THE EFFECT OF PHENOXYBENZAMINE ON THE RELATIONSHIP BETWEEN GLYCEROL RELEASE AND CYCLIC AMP LEVELS IN THE PRESENCE OF INSULIN IN EPINEPHRINE-STIMULATED RAT ADIPOCYTES

Ellen H. A. WONG+, Ernest G. LOTEN*, John H. EXTON and Charles R. PARK

Department of Physiology, Vanderbilt University, School of Medicine, Nashville, TN 39232, USA, +Department of Biochemistry, University of Singapore, College Road, 0316 Singapore and **Department of Clinical Biochemistry, University of Otago, Dunedin, New Zealand*

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1. Introduction

Insulin does not affect the relationship of cellular cyclic AMP levels to glycerol in rat adipocytes stimulated with corticotropin [1]. The inhibitory action of insulin on lipolysis was shown to fully account for the decrease in cyclic AMP. This was true, irrespective of whether measurements were made when cyclic AMP was on the upward rise after hormone stimulation, or on the decline. The curves obtained with and without insulin were superimposable. On the other hand, in the presence of epinephrine, a mixed α- and β-agonist, the drop in glycerol production due to insulin, has been shown to be greater than expected from the observed fall in cyclic AMP levels [2].

Phenoxybenzamine, an α-adrenergic blocker, in the concentration range 1–10 μM, has been shown to have a potentiating effect on epinephrine-stimulated lipolysis in rat fat cells [3]. We investigate here whether this effect of phenoxybenzamine could modify the effects of epinephrine on lipolysis and cyclic AMP levels to alter their relationship sufficiently so that the decrease in lipolysis due to insulin can be fully accounted for by the observed fall in cyclic AMP.

2. Materials and methods

Most chemicals including bovine serum albumin (fraction V), and L-epinephrine were purchased from Sigma; D,L-isoproterenol HCl was from K and K; and collagenase from Worthington. Phenoxybenzamine HCl was a gift from Smith, Kline and French. Insulin was a 10-times recrystallized preparation given by Novo (Copenhagen, Denmark). Cyclic [^{3}H]AMP (37.7 Ci/mol) and [^{14}C]glycerol (133 mCi/mol) were from New England Nuclear.

Preparation of fat cells, determination of cell weight, experimental conditions and assay methods are as in [1,5]. In all experiments the cells were distributed into separate polyethylene vials, gassed with 95% O_2/5% CO_2 and preincubated for 20 min with or without insulin. The vials were always regassed if further additions were made. Incubations for 10 min at 37°C were carried out for all experiments.

Results from representative experiments are shown. Data are reported as the mean values of duplicate incubations. Duplicates were within 10% of each other in every case. All observations were confirmed by repetition on at least two other occasions using different batches of cells. The validity of expressing results in this manner, because of the variability in the degree of response from different batches of cells, has been discussed by others [6–8].

3. Results

3.1. *Choice of insulin concentration*

Varying concentrations of insulin have been found to have different effects on epinephrine-stimulated lipolysis and cyclic AMP levels in rat fat cells. Insulin had a biphasic inhibitory effect on adenylate cyclase in broken fat cell systems [9,10]. However, insulin, in the presence of norepinephrine, had a monophasic effect on cyclic AMP levels and a biphasic effect on lipolysis [8]. Experiments were therefore carried out

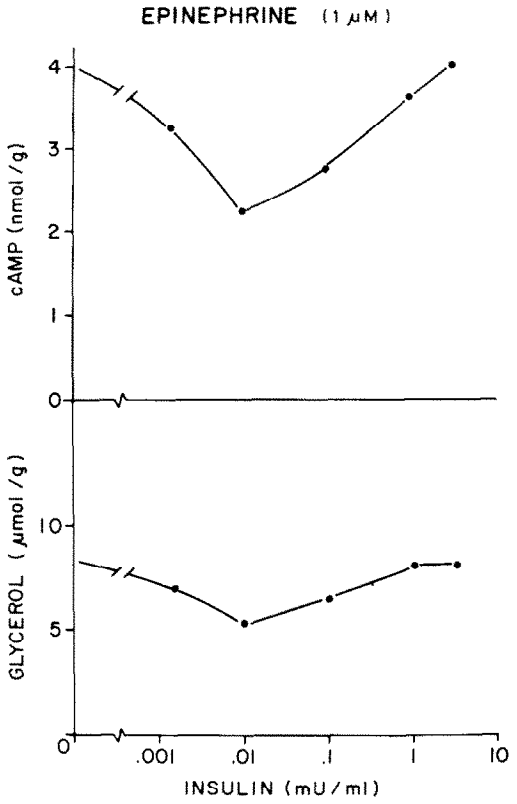

Fig.1. Effects of insulin concentration on lipolysis and cyclic AMP in the presence of epinephrine. Fat cells (11 mg/ml) were from fasted rats.

to determine the concentrations of insulin to be used for the present study. In the experiments shown in fig.1, insulin at low concentrations (0.001–0.1 mU/ml) exerted an antilipolytic effect on epinephrine-stimulated lipolysis which was lost at high concentrations (1–10 mU/ml). The biphasic concentration dependence of insulin effect on lipolysis was also observed for the cyclic AMP content of the fat cells which paralleled those changes in protein kinase activity ratio (–cAMP/+cAMP) as shown in fig.2.

3.2. *The effects of insulin in the presence of epinephrine and isoproterenol*

Experiments in fig.3 show that the relationship between epinephrine-stimulated lipolysis and cyclic AMP content fall on a smooth curve not observed when insulin was present. Insulin appeared to inhibit lipolysis to a greater degree than could be attributed to a corresponding decrease in the level of cyclic

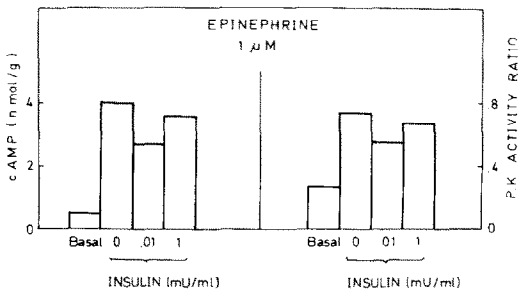

Fig.2. Effects of insulin concentration on cyclic AMP and protein kinase activity ratio (–cAMP/+cAMP) in the presence of epinephrine. Fat cells (10 mg/ml) were from fasted rats.

AMP. These results are in agreement with those in [2]. Insulin, however, did not modify the relationship between lipolysis and cyclic AMP content when fat cells were incubated with isoproterenol, which interacts predominantly with β-adrenergic receptors. The results of these experiments seem to suggest that the discrepency seen with insulin plus epinephrine could be due to an interaction of insulin with the α-mechanism of catecholamine action. If this was so, the observed discrepency would be corrected by the presence of an α-adrenergic blocker.

3.3. *The effect of phenoxybenzamine on insulin inhibition of epinephrine-stimulated lipolysis and cyclic AMP levels*

Phenoxybenzamine (1 and 10 μM) an α-adrenergic

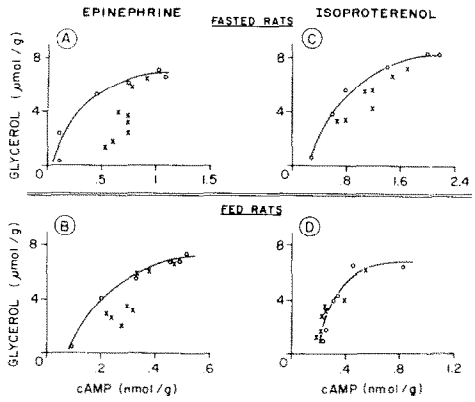

Fig.3. Relationship between cyclic AMP and lipolysis in the presence (x) and absence (o) of insulin. Cells were from fed (B:50 mg/ml; D:32 mg/ml) and fasted rats (A:37 mg/ml; C:35 mg/ml). Concentrations of hormones used were: 10 nM–1 μM epinephrine; 10–200 nM isoproterenol; 1–100 μU/ml insulin.

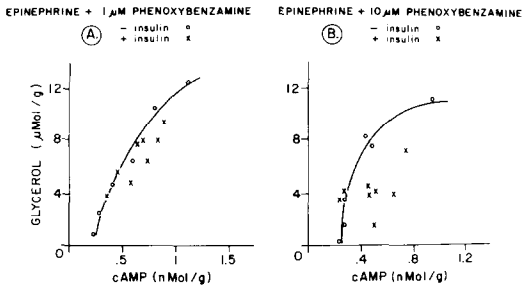

Fig.4. The effects of phenoxybenzamine on the relationship between cyclic AMP and lipolysis, activated by epinephrine, in the presence (x) and absence (○) of insulin. Cells from fasted rats (A: 23 mg/ml; B:40 mg/ml) were preincubated with phenoxybenzamine and insulin (0–100 μU/ml) for 20 min before the addition of epinephrine (0.01–1 μM) and incubated for 10 min.

blocker, has been shown [3] to have a potentiating effect on epinephrine-stimulated lipolysis in rat fat cells, not observed outside this concentration range. Experiments were therefore carried out with these two concentrations of phenoxybenzamine to determine whether its potentiating effects on epinephrine-stimulated lipolysis could ameliorate or abolish the decrease in lipolysis, due to insulin, not accounted for by a corresponding decrease in cyclic AMP levels. Results of these experiments are shown in fig.4. 1 μM appeared to be more effective than 10 μM phenoxybenzamine in modifying the effects of insulin on epinephrine-stimulated lipolysis and cyclic AMP content, to sufficiently prevent their relationship from being altered by the presence of insulin. Subsequent experiments showed that under our experimental conditions, 1 or 2 μM were more effective than higher concentrations of phenoxybenzamine, and that the optimum concentration varied from one cell preparation to another. Fig.5 shows 4 experiments carried out with 2 μM phenoxybenzamine on 4 different occasions. In expts. A and D, 2 μM phenoxybenzamine was more effective in completely preventing insulin from altering the relationship of cyclic AMP levels to lipolysis stimulated with epinephrine than in expts. B and C. For all these experiments, the protein kinase activity ratios were also determined and correlated with the cyclic AMP content, or glycerol release, in the presence and absence of insulin. The correlations for expt. D are shown in the right panel. Phenoxybenzamine appeared to effectively prevent insulin from altering the relationships between any of these parameters in epinephrine-stimulated fat cells.

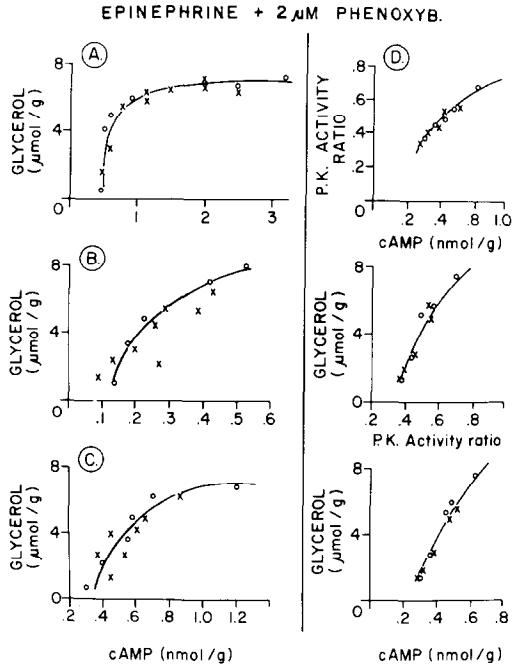

Fig.5. Relationship between cyclic AMP, protein kinase activity ratio and lipolysis activated by epinephrine and phenoxybenzamine, in the presence (x) and absence (○) of insulin. Cells from fed rats (35,34,25 and 25 mg/ml for A,B,C and D, respectively) were incubated under similar conditions as those for experiments shown in fig.4.

4. Discussion

Several conditions modify the effect of insulin on the accumulation of cyclic AMP induced by lipolytic hormones in adipocytes [1]. The biphasic concentration dependence of insulin effect could explain the failure of some investigators to observe a significant lowering of cyclic AMP levels when supraoptimal concentrations were used, e.g. 0.5 mU insulin/ml [11] failed to elicit any insulin effect on the elevations of cyclic AMP caused by epinephrine. At this concentration, insulin effects were minimal under our experimental conditions.

Paradoxical effects of high concentrations of insulin in increasing cyclic AMP levels and lipolysis in the presence of 100 μM or higher epinephrine concentrations have been reported [8,12]. These effects were not observed in the present study as epinephrine concentrations used were 1 μM or less. It was evident, however, that even at low epinephrine concentrations, insulin inhibited lipolysis by a mechanism in addition

to lowering cyclic AMP (fig.4). This is in agreement with other studies [2].

The experiments with isoproterenol, and the effects of phenoxybenzamine in modifying epinephrine-induced lipolysis, suggest that the cyclic AMP-dependent component of insulin inhibition might involve modulation of an α-adrenergic mechanism(s). It is well established that activation of α-receptors in human and hamster adipocytes reduces lipolysis [13,14]. The α-blocker, phenoxybenzamine, has also been shown to increase epinephrine-induced lipolysis in fat cells of hamster [15] and rat [2] and α-receptors are thought to mediate the inhibition of lipolysis in adipose tissues [16]. In the present study, phenoxybenzamine appeared to prevent the insulin inhibition of lipolysis which was cyclic AMP independent. This is consonant with the recent report of evidence for a second pathway of lipolysis activation in response to epinephrine, in addition to that involving a cyclic AMP-mediated increase in the state of phosphorylation of the hormone-sensitive lipase [17].

Ca^{2+} have been shown to mediate α-adrenergic effects in the liver [18] and have been suggested to be involved in α-responses in adipose tissue [19]. Insulin effects in lowering cyclic AMP content in fat cells in the presence of epinephrine, however, have been shown not to be dependent on Ca^{2+} [20]. Calcium was also not required for insulin action on adenylate cyclase [10,21] or on phosphodiesterase [22]. Thus it would appear that insulin effects on the cyclic AMP system were independent of the α-responses. On the other hand, the results of the present study show that insulin effects on lipolysis could be due to both α- and β-responses and that low concentrations of phenoxybenzamine provide for an effective α-blockade. Hence in the presence of phenoxybenzamine, the insulin effects on lipolysis correlated well with its effects on cyclic AMP. These findings are consistent with the recent observation that the inhibitory action of insulin on corticotropin-stimulated lipolysis can be fully accounted for by the decrease in cyclic AMP [1].

Acknowledgement

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