Ontogenic development of α -adrenergic receptors and responsiveness in white adipocytes

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The influence of age on adipocyte α -adrenergic receptors was studied by using the binding of [3 H]dihydroergocryptine to crude adipocyte membranes from hamsters of different ages (1–10 months). The number of α -receptor sites was found to increase with increasing age, but in contrast, their binding affinity remained unchanged. These changes in receptor concentrations, which were not related to cell-size differences, were also accompanied by parallel variations of the α -adrenergic responsiveness of the fat-cells.

Several studies have shown that the lipolytic response to catecholamines of adipocytes from different species varies with age and cell size (Goldrick & McLoughlin, 1970; Zinder & Shapiro, 1971; Nakano *et al.*, 1971; Jacobsson & Smith, 1972; Livingston *et al.*, 1974; Giudicelli *et al.*, 1977a). In rat fat-cells, the lipolytic activity of catecholamines is the result of a pure β -adrenergic stimulation (Fain, 1973). Therefore, we have examined the possibility that age and cell size might affect the number of β -adrenergic receptors in rat adipocytes (Giudicelli & Pecquery, 1978). These investigations have shown that the number of these receptors increases proportionally to the fat-cell surface area during completion of maturation and decreases during senescence. Moreover, these modifications were found to be accompanied by parallel variations in the sensitivity of adenylate cyclase to catecholamines (Giudicelli & Pecquery, 1978).

In human fat-cells, it is well established that the adrenergic response is the result of functionally opposed α - and β -adrenergic actions on adenylate cyclase and lipolysis (Burns *et al.*, 1971). Fat-cells from other species, such as the rabbit (Lafontan, 1979), the hamster (Hittelman *et al.*, 1973; Giudicelli *et al.*, 1977b) and the dog (Berlan & Dang Tran, 1978) elicit also a mixed α - and β -adrenergic response when exposed to catecholamines. It has been shown that the α -adrenergic responsiveness of adipocytes from the rabbit (Lafontan, 1979) and the dog (Berlan & Dang Tran, 1978) increases with fat-cell size and age. As the α -adrenergic receptors were not investigated in these studies, we have

examined the influence of age and cell size on the α -adrenergic receptors and responsiveness of hamster adipocytes in which we have identified specific binding sites for [3 H]dihydroergocryptine possessing all the characteristics expected of true physiological α -adrenergic receptors (Pecquery *et al.*, 1979a,b; Pecquery & Giudicelli, 1980).

Materials and methods

Preparation of 'crude adipocyte membranes'

Male golden hamsters (Charles River) of different ages (1–10 months) and weight (54–150 g) were fed *ad libitum* before being killed by decapitation. Epididymal fat-pads from five to 20 hamsters were pooled and isolated fat-cells were prepared by the rapid (7 min) collagenase-digestion isolation procedure of Hittelman *et al.* (1973). Isolated fat-cells were suspended in 15 ml of medium I (0.25 M-sucrose/1 mM-EDTA/10 mM-Tris/HCl, pH 7.4) and disrupted by six aspirations through a swinny filter holder fitted with a stainless-steel photo-etched support (xx 30 01 200; Millipore), as described by Avruch & Wallach (1971). After breakage, the suspension was immediately centrifuged at 20 000 g for 15 min at 4°C. The resulting pellet was redispersed in 10 ml of medium II (10 mM-MgCl₂, 50 mM-Tris/HCl), re-centrifuged and resuspended twice more in the same way. The final pellet ('crude' membranes) was resuspended in medium II, resulting in a suspension containing 1.8–2.5 mg of protein/ml, which was immediately used for the binding assays. Recovery of crude-membrane protein was 20–25% of the whole protein content of the fat-cell and was unaffected by age and cell size.

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Binding assays

Binding assays were performed by a slight modification (Pecquery *et al.*, 1979a,b) of the method of Williams *et al.* (1976). In brief, protein (150–200 µg) was usually incubated with [³H]-dihydroergocryptine in a total volume of 150 µl of medium II for 10 min with shaking at 37°C. Incubations were terminated by adding 4 ml of ice-cold buffer, followed by a rapid vacuum filtration of the suspension through a Whatman GF-C glass-fibre filter. Filters were rapidly washed with two 6 ml portions of buffer. Under these conditions, filtration and washing procedures required less than 10 s. Filters were dried and added to 10 ml of 'scintillation cocktail' (MI 96, Packard) and counted with an efficiency of 40%. Non-specific binding, determined by measuring the radioactivity retained on filters when incubations were performed with a large excess (10 µM) of phentolamine, was not significantly affected by age and cell size and averaged 20 ± 5% of the total counts bound at 8 nM [³H]-dihydroergocryptine. All values reported refer to specific binding, which is defined as total radioactivity bound minus non-specific binding.

Determination of α -adrenergic responsiveness

The α -adrenergic responsiveness of the fat-cell was assessed by measuring the increment of adrenaline-stimulated cyclic AMP synthesis induced by phentolamine. Fat-cells [(1–2) × 10⁵ cells/ml] were incubated at 37°C in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5 mM glucose and 2% bovine albumin, in the presence of (–)-adrenaline (50 µM) alone or in combination with phentolamine (0.5 mM), and, after 10 min, the total amount (cell plus medium) of cyclic AMP produced was determined (Brown *et al.*, 1971).

Other determinations

Protein was determined by the method of Lowry *et al.* (1951). Cell numbers were calculated as previously described (Giudicelli *et al.*, 1977a).

Chemicals

[³H]Dihydroergocryptine (sp. radioactivity 22 Ci/mmol), cyclic [³H]AMP and cyclic AMP-binding protein were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Fresh stock solutions of phentolamine (CIBA), [³H]dihydroergocryptine and (–)-adrenaline (Sigma) were prepared as previously described (Pecquery *et al.*, 1979a).

Results and discussion

The steady-state binding of [³H]dihydroergocryptine to adipocyte membranes from hamster of different ages was studied as a function of [³H]-dihydroergocryptine concentration. Except the

membranes from 0–1 month-old animals in which specific [³H]dihydroergocryptine binding could not be detected, binding was saturable in the different preparations studied and was variable with age, the highest binding values being observed in membranes from 10 month-old hamsters (results not shown).

To quantify the maximal binding capacity of the specific [³H]dihydroergocryptine-binding sites in these different membrane preparations, the above binding data were analysed by the method of Scatchard (1949). In reports from this laboratory (Pecquery *et al.*, 1979b; Pecquery & Giudicelli, 1980; Giudicelli *et al.*, 1980), Scatchard analysis of [³H]dihydroergocryptine binding to adipocyte membranes from young-adult hamsters has revealed the existence of two separate [³H]dihydroergocryptine-binding components differing in their respective affinity and binding capacity and that are each a mixed population of pharmacologically classified α_1 - and α_2 -receptor subtypes. Confirmation of the existence of these two apparent [³H]dihydroergo-

Fig. 1. Scatchard analysis of specific [³H]dihydroergocryptine binding to crude adipocyte membranes from hamsters of different age

Crude membranes were prepared from isolated adipocytes obtained from 1.5 (▲), 3 (■), 4 (○) and 10 (●) month-old hamsters. In each age group, membranes from five to 20 hamsters were pooled. Specific binding of [³H]dihydroergocryptine to these membranes was determined as described in the Materials and methods section and plotted by the method of Scatchard (1949). Each point is the mean of triplicate determinations from one representative experiment, each experiment being replicated at least twice.

cryptine-binding sites was presently provided by the data in Fig. 1. As can be seen, Scatchard analysis of the [³H]dihydroergocryptine binding to membranes from 1.5, 3, 4 and 10 month-old hamsters showed indeed similar shaped biphasic plots, indicating the persistency of these two [³H]dihydroergocryptine-binding components over the 1.5–10 month period of age. Moreover, although the affinity of both of these sites did not seem to be altered during growth (as attested by the parallelism of the different plots in Fig. 1), the maximal binding capacity of each of these sites increased with age. For purposes of comparison, the maximal number of [³H]dihydroergocryptine-binding sites (high- plus low-affinity sites) found in the different groups of age are shown in Table 1. Binding of [³H]dihydroergocryptine progressively increased with age, reaching the value of 1720 ± 210 fmol/mg of protein in membranes from 10 month-old hamsters.

In these experiments where changes in receptor concentration are measured in membrane preparations, it is important to ensure that the observed differences are not artefactual, but are a true reflection of changes in the intact cell. As α-adrenergic receptors of hamster fat-cells are almost exclusively localized in the plasma membrane (Pecquery & Giudicelli, 1980), this is particularly important when comparing these receptors in different populations of adipocytes for which the membrane recovery may be different. The fact that crude membrane protein per cell was relatively constant over the cellular age range studied here suggests that the observed modifications of α-adrenergic receptor number during growth reflect true modifications of these receptors in the intact cell.

From the data in Table 1, there seems to be no relationship between the age-related increase in the number of α-adrenergic receptors and the evolution of the fat-cell size during growth. In fact, the mean fat-cell diameter was found to increase up to 3 months of age, after which it remained constant. As a consequence of this cell-size evolution, the density of α-adrenergic receptors (expressed as the number of receptor sites present/mm² of cell-surface area) was not constant but increased with age. This situation is therefore different from that applying to rat adipocyte β-adrenergic receptors, which were found to increase proportionally to the fat-cell surface as long as the fat-cell enlarges (Giudicelli & Pecquery, 1978).

To determine whether the age-related changes in the number of α-adrenergic receptors had any influence on the fat-cell α-adrenergic responsiveness, the ability of the α-adrenergic antagonist phentolamine to increase further the adrenaline-stimulated cyclic AMP production was also studied as a function of age. As can be seen in Table 1, α-adrenergic responsiveness (estimated by sub-

Table 1. Influence of age on the number of α-adrenergic receptors and on the α-adrenergic responsiveness of hamster white fat-cells

Maximal number of α-adrenergic receptors presented are means ± s.e.m. of total specific [³H]dihydroergocryptine binding (high- plus low-affinity binding derived by Scatchard analysis for two to four separate experiments involving ten to 12 concentrations with binding determined in triplicate as described in the Materials and methods section. Cyclic AMP production was measured after 10 min incubation in the absence or presence of either 50 μM-adrenaline alone or in combination with 0.5 mM-phentolamine, under the conditions described in the Materials and methods section. Cyclic AMP values are means ± s.e.m. for five determinations. Abbreviation used: ND, not detectable.

Age (months)	Mean fat-cell		Maximum number of α-adrenergic receptors				Cyclic AMP production (pmol/10 ⁵ cells per 10 min)			α-Adrenergic responsiveness*
	Diameter (μm)	10 ³ × Surface area (mm ²)	(fmol/mg of protein)	(Sites/cell)	(Sites/mm ² of cell surface)		Basal	Adrenaline	Adrenaline + phentolamine	
1	60	11.3	ND	ND	ND		0.27 ± 0.03	4.62 ± 0.33	4.39 ± 0.05	None
1.5	68	14.5	180 ± 27	22 800 ± 3400	1.6 × 10 ⁶		1.31 ± 0.06	9.47 ± 0.25	11.30 ± 0.67	1.83 ± 0.47
3	85	22.7	910 ± 113	131 500 ± 16 300	5.8 × 10 ⁶		3.27 ± 0.11	28.1 ± 2.5	148.0 ± 6.9	119.9 ± 7.0
4	83	21.6	1100 ± 98	165 000 ± 14 700	7.8 × 10 ⁶		1.97 ± 0.12	39.7 ± 0.5	223.5 ± 8.0	183.8 ± 6.9
10	86	23.2	1720 ± 230	246 000 ± 32 900	10.6 × 10 ⁶		1.98 ± 0.45	34.5 ± 4.2	299.1 ± 18.7	264.6 ± 32.4

* α-Adrenergic responsiveness is estimated by subtracting the amount of cyclic AMP produced by adrenaline from the amount of cyclic AMP found in the presence of adrenaline plus phentolamine.

tracting the amount of cyclic AMP produced in the presence of adrenaline from the amount of cyclic AMP found in the presence of adrenaline plus phentolamine) also varied with age. Undetectable in adipocytes from 1 month-old animals, α -adrenergic responsiveness was slight (1.8 pmol) but significant in fat-cells from 1.5 month-old hamsters and was maximal (264 pmol) at the age of 10 months. Moreover, data in Table 1 also indicate that the age-induced variations of the fat-cell α -adrenergic responsiveness were closely parallel to the age-induced variations of the number of α -adrenergic receptors. Considering the fact that the α -adrenergic responsiveness was tested under conditions of maximal cyclic AMP response to adrenaline, these results indicate that the additional receptors developing in the adipocyte membrane during growth are functional receptors rather than spare ones.

Several factors may theoretically be responsible for the development of adipocyte α -adrenergic receptors with age. With the exciting new knowledge that certain hormones can regulate their own as well as other receptors it is tempting to postulate that the receptor changes reported here may be linked to modification in the secretion of some hormones during maturation. In the hamster, for instance, somatotropin secretion was shown to increase between 16 days and adulthood (Borer & Kelch, 1978). Considering the fact that this hormone promotes the catecholamine action on cyclic AMP synthesis in rat fat-cells (Fain, 1973), it seems conceivable that somatotropin may also modulate the α -adrenergic responsiveness of hamster adipocytes through a direct action at the α -adrenergic receptor level. Another group of hormones that might influence the number of α -adrenergic receptors during growth are the thyroid hormones. This suggestion is based on our recent observation (Giudicelli *et al.*, 1980) that these hormones decrease the number of α -adrenergic receptors in hamster fat-cells, and also on the fact that, at least in humans (Herrmann *et al.*, 1974), the rate of thyroid-hormone secretion progressively declines with age.

Regardless of the mechanism of this age-induced increase in the α -adrenergic receptor number in hamster adipocytes, the present study strongly suggests that the α -adrenergic responsiveness that progressively appears with aging and with cell enlargement in adipocytes from other species (Berlan & Dang Tran, 1978; Lafontan, 1979) is probably related to an increased number of α -receptor sites. Finally, the fact that in the present study both the α -adrenergic responsiveness and receptor number were highly dependent on the age of the hamster suggests that a detailed examination of such age-related changes in other species may resolve many discrepancies in the literature concern-

ing the overall adrenergic control of lipolysis in adipocytes. This could be particularly important in the case of the commonly used rat adipocytes. This could be particularly important in the case of the commonly used rat adipocytes, which seem to exhibit α -adrenergic responsiveness (Garcia-Sáinz & Fain, 1980).

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