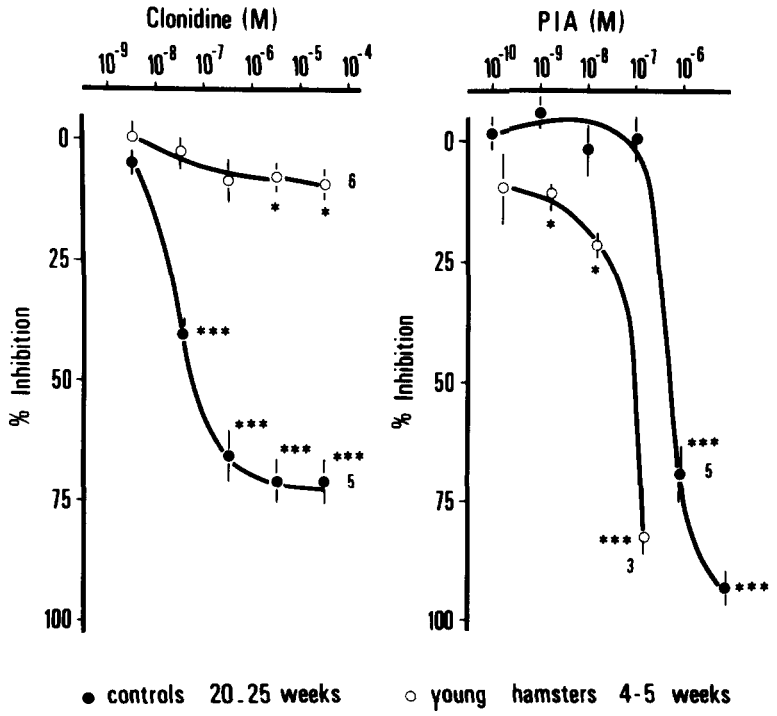


Fig. 1. Effects of increasing concentrations of clonidine (α-agonist) and phenylisopropyladenosine (PIA) on theophylline-induced lipolysis in fat cells of young and adult hamsters. The effects of the drugs are expressed as percent inhibition of theophylline-stimulated lipolysis. Values shown are means ± SEM of (n) different experiments indicated by the numbers. Statistical significance of the inhibition of lipolysis promoted by the agents was tested according to Student's paired *t* test. (*, $P < 0.05$; ***, $P < 0.001$).

at 10^{-8} M PIA, while in adipocytes from adults, the antilipolytic effect was only seen at 10^{-6} M PIA.

Effect of cold exposure on α-adrenergic response in adipocytes from adult hamsters

As shown in Table 1, the major consequence of a 6-week cold exposure was a significant reduction of hamster body weight. It is of interest to mention that, during the experimental period (from May to July), cold exposure did not promote hypothermia in the hamsters although a reduction of physical activity was observed in cold-exposed animals. In spite of over-feeding (food intake was increased 10–20%), this change in weight was brought about by a noticeable decrease in epididymal and perirenal rat pads and fat cell size. Thus, adult hamsters, of the same age as the controls, with a reduced fat cell size were obtained; mean fat cell size was similar to that found in younger hamsters. The ability of increasing concentrations of clonidine to inhibit lipolysis in adipocytes from control and cold-exposed hamsters is presented in **Fig. 2.** The lack of inhibitory effect of clonidine can be seen in cold-exposed hamster adipocytes even at high concentrations of the α_2-adrenergic agonist. On the other hand the antilipolytic effect of PIA was observed in control and cold-exposed hamster fat cells. The dose-response curves of PIA are superimposable, suggesting that the adenosine-inhibiting system is not altered in the adipocytes of cold-exposed animals.

Effect of isoproterenol on lipolysis of fat cells from young, adult, and cold-exposed adult hamsters

Since both α- and β-adrenoceptors exist in hamster fat cells, it was of interest to observe the effect of β-adrenergic stimulation on the adipocytes from the three groups of hamsters.

The study of the effect of increasing concentrations of isoproterenol (a β-adrenergic agonist) on the lipolytic activity of isolated fat cells was carried out in each group (**Table 2**). When the lipolytic potency of isoproterenol is expressed by the ratio of stimulated lipolysis:basal lipolysis, we obtain information on β-receptor efficiency. The dose-response studies showed that in the three groups, the maximal β-mediated response was obtained with 0.5 μM isoproterenol in each group.

However the lipolytic activity of the adipocyte in the different groups is expressed (on a per 10^6 cells or per 100 mg total lipid basis) (Table 2), the ratio is not sig-

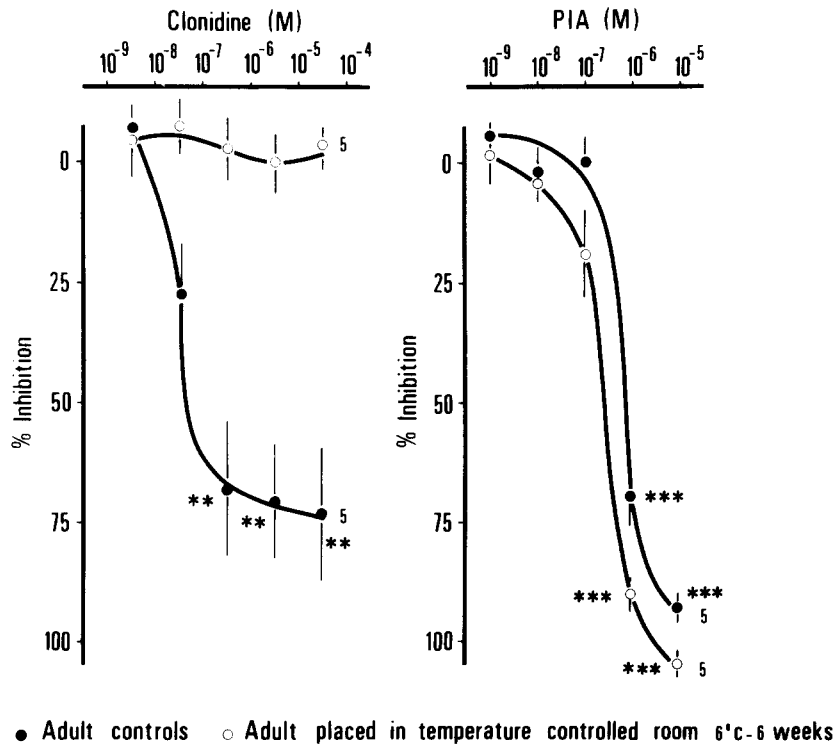


Fig. 2. Effect of increasing concentrations of clonidine and PIA on theophylline-induced lipolysis in fat cells of adult controls and adult hamsters with a reduced adipose tissue mass after cold exposure. The effects of drugs are expressed as percent inhibition of theophylline-stimulated lipolysis. Values are means ± SEM of (n) different experiments indicated by the numbers. Statistical significance of the inhibition of lipolysis promoted by the agents was tested according to Student's paired *t* test. (**, $P < 0.01$; ***, $P < 0.001$).

nificantly different at any concentration. Thus, the β-adrenergic effect is not noticeably modified in adipocytes from young animals and from cold-exposed emaciated animals.

[^{3}H]Clonidine binding on fat cell membranes from young, adult, and cold-exposed adult hamsters

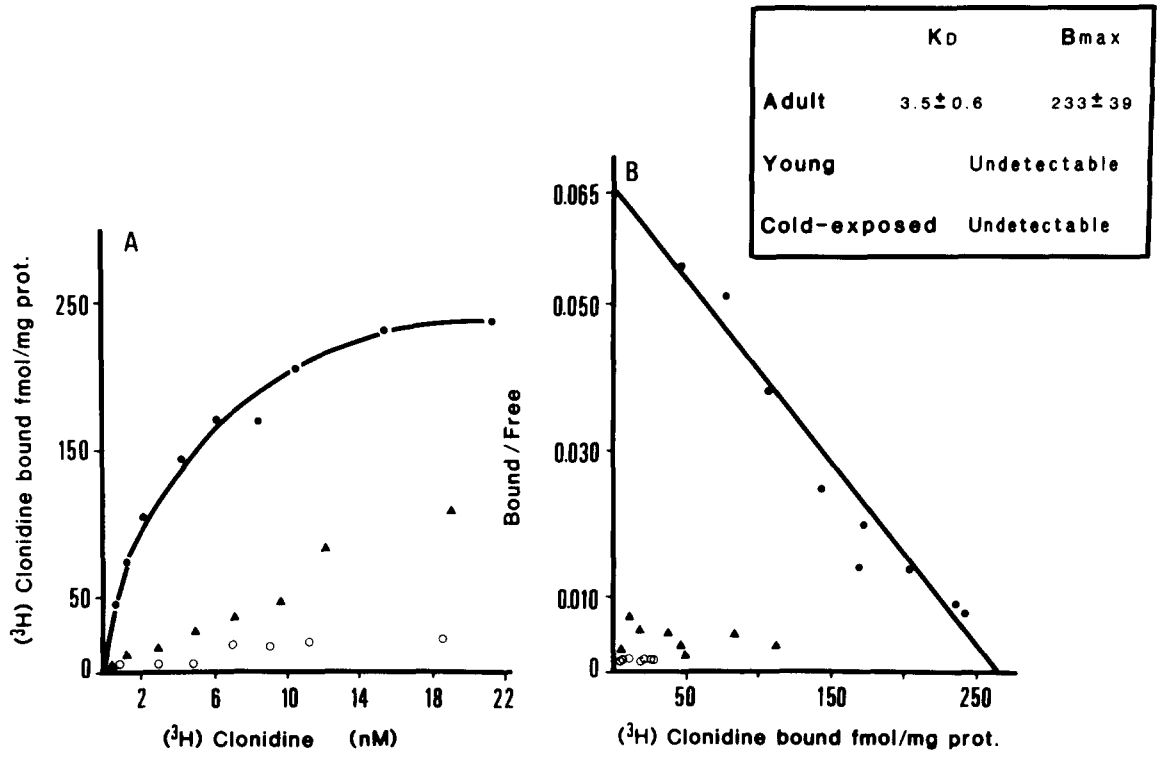
In order to assess the results obtained concerning the α_2-adrenergic response, we attempted to characterize the α_2-adrenoceptors using [^{3}H]clonidine on fat cell membranes from the three groups of animals.

The binding of [^{3}H]clonidine to hamster fat cell membranes was rapid. Half-maximal binding was reached in 2 min at 25°C. The association of the radioligand to its binding sites reached equilibrium within 10–15 min and was stable for at least 60 min. **Fig. 3** shows a typical saturation curve of the specific binding of [^{3}H]clonidine as a function of [^{3}H]clonidine concen-

TABLE 2. Comparison of isoproterenol-stimulated lipolysis in isolated fat cells of young, adult control, and adult hamsters placed in a temperature-controlled room (6°C, 6 weeks)

			Isoproterenol (μM)			
	n[a]	Basal Lipolysis	0.005	0.05	0.5	5
Adult hamsters (20–25 weeks)	5	0.89 ± 0.21[b]	1.16 ± 0.27	3.07 ± 0.52	4.98 ± 0.51	5.27 ± 0.44
		2.22 ± 0.53[c]	2.91 ± 0.69	7.67 ± 1.30	12.46 ± 1.64	13.19 ± 1.09
Young hamsters (4–5 weeks)	5	1.48 ± 0.26	1.67 ± 0.69	3.66 ± 0.41	7.31 ± 0.63	7.69 ± 0.45
		0.33 ± 0.04	0.42 ± 0.09	0.92 ± 0.10	1.89 ± 0.16	1.95 ± 0.11
Adult hamsters placed in temperature controlled room (6°C, 6 weeks)	5	1.14 ± 0.22		2.78 ± 0.52	5.36 ± 0.80	6.91 ± 0.95
		0.33 ± 0.03		0.70 ± 0.34	1.76 ± 0.41	1.82 ± 0.42

[a] Results are means ± SEM of (n) different experiments.
[b] Lipolysis is expressed as μmol glycerol/100 mg lipid per 90 min.
[c] Lipolysis is expressed as μmol glycerol/10^6 cells per 90 min.
However the lipolytic activity is expressed, the ratio of stimulated lipolysis/basal lipolysis is not significantly different. Moreover, there are no significant differences in the ratio (at corresponding concentrations) between the experimental groups.



	KD	Bmax
Adult	3.5±0.6	233±39
Young		Undetectable
Cold-exposed		Undetectable

Fig. 3. An example of specific binding of [^{3}H]clonidine to fat cell membranes from adult hamsters (●), young hamsters (○) and adult hamsters with reduced adipose tissue after cold exposure (▲). Panel A, as function of [^{3}H]clonidine concentration. Fat cell membranes were incubated with increasing concentrations of [^{3}H]clonidine for 15 min at 25°C and specific binding was determined as described in Materials and Methods. Panel B, Scatchard plot of specific binding of [^{3}H]clonidine derived from the saturation curves (Panel A). The ratio (bound/free) of bound ligand to free ligand (nM) is plotted against bound ligand (fmol/mg protein). The slope of the plot ($-1/K_D$) was determined by linear regression analysis. The number of binding sites (B_{max}) is calculated from the x intercept of the regression line. Inset table, [^{3}H]clonidine binding characteristics in fat cell membranes of adult hamsters (n = 6), young hamsters, and adult hamsters with reduced adipose tissue after cold exposure (6°C, 6 weeks) (cold-exposed). Parameters of binding cannot be calculated in either young or cold-exposed hamster fat cell membranes since the ratio bound/free is constant whatever the concentration of [^{3}H]clonidine used. The term "undetectable" indicates that binding parameters could not be determined. The experiments were repeated three times for young and cold-exposed hamsters (fat cell membranes pooled from 25–30 hamsters).

tration. Nonspecific binding represented 20–25% of the total binding in adult control fat cell membranes and 80% in the young and cold-exposed adult groups at 6 nM [^{3}H]clonidine. The binding exhibited saturation and Scatchard analyses indicated that there was only one class of binding site (n Hill = 1). The relative order of potency of antagonists in competition with the [^{3}H]clonidine binding sites was that expected for an α_2-adrenoceptor (not shown, see Ref. 19). The calculated B_{max} and K_D values obtained from Scatchard plots of various saturation curves are given in the inset in Fig. 3. It is noticeable that in young hamsters or adult hamsters submitted to cold exposure typical α_2-adrenoceptor binding was not detectable. Nonspecific binding values were higher than in the controls. Bound/Free values of Scatchard plots were low and there was no possibility of conducting linear regression analysis of the data (Fig. 3). Thus, under our experimental conditions, [^{3}H]clonidine binding in young or cold-treated hamsters cannot be accurately quantified and is very low. The large number of animals needed for a determination, 25–30, did not allow the binding experiments to be repeated more than three times. Nevertheless, it is noticeable that the binding results are in good agreement with lipolysis studies.

DISCUSSION

The present studies on isolated fat cells of hamsters were carried out to extend our earlier results on α-adrenergic responses in fat cells (22, 23) and to investigate the relationship between aging, fat cell enlargement, and cellular responses to catecholamines. In the rabbit and the dog, the increased α_2-adrenergic responses, which are concomitant to fat cell enlargement, could explain, in part, the age-related decrease in the lipolytic effect of physiological amines (epinephrine and norepinephrine) previously reported (22, 23).

The present results demonstrate that fat cell enlargement as a result of their maturation, occurring during

the growth of the hamsters (from 4–5 to 20–25 weeks), is associated with an increment of α_2-adrenergic response. The latter part of the life span will not be considered here. The occurrence and extent of this effect is not associated with a parallel variation (increment or decline) in β-adrenergic response (Table 2) as described previously in dog or rabbit adipocytes (22, 23). So the physiological amines, acting on the two sites, stimulate more α_2-adrenoceptors in the larger fat cells of 20–25-week-old hamsters than in younger animals.

In parallel to α_2- and β-adrenoceptor-mediated responses, we also investigated the adenosine-dependent inhibition of lipolysis to compare it with the α_2-mediated inhibiting effect. Since adenosine receptors and α_2-adrenoceptors are coupled to plasma membrane adenylate cyclase, act through an inhibition of the enzyme (30–33), and share a common pathway for their antilipolytic actions (34), it was of interest to check, by indirect means, if the enzyme was able to be inhibited by these two mechanisms under the various experimental conditions, especially when α_2-adrenergic responses were lacking. It is clear (Fig. 1) that the adenosine-dependent inhibition system is always functional and not subjected to noticeable variations during the development of hamster adipose tissue. Such a result supports the idea that the lack of α_2-adrenergic responses is not linked to the fact that adenylate cyclase cannot be inhibited and suggests investigation of the binding site variation.

Studies on α_2-adrenoceptor sites ([^{3}H]clonidine binding sites) revealed that these receptors are not detectable in small fat cells of young animals (Fig. 3). Thus, it appears that during fat cell development, α_2-adrenergic sites mature and α_2-adrenergic responses takes place concomitantly. A determination of β-adrenoceptor sites and adenosine sites was not carried out since their biological effects are not strikingly modified in the various groups. Thus, it would appear that during adipocyte growth and maturation, the fat cell plasma membrane undergoes structural changes mainly involving α_2-adrenergic sites and their probable coupling to adenylate cyclase. The β-adrenergic responses and adenosine-dependent effects indicate that adenylate cyclase can be either stimulated or inhibited, respectively, by the two agents. Such a result is quite provocative, since decline of various hormone receptors generally occurs during fat cell aging or maturation (35–38), and probably explains the decrease of glucagon (36) or ACTH-dependent adenylate cyclase activation (17).

Since the occurrence of α_2-adrenergic responsiveness can be causally related to aging or fat cell enlargement, we attempted to separate the influence of age from that of fat cell size and to establish the major determinant in the maturation of α_2-adrenergic responsiveness. Experiments were carried out to compare two groups of hamsters of similar ages with clear-cut differences in their adipose tissue mass. Instead of submitting animals to caloric restriction, as previously done in the rabbit (39), the reduction of adipose mass was promoted by exposure of hamsters to a cold environment (6°C) for 6 weeks. Under such conditions, a significant weight loss associated with a noticeable fat cell size reduction was observed; the mean fat cell diameter of cold-exposed adult hamsters equalled that of 4–5-week-old hamsters (Table 1). Fat cell size reduction was associated with a complete disappearance of α_2-binding sites (Fig. 3) and α_2-adrenergic responsiveness (Fig. 2).

No noticeable modifications in the sensitivity of the adipocytes to the β-agonist (Table 2) or PIA (Fig. 2) were found. The increment of α_2-adrenergic responsiveness observed during fat cell maturation and enlargement was completely reversed after weight loss and fat cell size reduction. Thus, the appearance and disappearance of the α_2-adrenoceptors of the adipose tissue seems to be correlated with the extent of perirenal and epididymal fat stores. The striking differences, observed under our experimental conditions, in α_2-, β-, and adenosine-receptor modifications suggest that the regulation of these receptors in the adipose tissue is partly unrelated. Direct measurements of the senstivity of isoproterenol-stimulated and PIA-inhibited adenylate cyclase activities associated to the determination of β- and adenosine-receptor sites will probably improve our data on adenosine- and β-adrenoceptor-mediated effects.

Among the mechanisms that can be advanced to explain the decrease in the α_2-adrenergic response in the adipocytes of cold-exposed hamsters, the hormonal changes occurring during cold adaptation could play a critical role in the control of adrenoceptor-mediated effects. It is well known that catecholamine excretion (mainly norepinephrine) is increased by cold exposure; this adaptative mechanism enhances cold resistance in nonhibernating animals (40–42). The chronic exposure of various cells to catecholamines is well known to induce hyporesponsiveness of the cells to the amines, a phenomenon commonly known as desensitization (43). Is α_2-adrenoceptor depletion due to a desensitization promoted by higher catecholamine levels resulting from cold-exposure? The absence of apparent desensitization of β-adrenergic receptors makes this hypothesis rather difficult to uphold. β-Adrenoceptor desensitization has been shown to exist in fat cells (43–45), while α_2-adrenoceptor desensitization is still questionable (45). This aspect requires further investigations and catecholamine determinations; moreover, the effect of a shorter cold exposure should also be investigated. Another hormonal factor able to play a noticeable role in the control of α_2-adrenergic responsiveness in adipocytes of cold-exposed hamsters is thyroxine. Radioimmunoassay tech-

niques have revealed high plasma triiodothyronine (T_3) levels in cold-acclimated rats without any symptom of hyperthyroidism (41, 42). If we refer to Garcia-Sainz et al. (46), α_2-adrenergic sensitivity of hamster adipocytes is unaltered by thyroid status while a conflicting result showed that hypothyroidism increases the α_2-antilipolytic response (47). This aspect is still unsolved.

Our data do not provide answers with respect to the causes of the increase and decrease in fat cell α_2-adrenoceptors and α_2-adrenergic responses, but they provide a useful model for further investigations into the interplay between the various families of receptors involved in the control of lipolytic activity of the fat cell. More research is needed to shed light on these problems. Nevertheless, in order to find the cause of α_2-adrenoceptor variations and to gain further insight into α_2-adrenoceptor regulation, adipose tissue has the unique capacity, among the other body tissues, to alter its mass under various conditions depending on age, endocrine status, and energy balance.

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