Evidence of functional α_2 -adrenergic receptors in adult-rat adipocytes by using the agonist UK 14304

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The aim of this study was to re-assess whether α_2 -adrenergic receptors were present in rat adipocytes, by using UK 14304, a new and very selective α_2 -agonist. The following observations demonstrate the presence of functional α_2 -adrenoceptors in rat adipocytes. (1) Adipocyte lipolysis was dose-dependently inhibited by UK 14304 (maximal effect 80 % at 1 μ M-UK 14304, 45 % at 10 μ M-UK 14304, under basal or theophylline-stimulated conditions respectively). (2) UK 14304 bound specifically to purified plasma membranes, with $B_{\max}$ = 744 fmol/mg of protein and K_D = 9 nM. (3) The effect of UK 14304 was suppressed by α_2 -antagonists. (4) Adrenaline inhibited lipolysis upon β -adrenergic blockade (propranolol). (5) The anti-lipolytic effect of UK 14304 was modulated by the age of the rats.

INTRODUCTION

There is a large body of evidence to suggest that catecholamines interact with adipocytes through two classes of receptors that mediate opposite effects on lipolysis: stimulation through β -adrenoceptors and inhibition through α_2 -adrenoceptors. This dual control of lipolysis, which gives the cell greater possibility of adapting to physiological needs, seems to be a general feature of adipocytes from all species. First evidenced [1,2] and well established [3,4] in man, the presence of both β - and α_2 -adrenoceptors has also been shown in adipocytes from hamster [5–7], rabbit [8] and dog [9]. In contrast, in rat adipocytes, the fat-cells most commonly used in the laboratory, the presence of an α -adrenergic anti-lipolytic response remains controversial, as it has been observed in some studies [10,11], but not in others [2,7,12]. The conclusion that the rat adipocyte was devoid of α_2 -adrenoceptors was based mainly on studies using clonidine, a poorly selective α_2 -agonist. Therefore the aim of the present work was to re-examine this issue by using UK 14304 [5-bromo-6-(2-imidazolin-2-yl-amino)quinoxaline], a relatively new pharmacological tool, which has the advantages over clonidine of being a very selective and potent α_2 -adrenoceptor agonist [13].

The data reported below provide clear evidence that α_2 -adrenoceptors, as assessed with UK 14304, are present and functional in rat adipocytes. Moreover, they mediate an anti-lipolytic effect of adrenaline when its β -component is blocked.

EXPERIMENTAL

Methods

Male Wistar or Zucker (heterozygous lean *Fa/fa*) rats were used at 10 weeks of age or as otherwise stated. They were fed *ad libitum* on laboratory chow (UAR, Epinay sur Orge, France) (carbohydrate 65 %, protein 24 % and fat 11 % of total energy) until the time of the experiments between 09:00 and 10:00 h.

Epididymal adipocytes were isolated by the method of Rodbell [14] with the modifications previously described [15].

For studies of lipolysis, duplicate samples of adipocytes (~ 150 000) were suspended in 2.5 ml of Krebs–Ringer bicarbonate buffer [16] containing 10 mM-Hepes, pH 7.4, 5 mM-glucose and 3 % (w/v) bovine serum albumin and incubated at 37 °C in an atmosphere of O₂/CO₂ (19:1) in stoppered plastic vials for 60 min. Then a portion (1 ml) of the infranatant incubation medium was removed, acidified (trichloroacetic acid), neutralized (KOH) and assayed in duplicate for glycerol content by an enzymic method (triacylglycerol kits from Boehringer Mannheim).

Adipocyte plasma membranes were prepared as previously described [17]. Briefly, adipocytes, pooled from 9 to 14 10-week-old Zucker rats for each experiment, were washed three times with Krebs–Ringer bicarbonate buffer and resuspended in 0.25 M-sucrose/1 mM-EDTA/10 mM-Tris/HCl buffer, pH 7.4, homogenized in a glass Potter/Elvehjem homogenizer and centrifuged at 16 000 *g* for 15 min. The pellet was then rapidly fractionated by the method of Belsham *et al.* [18], by using density-gradient centrifugation in Percoll. After washing in 0.15 M-NaCl/1 mM-EDTA/10 mM-Tris/HCl, the purified plasma membranes were centrifuged at 25 000 *g* for 20 min, then suspended in 50 mM-Tris/HCl/10 mM-MgCl₂ buffer, pH 7.4 (Tris/Mg buffer), and frozen. The protein content was determined by the Bio-Rad protein assay (Bradford [19]), with bovine serum albumin as standard.

[³H]UK 14304 binding was measured by the method previously described [12] with slight modifications. Samples of membrane proteins (~ 20 μ g) were incubated in duplicate at 25 °C for 20 min in 0.4 ml of Tris/Mg buffer containing the required concentration of [³H]UK 14304. After incubation, the membranes were separated from the medium by vacuum filtration through Whatman GF/C glass-fibre filters. The filters were washed with 3 $\times$ 5 ml of ice-cold Tris/Mg buffer and then

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Fig. 1. Anti-lipolytic effect of α_2 -agonists: clonidine or UK 14304 on unstimulated (a) or theophylline-stimulated lipolysis (b)

Adipocytes from 10-week-old rats were incubated without (0 ADA) or with 1.6 μ g of adenosine deaminase/ml alone (a) or in combination with 3.3 mM-theophylline (b) as described in the Experimental section. Release of glycerol in incubation medium was measured in the presence of increasing concentrations of UK 14304 (●, ○) or clonidine (■) in Wistar (○) or Zucker rats (●, ■). Each point represents the mean $\pm$ S.E.M. of three to seven experiments.

counted for radioactivity in MP scintillation fluid (Beckman). All data were corrected for non-specific binding, which was determined by measurement of radioactivity when binding assays were performed in the presence of 10 μ M unlabelled idazoxan, a selective α_2 -antagonist [20] which exhibits a noteworthy high potency on the adipocyte post-synaptic α_2 -receptor [21].

Materials

[3 H]UK 14304 (3TBq/mmol) was purchased from N.E.N. (Boston, MA, U.S.A.) and unlabelled UK 14304 from Pfizer (Sandwich, Kent, U.K.); idazoxan was generously given by Dr. H. Dabiré (Paris).

RESULTS AND DISCUSSION

The data illustrated in Figs. 1–5 were obtained by using adipocytes from 10-week-old rats.

Fig. 1 shows that, under our experimental conditions, lipolysis was nearly completely inhibited by the adenosine released in the medium. The addition of adenosine deaminase at a concentration of 1.6 μ g/ml (0.32 unit/ml) was sufficient to remove the inhibitory action of adenosine completely, since further increase in adenosine deaminase had no additional action (results not shown). It is clear that clonidine had a very small effect on unstimulated lipolysis (Fig. 1a) and virtually no effect on theophylline-stimulated lipolysis (Fig. 1b) in Zucker-rat adipocytes, in agreement with previous observations in Sprague–Dawley-rat adipocytes [12]. In contrast, a dose-dependent inhibition of lipolysis (Fig. 1a) was observed in those adipocytes in response to the addition of the more selective α_2 -agonist UK 14304, which was able to decrease lipolysis by nearly 80% at the maximally effective dose. Half-maximal and maximal inhibitions of lipolysis were achieved at UK 14304 concentrations of 7 nM (ED_{50}) and 1 μ M respectively. Moreover, Fig. 1(a)

Fig. 2. [3 H]UK 14304 binding to adipocyte plasma membranes from 10-week-old Zucker rats

Saturation curves are shown for UK 14304 binding to adipocyte plasma membranes from 10-week-old Zucker rats. Membranes were incubated with increasing concentrations of [3 H]UK 14304 for 20 min in the absence or presence of 10 μ M unlabelled idazoxan. Total (●), specific (○) and non-specific (▲) binding were determined as described in the Experimental section. The inset shows a Scatchard plot of the equilibrium binding data. Each point represents the mean of triplicate samples. The data presented are from one representative experiment; four experiments were performed.

Fig. 3. Action of α_2 -adrenergic antagonists on the anti-lipolytic effect of UK 14304

Adipocytes from 10-week-old Zucker rats were incubated with 1.6 μ g of adenosine deaminase/ml. The anti-lipolytic effect of increasing concentrations of UK 14304 was tested alone (●) or in the presence of 10 μ M-idazoxan (○), -yohimbine (▲) or -phentolamine (△). Each point represents the mean $\pm$ S.E.M. of three to four experiments.

shows that lipolysis in adipocytes from Wistar rats displayed a dose/response curve to UK 14304 which was superposable on that of Zucker-rat adipocytes, demonstrating that the UK 14304 inhibition of lipolysis was independent of the rat strain. Under theophylline-stimulated conditions (Fig. 1b), adipocytes also displayed a dose-dependent response to UK 14304. However, the dose/response curve was shifted to the right as compared with that observed under unstimulated conditions and, at maximally effective concentration (about 10 μ M-UK 14304), the inhibition of lipolysis was only 45%. This decreased effect of UK 14304 in the presence of theophylline cannot be explained by an interaction of UK 14304 and theophylline at adenosine receptors, since we have checked in complementary experiments (results not shown) that UK 14304 does not bind at adenosine receptors. The effects of UK 14304 may be less against theophylline-stimulated lipolysis than against lipolysis with adenosine deaminase, simply because of the higher lipolytic rate in the presence of theophylline. It is noteworthy that the maximal absolute effect of UK 14304 was higher in the presence than in the absence of theophylline.

The presence of α_2 -adrenoceptors in Zucker-rat adipocytes was also assessed by radioligand-binding studies using [3 H]UK 14304 and purified plasma membranes. The results illustrated in Fig. 2 provide evidence of a specific and saturable binding of [3 H]UK 14304. Scatchard analysis of the binding data yielded a linear plot, suggesting the presence of a single class of [3 H]UK 14304-binding sites (Fig. 2, inset). The receptor density (B_{max}) calculated from this plot was 744 ± 101 fmol/mg of protein, and the dissociation constant (K_D) was 9.0 ± 1.0 nM (four experiments). These data demonstrated

Fig. 4. Effect of adrenaline on lipolysis upon complete β -adrenergic blockade

Adipocytes from 10-week-old Zucker rats were incubated with 1.6 μ g of adenosine deaminase (ADA)/ml without or with propranolol (10 μ M) and increasing adrenaline concentrations. Each point represents the mean $\pm$ S.E.M. of three experiments.

that the binding and the biological action of UK 14304 occurred over the same range of UK 14304 concentrations suggesting that there were no spare UK 14304 receptors.

In order to document further the pharmacological profile of the UK 14304-mediated inhibition of lipolysis in rat adipocytes, we examined the effect of α_2 -adrenergic antagonists on the biological action of UK 14304. The results depicted in Fig. 3 show that yohimbine, a very selective α_2 antagonist, totally abolished the inhibitory effect of UK 14304 on lipolysis, and that phentolamine and idazoxan caused a large decrease in both the responsiveness and the sensitivity of adipocytes to UK 14304. Altogether, the results of these competition experiments were in good agreement with the definition of an α_2 -adrenoceptor-mediated response.

Another approach to address the question of the presence of α_2 -adrenoceptors in rat adipocytes was to examine whether adrenaline was able to inhibit the lipolysis of these cells when β -adrenoceptors were blocked. Fig. 4 shows that, upon complete β -adrenergic blockade, using 10 μ M-propranolol, a dose-dependent inhibition of glycerol release was observed in response to adrenaline, demonstrating the presence of a dual adrenergic regulation of lipolysis in rat adipocytes.

Age-related changes in α_2 -adrenergic responsiveness have been observed in adipocytes from many species [8,9,22]. This prompted us to examine the influence of age on the response of rat adipocytes to UK 14304. As shown in Fig. 5(a), lipolysis in adipocytes from 5-week-old rats was poorly sensitive to UK 14304, which elicited a maximal inhibition of 20% only. Under theophylline-stimulated conditions, no significant UK 14304 effect was detectable before 6 weeks of age (Fig. 5b). This developmental pattern of α_2 -adrenergic responsiveness in rat adipocytes is very similar to that described in hamster adipocytes by Pecquery & Giudicelli [22].

In conclusion, the present findings that (1) UK 14304, a very selective α_2 -adrenergic agonist, binds to plasma

Fig. 5. Influence of rat age on the inhibitory effect of UK 14304 on unstimulated (a) or theophylline-stimulated (b) lipolysis

Adipocytes from lean Zucker rats aged 5 weeks (○), 6 weeks (▲) or 10 weeks (●) were incubated in the presence of 1.6 μ g of adenosine deaminase/ml alone (a) or in combination with 3.3 mM-theophylline (b) and increasing concentrations of UK 14304. Each point is the mean of two or three experiments.

membranes of rat adipocytes and, over the same range of concentrations inhibits lipolysis in these adipocytes, (2) the effect of UK 14304 is suppressed by selective α_2 -antagonists, (3) adrenaline is able to depress lipolysis when β -adrenergic receptors are blocked, are convergent pieces of evidence that α_2 -adrenoceptors are present and functional in rat adipocytes as in adipocytes of other species, including man. The distinctive unresponsiveness of rat adipocytes to clonidine, an α_2 agonist which has been shown to be active in fat-cells from hamster [7], dog [9] and man [3,4,23], supports the notion of species differences in the characteristics of α_2 -adrenergic receptors [24], stressing the need for using multiple pharmacological tools to test their presence. The indication provided here that α_2 -adrenoceptors in rat adipocytes are subject to physiological regulation, such as age, is supported by our observations of a marked increase in UK 14304-binding sites in adipocytes from obese Zucker rats (M.-C. Rebourcet, C. Carpené, M. Lafontan & M. Lavau, unpublished work). Therefore rat adipocytes provide a suitable model in which to study the pharmacological properties and the pathophysiological regulation of α_2 -adrenergic receptors.

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