INFLUENCE OF α-ADRENERGIC BLOCKING AGENTS ON CYCLIC AMP, CYCLIC GMP AND LIPOLYSIS IN HAMSTER WHITE FAT CELLS

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1. Introduction

Recent studies, showing that phentolamine potentiates the catecholamine-induced cyclic AMP (cAMP) accumulation in hamster fat cells [1], provided evidence for the existence of α-adrenergic receptors in these cells. Despite this effect, however, phentolamine was recently reported to inhibit catecholamine-induced lipolysis in hamster fat cells [2].

The present investigation was carried out to further characterize the mechanism of this paradoxical action of phentolamine. The influence of phentolamine and of another α-blocking agent, phenoxybenzamine, on the synthesis and release of cAMP, as well as on the lipolytic response of hamster fat cells to agents acting before and beyond cAMP synthesis, were compared. In addition, the previously suggested possibility [3] that cyclic GMP (cGMP) metabolism could be sensitive to α-adrenergic stimulation was also investigated.

This report shows that the opposite effects of phentolamine or phenoxybenzamine on catecholamine-induced cAMP synthesis and lipolysis involve two different mechanisms localized before and beyond cAMP synthesis, respectively. It shows, furthermore, that α-adrenergic stimulation has no influence on the cGMP level in hamster adipocytes.

2. Material and methods

Epididymal adipose tissue was obtained from fed golden hamsters (IRSC, Villejuif) weighing 90–110 g. Adipocytes were isolated following a slight modification of the procedure of Hittelman et al. [1] involving the suspension of minced pads in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 1.27 mM calcium and 2% bovine serum albumin. Crude collagenase (1.67 mg/ml) was added for digestion and the vials were vigorously shaken in a water bath at 37°C. After 7 min digestion, the isolated fat cells were separated from the remaining tissue fragments and washed as previously described [4].

The isolated washed fat cells (50–70 mg cell lipid) were suspended in 2 ml of the above-described medium with 5 mM glucose added and incubations were carried out for different periods at 37°C. Unless otherwise stated, all agonists and antagonists were added at the start of the incubations. Reactions were terminated by separating the fat cells from the medium and lipolysis was determined and expressed as described [5].

Cyclic AMP present in the medium and in the fat cells was extracted and determined following the radiocompetitive protein-binding assay of Brown et al. [6] as described [7]. In order to avoid the possible interference of albumin [8] on cAMP determination, the blanks and standards used in the assay of cAMP released into the medium were carried out on aliquots of the incubation buffer. Overall recovery of synthetic cAMP added to control samples was between 90% and 95%. Cyclic GMP was extracted and determined following the radioimmunological assay of Steiner et al. [9]. Under these conditions, even a 5000-fold cAMP excess over the cGMP content had no detectable interference on the assay of cGMP whose overall recovery was between 75–80%.

Metabolic data are expressed on the basis of fat cell lipid dry weight. Results are given as mean values ± SE and Student's '*t*' test was used for comparison of mean values.

Phenoxybenzamine was a preparation from Smith, Kline and French and was kindly supplied by Dr Mention from the laboratoires Gremy-Longuet. Phentolamine was a generous gift from Ciba Pharmaceutical Company. Cyclic [^{3}H]AMP, cyclic [^{3}H]GMP and the reagents used in cyclic nucleotide determinations were purchased from the Radiochemical Centre (Amersham). The origin of all other chemicals used has been described elsewhere [7].

3. Results

As shown in fig.1, norepinephrine (5×10^{-5} M) induced a marked increase in the intracellular cAMP level which reached a plateau after 30 min incubation. At this time, the high levels attained (about 17-fold the basal values) far exceeded those usually reported in rat fat cells under the present experimental conditions (absence of phosphodiesterase inhibitor). As suggested by Hittelman et al. [1], most of this peculiar high reactivity of hamster adipocytes is related to the briefness of the cell isolation procedure used: indeed, the cAMP levels found, under the same conditions of stimulation, in cells isolated following the method of Rodbell [10] attained only 2.5–3 times the basal values (data not shown).

As expected, phentolamine (5×10^{-4} M) enhanced the stimulating effect of norepinephrine on cAMP synthesis (fig.1). This effect was biphasic: the maximal level was reached within the first 10 min (7–8-times the level found with norepinephrine alone), after which the cAMP content slowly fell and returned after 30 min to levels similar to those found with norepinephrine alone. Under these conditions, phentolamine also enhanced the amount of cAMP released from the fat cell, showing thus that the modifications of cAMP metabolism induced by phentolamine are

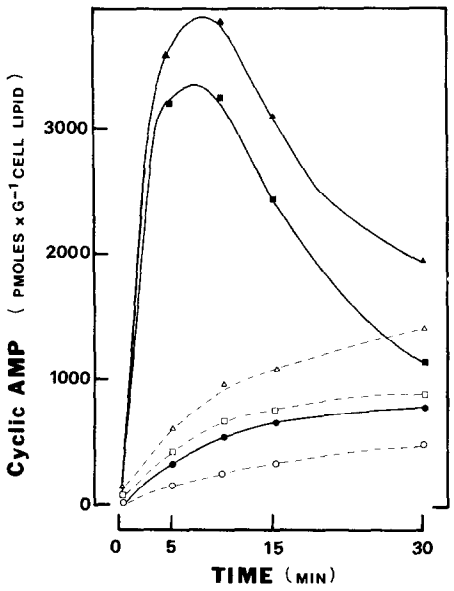

Fig.1. Influence of phentolamine on the time-course of norepinephrine-induced cyclic AMP accumulation and release in hamster fat cells. After preincubation for 30 min, either norepinephrine (5×10^{-5} M) (broken lines) or norepinephrine (5×10^{-5} M) plus phentolamine (5×10^{-4} M) (solid lines) were added. Cyclic AMP was determined just before and at different times after these additions. (□- - -□; ■—■) Intracellular cAMP; (○- - -○, ●—●) extracellular cAMP: (△- - -△; ▲—▲) total (intra + extracellular) cAMP. Each point represents the mean value of 4–8 determinations.

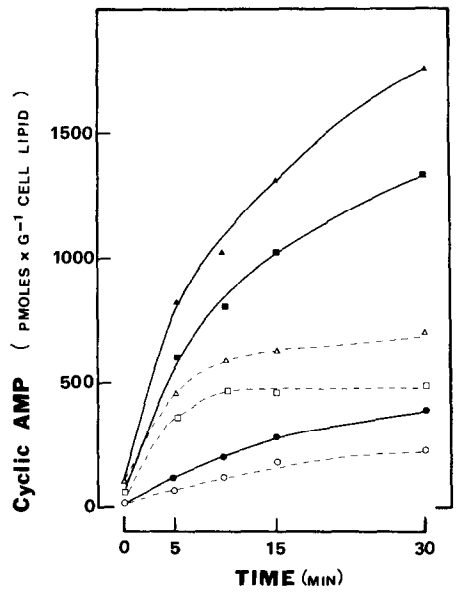

Fig.2. Influence of phenoxybenzamine on the time-course of norepinephrine-induced cyclic AMP accumulation and release in hamster fat cells. Experimental conditions and symbols are the same as those described in fig.1, except that phenoxybenzamine (5×10^{-4} M) was added in place of phentolamine.

Table 1
Inhibition of norepinephrine-induced lipolysis by phentolamine and phenoxybenzamine in hamster adipocytes

Addition to the medium	Glycerol release (μmol. g^{-1} cell lipid. h^{-1})	Inhibition of norepinephrine-induced lipolysis[a] (%)
0	2.9 ± 0.5	100
Norepinephrine (5×10^{-5} M)	43.5 ± 1.6	0
Norepinephrine (5×10^{-5} M) + phentolamine (5×10^{-4} M)	4.9 ± 0.8 ($P < 0.001$)	95
Norepinephrine (5×10^{-5} M) + phenoxybenzamine (5×10^{-4} M)	28.4 ± 1.8 ($P < 0.001$)	37

[a] Defined as = 100−100 (NT−B/N−B), where NT, N and B represent the glycerol released by cells incubated in the presence of norepinephrine plus test compounds, in the presence of norepinephrine alone and in the basal state, respectively

Each value is the mean ± SE of 5−10 determinations. Statistical significance refers to the results found with norepinephrine alone

not due to an inhibition of the transfer of cAMP from the fat cell into the medium.

When phenoxybenzamine (5×10^{-4} M) was added in place of phentolamine, the norepinephrine-induced cAMP accumulation in and out the fat cell also increased (fig.2). Although the maximal effect was of the same order of magnitude as that induced by phentolamine, cAMP accumulation followed a different time-course, the maximal increase occurring between 30 min and 60 min incubation.

Data in table 1 show that both α-adrenolytic drugs (5×10^{-4} M) caused an inhibition of catecholamine-induced lipolysis. Phentolamine was more potent than phenoxybenzamine, the rate of inhibition induced by both compounds being 95% and 37% respectively. Lipolysis stimulated by dibutyryl cyclic AMP (DcAMP) (10^{-3}M) was also antagonized by both blocking agents, action which was significant with concentrations above 10^{-5} M, in the case of phentolamine (table 2); at the concentration of 5×10^{-4} M, furthermore, the

Table 2
Inhibition of dibutyryl cyclic AMP-induced lipolysis by phentolamine and phenoxybenzamine in hamster adipocytes

Addition to the medium	Glycerol release (μmol. g^{-1} cell lipid. h^{-1})	Inhibition of DcAMP-induced lipolysis (%)
0	3.4 ± 0.2	100
DcAMP (1×10^{-3} M)	8.5 ± 0.7	0
DcAMP (1×10^{-3} M) + phentolamine (1×10^{-5} M)	7.2 ± 0.9 ($P > 0.05$)	25
DcAMP (1×10^{-3} M) + phentolamine (1×10^{-4} M)	4.8 ± 0.7 ($P < 0.001$)	73
DcAMP (1×10^{-3} M) + phentolamine (5×10^{-4} M)	4.6 ± 1.0 ($P < 0.001$)	76
DcAMP (1×10^{-3} M) + phenoxybenzamine (5×10^{-4} M)	6.1 ± 0.9 ($0.001 < P < 0.01$)	47

Experimental conditions and symbols as in table 1

Table 3
Failure of norepinephrine and phenoxybenzamine to modify the cyclic GMP accumulated in and released from hamster fat cells

	Intracellular cyclic GMP (pmol. g^{-1} cell lipid)				Extracellular cyclic GMP (pmol released. g^{-1} cell lipid)			
Time (min) following the addition of:	0	5	10	30	0	5	10	30
Norepinephrine (5×10^{-5} M)	4.8 ± 1.3	7.1 ± 2.8	5.2 ± 1.5	5.2 ± 1.1	4.3 ± 1.9	3.0 ± 0.7	4.7 ± 1.8	6.4 ± 1.1
Norepinephrine (5×10^{-5} M) + phenoxybenzamine (5×10^{-4} M)	4.8 ± 1.3	8.7 ± 1.7	9.2 ± 3.8	4.6 ± 5.4	4.3 ± 1.9	4.2 ± 1.3	6.5 ± 0.6	5.6 ± 0.9
		($P > 0.1$)	($P > 0.1$)	($P > 0.1$)		($P > 0.1$)	($P > 0.1$)	($P > 0.1$)

Conditions of incubation as in fig.1. Each value is the mean ± SE of 4 determinations. Statistical significance refers to the results found with norepinephrine alone

rate of inhibition induced by both blocking agents was similar to that found with norepinephrine-induced lipolysis.

The influence of α-adrenergic stimulation and inhibition on the cGMP accumulated in and released from hamster fat cells was also examined (table 3). As can be seen, no significant modification was encountered between the cGMP levels found after exposure to norepinephrine alone or to norepinephrine plus phenoxybenzamine. Moreover, these levels were not statistically different from those found in the basal state (data not shown).

4. Discussion

In fat cells, the first evidence confirming the coexistence of functionally opposed α- and β-adrenergic receptors as regulatory subunits of adenylate cyclase [11] was obtained with human adipocytes [12]. However, it was rapidly felt that this concept could not be extended to the fat cells of all species studied and particularly to the commonly used rat fat cells [13].

Recent studies have shown that hamster white fat cells have a peculiar behaviour which set them apart from rat adipocytes [1]. Indeed, experiments testing the influence of catecholamine and phentolamine on cAMP metabolism provided some evidence for the existence of α-adrenergic sensitivity in these cells, suggesting that hamster adipocytes may be an appropriate model to study some aspects of the hormonal control of human fat cells [1].

Despite its action upon cAMP synthesis, it was recently reported that phentolamine at concentrations higher than 5×10^{-5} M inhibits catecholamine-induced lipolysis in hamster fat cells [2]. To explain this paradoxical effect, we have presently investigated and compared the influence of phentolamine and of another α-adrenolytic drug, phenoxybenzamine [14], on both the time-course of norepinephrine-induced cAMP synthesis and on some of the lipolytic responses of hamster fat cells.

The present results showing that both drugs increase to a similar extent, but with different time-courses, the catecholamine-induced cAMP accumulation in and release from hamster adipocytes, strengthen the concept of the existence of α-receptors in these cells. In addition, the marked inhibitory effect of phentolamine against norepinephrine-induced lipolysis was confirmed [2], an effect which was also observed, although to a slighter extent with phenoxybenzamine. This rather low antilipolytic action of phenoxybenzamine could be explained by the peculiar long-lasting action of this drug on catecholamine-induced cAMP accumulation. In fact, this action still persisted when lipolysis was determined, suggesting that phenoxybenzamine, contrarily to phentolamine [13], may inhibit phosphodiesterase.

On the other hand, this study also shows that when used under conditions reported to increase the lipolytic response of hamster fat cells to catecholamines (low concentrations) [2], phentolamine has no significant effect on DcAMP-induced lipolysis. On the contrary, at concentrations inhibiting catecholamine-stimulated lipolysis, both phentolamine and phenoxybenzamine depress the DcAMP-stimulated one, with phentolamine being here again the most potent antagonist. As DcAMP mimicks the effects of endogenous cAMP [15], these data led us to conclude that the antagonistic effect of these two α-adrenolytic drugs against catecholamine-induced lipolysis is linked to their inhibitory action on one of the distal steps of the lipolytic process, i.e., cAMP binding to protein kinase, protein kinase or triglyceride lipase activation [16]. Therefore and because of the intracellular localization of the latter enzymes [16], it is also possible that the rather modest antilipolytic action of phenoxybenzamine is linked to a lower fat-cell membrane permeability to this compound than to phentolamine.

Finally, the present study also indicates that the hypothesis previously formulated [3], according to which α-adrenergic stimulation may increase cGMP, is not valid, at least for hamster fat cells. As acetylcholine has been shown to increase the intracellular cGMP levels [17], it is therefore likely that the rise in plasma cGMP reported by Ball et al. [3] after infusion of α-adrenergic agents is due to the parasympathic stimulation which occurs as a reflexive consequence of the pressor effect of α-adrenergic stimulation rather than to a direct response to α-adrenergic activity.

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