INHIBITION BY INSULIN OF THE ADRENALINE-STIMULATED ADENYLATE CYCLASE IN RAT ADIPOSE TISSUE

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1. Introduction

In a precedent report [1] we have described results suggesting that insulin exerts its antilipolytic activity at the level of both synthesis and degradation of cyclic AMP. Our experiments concerned the release of glycerol and free fatty acid by fat pad tissue, but were conceived to eliminate the well known effect of insulin on the low K_m phosphodiesterase activity [2–8] and possibly to show an effect on the adenylate cyclase activity. In the presence of 1-methyl-3-isobutylxanthine (10^{-3} M), a concentration which maximally stimulates lipolysis, insulin (100 μU/ml) has lost its antilipolytic action, most likely because it is unable to counteract the inhibitory effect of the methylxanthine. In this case, the stimulation of adenylate cyclase by 1 μg/ml adrenaline (5×10^{-6} M) brings an increment in lipolysis which can be suppressed by insulin (100 μU/ml). We have interpreted this fact as the consequence of an insulin action on the cyclic AMP synthesis rate. In this note we confirm the soundness of our previous interpretation concerning distinct effects of insulin on the synthesis and degradation rate of cyclic AMP.

2. Materials and methods

Crystalline beef insulin B-grade (25.9 IU/mg) was obtained from Calbiochem. L-Adrenaline bitartrate no. E-4375 was supplied by Sigma. IMBX lot 1353, was obtained from Regis. Bovine serum albumin Pentex (fraction V) was purchased from Miles and purified from fatty acids by the method in [9]. D,L-propranolol hydrochloride, lot 377, was kindly donated by ICI Pharma (Reims). 8-[^{3}H]adenosine 3′,5′-cyclic phosphate (21 Ci/mmol) was obtained from the Radiochemical center, Amersham. Male Wistar rats (180–230 g), from Animalabo (Paris) had free access to food and water.

The rats were decapitated and the epididymal fat pads removed, rinsed in ice-cold physiological saline (0.9% NaCl (w/v)) and placed at 4°C in Krebs-Ringer bicarbonate buffer (pH 7.4) with 1.3 mM Ca^{2+} and 4% (w/v) albumin free of fatty acid. This medium was used for all experiments. To study the time-course of cyclic AMP disposal, small fat pad fragments (80–100 mg) were preincubated 5 min at 37°C with adrenaline (1 μg/ml), then rapidly transferred to a medium containing propranolol (10^{-3} M) and according to assays insulin (100 μU/ml), IMBX (10^{-3} M) or both. At the end of the incubation, tissue fragments were rapidly dropped into cold perchloric acid (1 N) and cyclic AMP extracted. In another experiment, the fat pad fragments were preincubated 5 min in the presence of 1 μg/ml adrenaline and then incubated 10 min with propranolol (10^{-3} M) and cyclic-[^{3}H]AMP (1.8 μCi/assay), in the presence of IMBX (10^{-3} M), insulin (100 μU/ml) or both. The reaction was stopped by adding 20% (w/v) cold trichloracetic acid (final conc. 5%, w/v). The tissues were homogenized, the supernatant was extracted 5 times with water saturated diethylether and chromatographed on thin-layer

Abbreviations: cyclic AMP, adenosine 3′,5′-cyclic monophosphate; 5′-AMP, 5′ adenosine-monophosphate; IMBX, 1-methyl-3-isobutylxanthine

* The counter has a counting efficiency of 30%

chromatographic plates (silica gel F254, Merck AG) in the solvent: 95% ethanol/1 M ammonium acetate (13:7, v/v) according to [10] with cold markers. Individual zones corresponding to 5'-AMP, cyclic AMP and adenosine were visualized under ultraviolet light. Spots were outlined, scraped off and counted by scintillation counting technique (counter model 3320 Packard*). To study the cyclic AMP accumulation rate, fad pad fragments were incubated for various times with adrenaline (1 μg/ml) and IMBX (10^{-3} M), in the presence or absence of insulin (100 μU/ml).

The cyclic AMP content was measured by the radioimmunological method [11] except that bound ligand was separated from free ligand by filtration on Millipore filters (HAWP 0.45 μm) instead of equilibrium dialysis.

The results are the means of triplicate experiments expressed with standard errors.

3. Results

The rate of cyclic AMP disposal after stimulation by adrenaline of rat adipose tissue was studied by two methods: by measuring the appearance of metabolites from added cyclic[^{3}H]AMP; by radioimmunoassay directly.

Figure 1 represents the repartition of the radio-

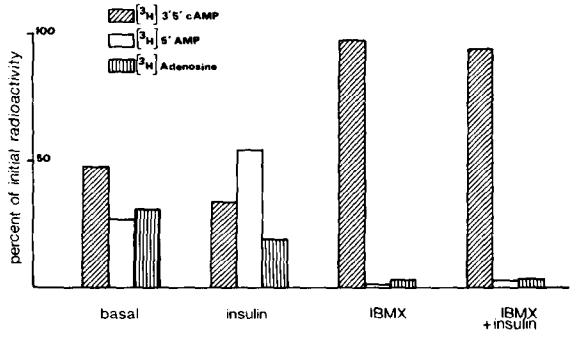

Fig.1. Disappearance of added cyclic[^{3}H]AMP from medium containing rat fat segments. Effect of insulin (100 μU/ml) on the degradation of the cyclic nucleotide in the presence or in the absence of 1-methyl-3-isobutylxanthine (10^{-3} M). Separation of 3',5'-cyclic[^{3}H]AMP metabolites was by thin-layer chromatography (see section 2).

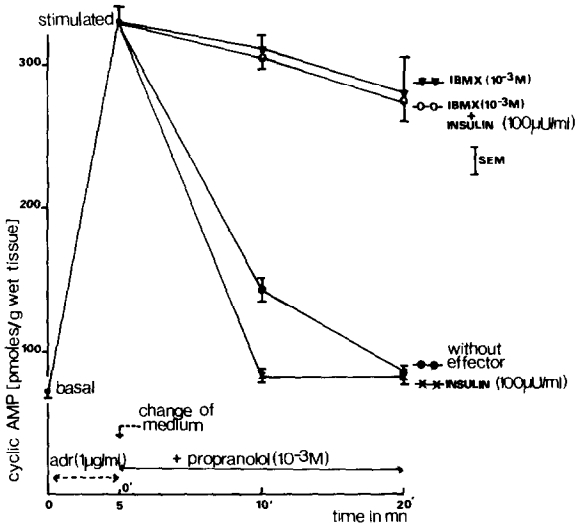

Fig.2. Time course of cyclic AMP content in the rat fat segments in which the cyclic nucleotide level was pre-elevated by adrenaline (1 μg/ml). Effect of insulin (100 μU/ml) in the absence or in the presence of IMBX (10^{-3} M). (X——X) insulin; (▼——▼) IMBX; (○——○) insulin plus IMBX. Experimental conditions are described in the text. Each value represents the mean ± SEM of 3 different experiments.

activity of added cyclic[^{3}H]AMP (0.9×10^{-10} mol/assay) between the cyclic nucleotide and its degradation products, mainly [^{3}H]adenosine-5'-phosphate and [^{3}H]adenosine. Insulin alone (100 μU/ml) decreases the percentage of cyclic[^{3}H]AMP remaining after 10 min incubation. IMBX (10^{-3} M) prevents the cyclic[^{3}H]AMP degradation and suppresses the stimulatory action of insulin.

Figure 2 depicts the time-course of the cyclic AMP content in rat fat segments in the presence of insulin and/or IMBX. Intracellular cyclic AMP content measured by radioimmunoassay, following adrenaline (1 μg/ml) stimulation over 5 min is significantly enhanced in comparison with the basal value (300 pmol/g wet tissue versus 80 pmol/g wet tissue, $P < 0.001$). In the presence of insulin (100 μU/ml), there is a rapid increase of the degradation rate of endogenous cyclic AMP. In the presence of IMBX (10^{-3} M) no significative degradation of cyclic AMP is observed over 20 min incubation whether insulin was present or not, showing that the positive effect of insulin on phosphodiesterase activity, is prevented when this enzyme is completely inhibited.

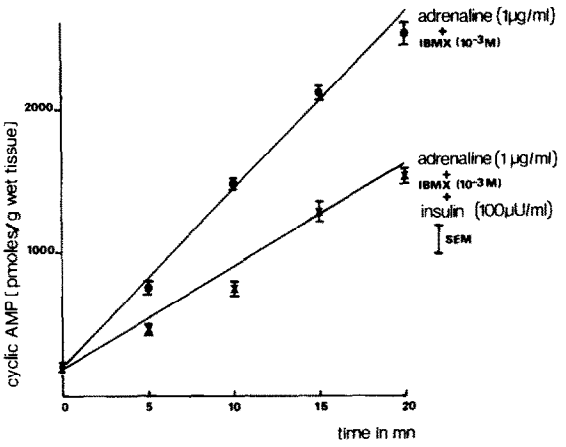

Fig.3. Effect of adrenaline (1 µg/ml) in the presence of IMBX (10^{-3} M) with or without insulin (100 µU/ml) on cyclic AMP synthesis in rat fat segments. (×——×) with insulin; (•——•) without insulin. Each point represents the mean values of 3 different experiments with SEM.

Figure 3 represents the accumulation rate of cyclic AMP in fat pad segments when adenylate cyclase activity is stimulated by adrenaline (1 µg/ml) and phosphodiesterase activity completely inhibited by IMBX (10^{-3} M). The efficiency of this inhibition, shown in fig.1,2, is confirmed by the linear character of the cyclic AMP accumulation process. In the presence of IMBX (10^{-3} M) and insulin (100 µU/ml), cyclic AMP accumulation rate is always constant but is less important than in the absence of insulin, this result may be attributed to a decreased rate of cyclic AMP synthesis in the presence of hormone.

4. Discussion

There is a general acceptance of the fact that insulin may be antilipolytic by increasing the low K_m phosphodiesterase activity [2–8]. The results concerning an action at the adenylate cyclase level are less convincing, since only indirect evidence is presented. The last one concerns the role of calcium which is necessary for the activation of the phosphodiesterase activity. Nevertheless, in its absence, the antilipolytic effect of insulin may occur, suggesting an other site of action of the hormone [12]. The controversial character of the reported effects of insulin on intracellular cyclic AMP content [13–16] may be explained by the complex evolution of the cyclic AMP content in a tissue stimulated by a catecholamine when the phosphodiesterase activity is operative. Whatever the time of the assay, the concentration of the cyclic nucleotide is not representative of the amount present during the stimulation. This situation is complicated again by the addition of insulin which modifies the degradation rate constant and the location (in time) of the stimulated steady state.

We think that a simple solution to this problem is to completely inhibit the degradation rate and to study the synthesis rate alone. This is what we have done. Our results show that 1 methyl-3-isobutylxanthine (10^{-3} M) inhibits the phosphodiesterase activity and the insulin effect on this enzyme. In these conditions insulin is able to decrease significantly the synthesis rate of cyclic AMP.

Acknowledgements

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