GUANINE NUCLEOTIDES REGULATE SPECIFICALLY AGONIST-BINDING AFFINITY OF THE α_2-ADRENERGIC RECEPTOR IN WHITE ADIPOCYTES

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1. Introduction

Recent studies have provided evidence that GIP may play an important regulatory role in the modulation of the binding affinity of some α_2-adrenergic receptors [1–5]. However, this effect of GTP, which seems analogous to its now well established action on agonist-binding to the β-adrenergic receptors of different cells including adipocytes [6–10], has been so far shown to apply to the α_2-adrenergic receptors of rather limited examples to allow generalization to all cellular systems involving α_2-adrenergic receptors.

Recent reports from this laboratory [11–12] and others [13] have demonstrated by direct binding experiments the existence of α-adrenergic receptors in hamster fat cells. Since these studies have shown that these receptors were predominantly constituted by the α_2-adrenergic subtype [12,13], the aim of the present investigation was to determine whether GTP may also specifically regulate the affinity of these receptors towards their agonists. This was achieved by studying the influence of the non-hydrolyzable analog of GTP, guanosine 5′-(β,γ-imido)triphosphate (Gpp(NH)p) on the binding of the α-antagonist [^{3}H]dihydroergocryptine and of the α-agonist [^{3}H]norepinephrine as well as on the displacement of [^{3}H]dihydroergocryptine by various α-agonists and antagonists. This report shows that, Gpp(NH)p does not affect the binding of the α-antagonist [^{3}H]dihydroergocryptine nor its displacement by the α-antagonists phentolamine and yohimbine. However, Gpp(NH)p severely decreases the overall binding affinity of the α-adrenergic receptors of hamster adipocytes for the α-agonists (–)-epinephrine and [^{3}H]norepinephrine and for the α_2-selective agonist clonidine, an effect which appears to result from the conversion of the high affinity state of the adipocyte α_2-receptor for agonists into the low affinity one.

2. Material and methods

Binding assays were performed on crude adipocyte membranes prepared from isolated hamster adipocytes and then thoroughly washed (to reduce endogenous GTP) as described in detail elsewhere [11]. When [^{3}H]norepinephrine binding was studied, membranes (150–200 μg of protein) were incubated in Tris–HCl (50 mM), $MgCl_2$ (5 mM), ascorbate (0.8 mM), pyrocatechol (1 mM), (±)-propranolol (10 μM) (pH 7.4 at 25°C) to suppress binding to the β-receptors and with various concentrations of [^{3}H]norepinephrine (spec. activity 38 Ci mmol^{-1}, Radiochemical Centre, Amersham), in a total volume of 120 μl. Incubations were performed for 30 min at 25°C (optimal conditions of binding) [14] and were terminated by adding 5 ml incubation buffer, followed by a rapid vacuum filtration of the suspension through Whatman GF/C glass fiber filters. Filters were washed with 10 ml of buffer, were dried, were added to scintillation cocktail (PCS, Amersham) and were counted. Specific binding defined as binding displaceable by 10 μM phentolamine (Ciba) averaged 55–70% of total binding at 40 nM [^{3}H]norepinephrine.

When [^{3}H]dihydroergocryptine binding was studied, membranes (200 μg of protein) were incubated with [^{3}H]dihydroergocryptine (spec. act. 22 Ci mmol^{-1}, Radiochemical Centre, Amersham) (usually 9–10 nM) in Tris–HCl (50 mM), $MgCl_2$ (10 mM) buffer (pH 7.4 at 37°C) in a total volume of 150 μl. After 10 min, incubations were terminated and radioactivity bound to the filter was determined as pre-

viously described [11]. Specific binding (binding displaceable by 10 μM phentolamine) was 75% of total binding at 10 μM [^{3}H]dihydroergocryptine. Origin of the chemicals used are described elsewhere [11,12].

3. Results and discussion

As shown in fig.1A, the binding of [^{3}H]dihydroergocryptine was unaffected by the presence of 100 μM Gpp(NH)p (Boehringer, Mannheim), a concentration which altered neither the total nor the non-specific binding of [^{3}H]dihydroergocryptine. Competition curves of phentolamine (a mixed α_1- and α_2-antagonist) [15] and of yohimbine (a preferential α_2-antagonist) [16] in displacing [^{3}H]dihydroergocryptine were also unaltered by the addition of Gpp(NH)p (fig.1B).

Fig.2A shows the effects of Gpp(NH)p on the ability of (–)-epinephrine to displace [^{3}H]dihydroergocryptine. As can be seen, the competition curve in the absence of the nucleotide was biphasic (log-logit transformation of the data yielding a slope value n_H = 0.56), suggesting the existence of two affinity states of the adipocyte α-receptor for (–)-epinephrine with respective apparent EC_{50} values of 80–100 nM and 3500–4000 nM. This is consistent with previous data from this laboratory [14] and from the present study (see fig.2B) showing by direct equilibrium binding studies the presence of two different binding components for [^{3}H]norepinephrine in hamster adipocyte membranes. Contrasting with the failure of Gpp(NH)p to alter [^{3}H]dihydroergocryptine, phentolamine and yohimbine binding, this nucleotide markedly affected the ability of (–)-epinephrine to displace [^{3}H]dihydroergocryptine. In fact, the (–)-epinephrine competition curve was steeper and shifted to the right in the presence of Gpp(NH)p. Log-logit transformation of these data yielded a slope n_H = 0.97 which is consistent with displacement at a single class of binding sites and an EC_{50} value of 2500 nM, which is close to the EC_{50} value found in the absence of Gpp(NH)p for the sites displaced with low affinity by (–)-epinephrine. This suggests that the guanine nucleotide may induce a conversion of the high affinity state of the α-receptor for catecholamine into the low affinity one. Consistent with this hypothesis is the fact that in the presence of Gpp(NH)p, no specific binding of [^{3}H]norepinephrine could be detected at concentrations less than 20 nM (fig.2B) an effect which reflects a selective

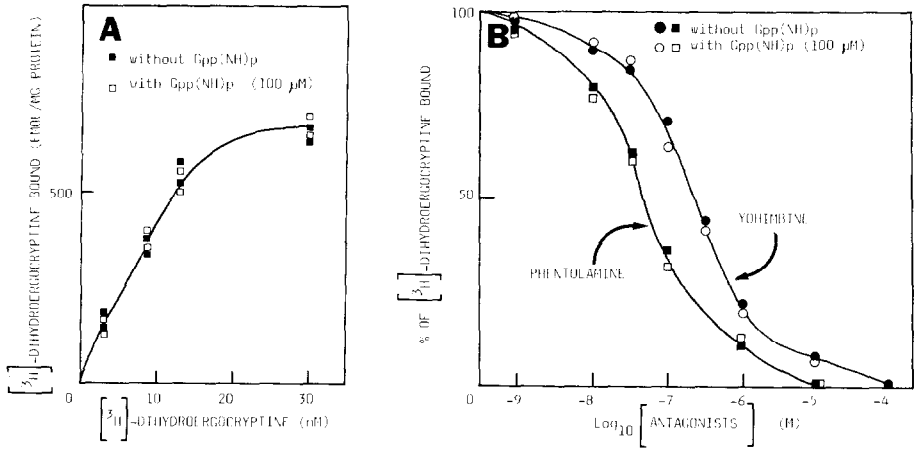

Fig.1. Influence of Gpp(NH)p on the specific binding of α-adrenergic antagonists to the α-adrenergic receptors of hamster adipocyte membranes. A. Concentration dependence curve for specific binding of [^{3}H]dihydroergocryptine. Membranes were incubated at 37°C with various concentrations of [^{3}H]dihydroergocryptine, in the absence or presence of 100 μM Gpp(NH)p. After 10 min, the amount of radioligand specifically bound was determined. Each point represents the mean of triplicate determinations from one experiment. B. Displacement of [^{3}H]dihydroergocryptine specifically bound to hamster adipocyte membranes by phentolamine and yohimbine. Membranes were incubated at 37°C with 9 nM [^{3}H]dihydroergocryptine and various concentrations of phentolamine and yohimbine, in the absence or presence of 100 μM Gpp(NH)p. After 10 min, the amount of radioligand remaining specifically bound was determined. Results are expressed as percent of [^{3}H]dihydroergocryptine specifically bound in the absence of phentolamine or yohimbine. Each point is the mean of two different experiments performed in triplicate.

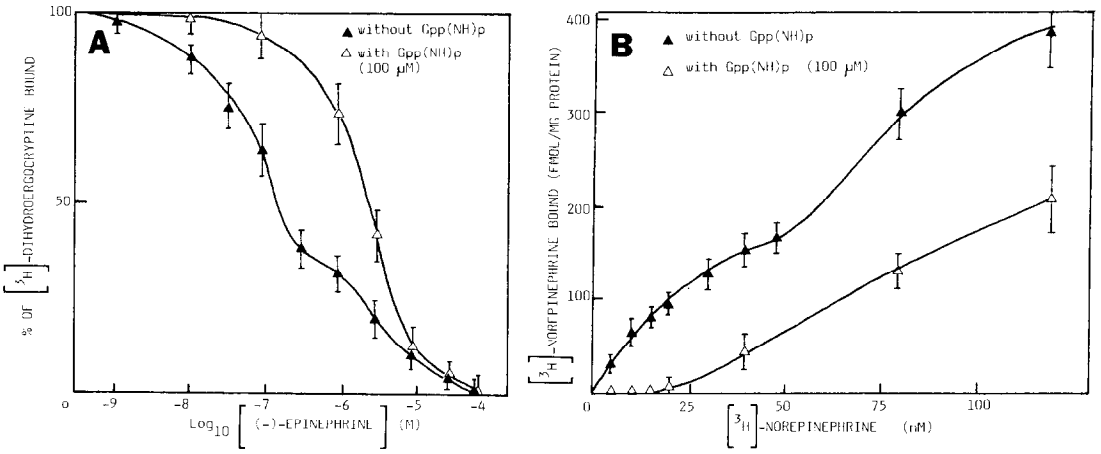

Fig.2. Influence of Gpp(NH)p on the specific binding of catecholamines to the α-adrenergic receptors of hamster adipocyte membranes. A. Displacement of [^{3}H]dihydroergocryptine specifically bound to hamster adipocyte membranes by (−)-epinephrine. Experimental conditions are as described under fig.1B. Each point is the mean of three different experiments performed in triplicate and the vertical lines represent 2 S.E.M. B. Concentration dependence curve for specific binding of [^{3}H]norepinephrine to hamster adipocyte membranes. Membranes were incubated with increasing concentrations of [^{3}H]norepinephrine at 25°C for 30 min in the absence (total binding) or presence of 10 μM phentolamine (non-specific binding), under the conditions described in the text. Gpp(NH)p (100 μM) was present in half of the assays. Data points represent the mean of triplicate determinations from one representative experiment and the vertical lines 2 S.E.M.

abolition of the binding of [^{3}H]norepinephrine to its high affinity binding component.

As the α-receptor population of hamster fat cells is almost completely constituted by the α_2-adrenergic receptor subtype [12,13], it seemed likely that the effects of Gpp(NH)p on catecholamine binding resulted from an effect of the guanine nucleotide on agonist-binding to the α_2-receptor subtype. This hypothesis was investigated by studying the influence of Gpp(NH)p on the competition curve of the selective α_2-agonist clonidine [17] in displacing [^{3}H]dihydroergocryptine. As shown in fig.3, the competition curve in the absence of the nucleotide was shallow yielding logit slope value n_H = 0.66 and EC_{50} value of 70–90 nM. In the presence of Gpp(NH)p, the clonidine competition curve was steeper (n_H = 0.94) and shifted to the right resulting in a 5–6-fold decrease in the receptor-binding affinity for this α_2-agonist. These data are thus consistent with the hypothesis that the α_2-adrenergic receptor of hamster fat cells has one affinity state for α-antagonists, such as phentolamine and yohimbine, whether guanine nucleotides are present or not, whereas it has two different affinity states for α_2-agonists such as clonidine, the proportion of which is dependent on the presence of these nucleotides: in fact, in the presence of Gpp(NH)p, α_2-agonists appear to bind to a homogeneous and low affinity state of the receptor, whereas in the absence of Gpp(NH)p, these agonists bind to both the high and low affinity states of the α_2-receptor subtype.

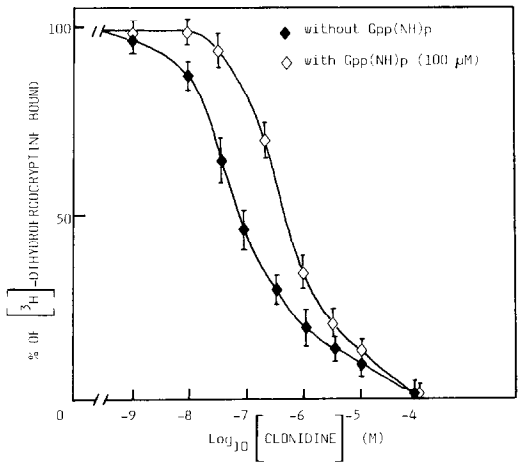

Fig.3. Influences of Gpp(NH)p on the displacement of [^{3}H]dihydroergocryptine specifically bound to adipocyte membranes by clonidine. Experimental conditions were the same as in fig.2A, except that clonidine was used instead of (−)-epinephrine. Each point is the mean of three different experiments performed in triplicate and the vertical lines represent 2 S.E.M.

These experiments thus suggest that in hamster fat

cells, as in platelets [2,4], uterus [4] and brain [1], GTP plays an important role in the regulation of agonist binding to the α_2-adrenergic receptor, by converting the high affinity state into the low affinity form of the receptor. Such a regulatory role of GTP has already been postulated for adenylate cyclase-coupled receptors in which agonist binding results not only in cyclase activation (for instance, β-adrenergic receptors of different cells including adipocytes) [6–10], but also in cyclase inhibition [2,18]. This is especially the case in hamster fat cells, in which Aktories et al. [19] have recently shown the direct inhibition of adenylate cyclase by α-adrenergic agonists. As in the case of human platelets [16], this inhibition of adipocyte adenylate cyclase appears to be mediated by the α_2-adrenergic receptor subtype [13,14,20,21]. It seems thus possible that the α_2-receptors of the fat cell interact, like the β-receptors, with a membrane-bound nucleotide-regulatory protein [22], and that this interaction is important in the guanine nucleotide-mediated modulation of agonist affinity states. In analogy with the β-adrenergic receptors of rat adipocytes [8–10], the existence of such an interaction would also suggest the possibility that the α_2-adrenergic receptors of hamster fat cells may be desensitizable by catecholamines.

In conclusion, this study shows that the α_2-receptor of hamster adipocyte is, beside the rather few systems so far studied, an additional example of a post-synaptic and inhibitory adenylate cyclase-coupled α_2-receptor in which agonist-binding is regulated by guanine nucleotides. Examination of other examples of α_2-receptors will determine whether this nucleotide regulation can be generalized to other tissues and species. This might be of particular interest for human adipose tissue, in which the α-adrenergic receptors have been recently recognized as belonging to the α_2-subtype [23] and in which catecholamines appear to be the only hormones exerting a physiological role in the regulation of lipolysis [24].

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