A postsynaptic α 2-receptor: the alpha-adrenergic receptor of hamster white fat cells[1]

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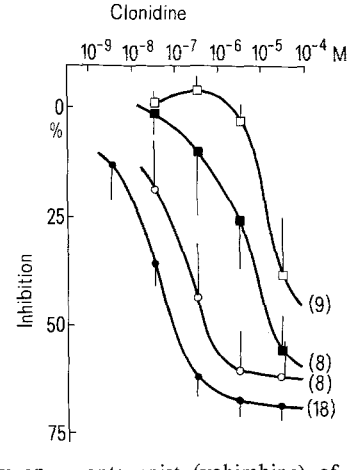
Summary. The effects of selected α-agonists and α-antagonists on theophylline-induced lipolysis were investigate in isolated hamster white fat cells. α_2-Agonists (tramazoline, clonidine) inhibited theophylline-induced lipolysis while an α_1-agonist (methoxamine) was without any effect. The inhibitory effect of α_2-agonists was suppressed by yohimbine (α_2-antagonist), whereas α_1-antagonists were inefficient. This result implies that the α-adrenergic receptor of hamster fat cells is of the α_2-type, although located postsynaptically.

Several authors have demonstrated the existence of α-adrenergic sensitivity in hamster adipocytes[2-4]. Direct biochemical evidence for the existence of α-adrenergic receptors in hamster white fat cell membranes has recently been reported[5]. However, a more precise pharmacological definition of the α-adrenergic receptor of hamster adipocytes has not been proposed until now. Since the definition of 2 subtypes of α-receptor (α_1 and α_2) seems currently to be accepted[6-8], in the present study we investigated, using selected agonists and antagonists, the type of the α-receptor responsible for the inhibition of lipolysis in isolated hamster fat cells. The experiments were undertaken to determine whether selected α-adrenergic agents affect the magnitude of the lipolytic response promoted by theophylline since stimulation of α-adrenergic receptors induces the inhibition of theophylline-stimulated lipolysis in hamster[4] or human fat cells[9].

Materials and methods. Male golden hamsters (commercial breeding centre Evic Ceba, Zone industrielle F-33290 Blanquefort) weighing between 100 and 145 g were housed at 20–22 °C and fed ad libitum for at least 1 month before use. They were killed by decapitation after overnight fasting. Epididymal adipocytes from 2 or 3 hamsters were prepared following the procedure of Hittelman et al.[2]. Epididymal adipose tissue (3–4 g) was digested with crude bacterial collagenase (Worthington Biochemical Corporation, Freehold, N.J.) at a concentration of 1.7 mg/ml in 6 ml of Krebs-Ringer bicarbonate buffer (KRB) for 8–10 min. The adipocytes were washed 3 times and samples (0.5 ml) of cell suspension were randomly distributed among plastic scintillation vials containing 1.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) with 35 mg/ml bovine serum albumin (Sigma fraction V) and 6 μmoles/ml glucose. The cells were incubated at 37 °C for 90 min under an air atmosphere. The various drugs were added (20 μl) just before starting the incubation. Incubations were done in duplicate. At the end of the incubation, the samples were placed in an ice bath to stop the metabolic activity of the cells. An aliquot (200 μl) of the medium was used to determine enzymatically the amount of glycerol[10] released from the adipocytes. The triglyceride content was estimated gravimetrically after extraction of total cell lipid according to the method of Dole and Meinertz[11] and used for the expression of metabolic activities. Generally 30–40 mg of cell lipid were added to each vial (2 ml of incubation medium). The following drugs were used: clonidine hydrochloride (Boehringer Ingelheim), methoxamine hydrochloride (Burroughs Welcome), yohimbine (Houdé), prazosin (Pfizer Laboratories). Tramazoline and AR-C 239 were gifts from H. Schmitt (Dept. Pharmacology, Paris). Drugs were dissolved in saline immediately before use. Concentrations reported in the text refer to the base.

Results. In order to characterize the α-receptor of the hamster fat cells, the effect of selected α-adrenoceptor agonists was studied on theophylline-stimulated adipocytes which represent an accurate model for the exploration of the inhibitory effects reflecting α-receptor stimulation[12]. Non-stimulated isolated fat cells showed a low basal rate of lipolysis which did not allow the investigation of a possible inhibition of lipolysis. Theophylline (0.33 mM) stimulated hamster fat cell lipolysis (4–5 times) (0.642 ± 0.058 vs 3.210 ± 0.280 μmoles/100 mg lipid over 90 min). The effects of α-adrenoceptor agonists on theophylline-induced lipolysis are shown in table 1. Tramazoline and clonidine caused concentration-dependent inhibition of the lipolytic response induced by theophylline. Tramazoline, the most potent α_2-agonist according to Langer's classification[7], is approximately equipotent with clonidine itself on the adipocyte preparation. In contrast, methoxamine, a relatively pure α_1-agonist, had little or no inhibitory effect; a slight inhibitory effect appeared with fairly high concentrations compared with the α_2-agonists. Epinephrine, norepinephrine and phenylephrine, previously studied by others[3,4], have not been considered in detail in the present investigation. It is only shown (table 1) that epinephrine associated with propranolol (the β-adrenolytic drug is added in order to suppress the β-lipolytic effect of epinephrine) inhibited theophylline-induced lipolysis and that the concentration causing inhibition was 10 times as strong as that required with α_2-agonists. The high efficiency of α_2-agonists suggests that the α-receptor of hamster fat cells is of the α_2-type. In order to confirm the nature of the receptor obtained with agonist potencies, an investigation of the possible interactions between clonidine and several α-adrenoceptor antagonists was carried out. Yohimbine (α_2-antagonist) prevented clonidine inhibition of theophylline-induced lipolysis.



Antagonism by an α_2-antagonist (yohimbine) of the inhibitory effect of clonidine on theophylline-induced lipolysis in isolated hamster white fat cells. Clonidine alone: ●; clonidine + yohimbine: ○, 2.8 μM; ■, 14 μM; □, 140 μM. The results are expressed as the percentage of inhibition of theophylline-induced glycerol release/90 min/100 mg lipid. Number of determinations per clonidine concentration in parentheses.

Table 1. Percent inhibition by α_2- and α_1-adrenoceptor agonists of the theophylline-induced lipolysis in isolated hamster white fat cells

		α-Agonists concentration (μM) 0.05	0.5	5	50
Tramazoline	(8)	35.6±5.4	48.3±8.2	49.0±7.9	-
Clonidine	(18)	36.0±5.0	62.1±5.1	67.0±4.7	68.3±4.6
Methoxamine	(7)	4.2±3.0	4.9±2.0	11.6±8.6	11.7±3.2
Epinephrine and propranolol 40 μM	(4)	-	11.2±8.0	44.8±9.6	-

Isolated fat cells were incubated in the presence of 0.33×10^{-3} M theophylline and the indicated concentration of α-adrenoceptor agonists. Results are mean± SEM. Number of determinations per agonist concentration, in parentheses.

Table 2. Effects of α_1-adrenoceptor antagonists on the inhibitory effect of clonidine on the theophylline-induced lipolysis in isolated hamster white fat cells

		Clonidine (μM) 0.05	0.5	5	50
Clonidine alone	(18)	36.0±5.0	62.1±5.1	67.0±4.7	68.3±4.6
Clonidine and prazosine 13 μM	(8)	23.2±5.7	58.5±6.7	64.7±6.0	62.9±6.1
Clonidine and AR-C 239 24 μM	(7)	42.7±9.6	57.8±7.6	67.4±5.7	70.4±5.9

Isolated adipocytes were incubated in the presence of theophylline (0.33×10^{-3} M), clonidine and the indicated concentration of α_1-adrenoceptor antagonists. The results are mean± SEM of percentages as shown in table 1. Number of determinations per agonist concentration, in parentheses.

Yohimbine produced parallel concentration-dependent shifts to the right of the clonidine dose-response curve (figure). In contrast, α_1-antagonists such as prazosin and AR-C 239, did not significantly modify the responsiveness of theophylline-stimulated adipocytes to clonidine (table 2). In conclusion, the inhibitory effect of clonidine on theophylline-induced lipolysis was suppressed by the α_2-antagonist while the α_1-antagonists were without any noticeable effect.

Discussion. In previous investigations Schimmel[3] has shown that epinephrine associated with propranolol or phenylephrine partially inhibited lipolysis produced by high concentrations of isoproterenol, ACTH or methyl-xanthines, suggesting that the inhibition by α-adrenergic stimulation is only evident when the lipolytic activity has been increased. The data obtained show that clonidine and tramazoline were the most potent α-adrenergic agents able to inhibit theophylline-induced lipolysis in the hamster fat cell. They were efficient at concentrations 10 times lower than those required with epinephrine while methoxamine was practically inactive (table 1). If we refer to the subclassification of α-adrenoceptors proposed by Langer[7], the order of agonist potencies described in this work (tramazoline ⩾ clonidine > epinephrine > methoxamine) is consistent with the definition of an α_2-receptor. The α_2-receptor, originally described as a presynaptic α-receptor which mediates inhibition of norepinephrine release during nerve stimulation[6,7] has mostly been characterized in in vivo studies. It can be seen, in view of the activity of the agonists, that the α-receptor of the hamster fat cell, although localized postsynaptically, should be classified as α_2. The analysis carried out with α-antagonists (figure and table 2) clearly confirms the conclusion drawn from the studies with agonists. Yohimbine is considered to be a preferential blocking agent for α_2-adrenoceptors which mediate an inhibitory effect (reduction of norepinephrine release during nerve stimulation) and described as presynaptic α-adrenoceptors[7,8,13] while prazosin and AR-C 239 were highly effective against the α_1 postsynaptic receptor[8]. The higher efficiency of yohimbine in the antagonism of clonidine inhibition of theophylline-induced lipolysis is consistent with the α_2 type of the α-receptor of hamster fat cells. The absence of any effect of prazosin and AR-C 239 sustains the analysis conducted with the α_2-antagonist. In conclusion, the results obtained with selected agonists and antagonists indicate that the α-adrenergic receptor of hamster fat cells is an α_2-receptor although located postsynaptically in non-nervous tissue. Can α_2-receptors be located postsynaptically? Reports on rabbit platelet membranes[14], frog skin[15], and rabbit duodenum[16] indicate that α-receptors located postsynaptically may be of the α_2-type. Our results add further evidence for the existence of postsynaptic α_2-receptors. Such a result brings valuable information for further investigations upon the α sites of adipocytes. Moreover the results indicate that the in vitro system of isolated white fat cells of the hamster provide a test for activity, on the α-adrenoceptors, of selected agonists and antagonists.

1 Acknowledgments. This work was supported by grants from CNRS (ERA 412) and DGRST (grant No. 7871078). We thank M. Dauzats for excellent technical assistance. We thank Prof. H. Schmitt for tramazoline and AR-C 239 and for helpful discussion.

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