INSULIN ACTION ON THE ADENYL CYCLASE SYSTEM: ANTAGONISM TO ACTIVATION BY LIPOLYTIC HORMONES

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1. Introduction

The lowering of intracellular 3′,5′-AMP by insulin has been shown in liver [1] and adipose tissue [2, 3], both target organs in which the hormone is known to counteract a number of cyclic AMP-controlled metabolic pathways. From metabolic studies concerning the antagonism between glucagon and insulin in liver [1, 4] and of lipolytic hormones and insulin in adipose cells [5, 6], an interaction at a site controlling the formation of cyclic AMP could be inferred. Although effects upon adenyl cyclase [7] and cyclic nucleotide phosphodiesterase [8, 9] after treatment of intact cells with insulin have been reported, the issue has remained controversial [6, 10], especially since direct effects upon adenyl cyclase could not be observed [11–13].

We have recently reported a direct *in vitro* interaction between glucagon and insulin on adenyl cyclase activity in a particulate preparation from mouse liver [14]. Furthermore, we have shown that NSILA-S, a polypeptide with a similar molecular weight as insulin which is known to mimic most metabolic effects of the hormone [15] could also inhibit the glucagon effect over a physiologically relevant concentration range, but had no effect upon the fluoride-stimulated system. Inactivation of the substance by reduction and aminoethylation resulted in complete loss of its biological activity and at the same time abolished its effect upon the adenyl cyclase system [14]. The present report presents further findings with the mouse liver preparation and demonstrates a similar action of insulin upon adenyl cyclase in rat lipocyte "ghosts", a system which has been developed by Rodbell [16].

2. Experimental

Crude membrane preparations were obtained from fresh mouse livers after complete disruption of cells with a high-speed mechanical homogenizer. The assay and properties of adenyl cyclase activity in this system have been described [17]. Minor modifications include the use of creatin phosphate and creatin phosphokinase for ATP regeneration and the use of 5 mM 3′,5′-AMP as a trapping system [18] in order to prevent breakdown of labeled 3′,5′-AMP. Lipocyte "ghosts" were prepared according to the method of Rodbell [16], suspended in Tris buffer with 1% bovine albumin and assayed for adenyl cyclase activity according to a modification of the method of Krishna et al. [19]. The incubation system contained: 20 mM Tris-HCl, pH 7.4, 4.5 mM $MgCl_2$, 1 mM 3′,5′-AMP, 0.45 mM ^{32}P-ATP, 10 mM creatine phosphate, 300 μg creatinkinase, and hormones as indicated in a total vol of 0.1 ml. The reaction was started with the addition of ghost-suspensions (50–100 μg protein/test) and allowed to proceed for 10 min at 37°. Formation of 3′,5′-AMP was strictly linear with time and protein concentration. Phosphodiesterase activity in liver and ghost preparations as measured according to the method of Loten and Sneyd [9] was too small to account for a significant breakdown of 3′,5′-AMP under assay conditions.

3. Results and discussion

Preliminary studies suggested that the antagonism of glucagon and insulin was competitive with respect

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Table 1
Inhibition of glucagon effect upon adenyl cyclase activity of mouse liver by insulin.

Additions	No. of experiments	Adenyl cyclase activity (nmoles × g protein^{-1} × min^{-1})	Δ	p
None	11	8.8 ± 1.9		
Glucagon, 0.5 μg/ml	11	26.7 ± 3.9		
Glucagon, 0.5 μg/ml + insulin, 100 μU/ml	11	16.5 ± 3.8	10.2 ± 2.2	< 0.0005

Assay conditions as described [14]. Crystalline porcine insulin from the Novo Co. and crystalline bovine insulin from Farbwerke Hoechst were used. Values indicate mean ± S.E.M., significance of difference was calculated on the basis of paired comparison.

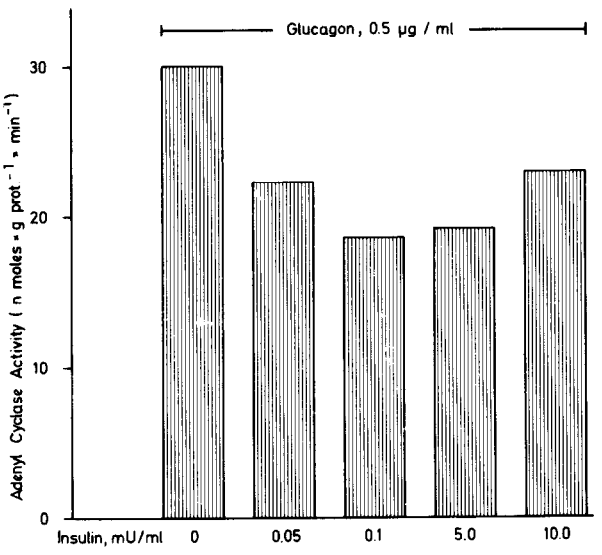

Fig. 1. Effect of increasing concentrations of crystalline insulin upon glucagon stimulated adenyl cyclase activity from mouse liver. Submaximal stimulation by 0.5 μg/ml of synthetic glucagon, crystalline bovine insulin from Novo, Copenhagen.

to activation of the liver enzyme. We therefore used submaximal concentrations of synthetic glucagon as shown in table 1. The table represents 11 experiments with different liver preparations performed over the period of 10 mon. Although considerable variation was observed due to differences in animals and quality of ^{32}P-ATP used, the effect of insulin was highly significant on the basis of paired comparison. Fig. 1

Table 2
Fluoride stimulation of mouse liver adenyl cyclase in the presence and absence of insulin.

Additions	Adenyl cyclase activity (nmoles × g protein^{-1} × min^{-1}) Experiment I	Experiment II
None	3.9	4.0
NaF, 10 mM	37.9	44.5
NaF, 10 mM + insulin, 100 μU/ml	43.6	40.0

shows that the effect was most pronounced at low concentrations of insulin, which lie in the physiological range, and decreased again when more hormone was added. When the system was stimulated by 10 mM NaF, no effect of insulin could be observed (table 2), suggesting insulin action at a step between the glucagon receptor and the catalytic unit of the adenyl cyclase system which is thought to be directly stimulated by fluoride [20]. The experiment provides also evidence against the possible stimulation of 3′,5′-AMP breakdown by insulin under assay conditions.

Studies with lipocyte ghosts showed that the effect of insulin is not restricted to the liver preparation. The effect of submaximal concentrations of norepinephrine, glucagon and ACTH could be significantly reversed by 50 μU/ml of crystalline insulin, a concentration which lies well within the physiological range (table 3). Again, insulin had no effect upon the fluoride-stimulated system and did not inhibit the basal

Table 3
Effect of crystalline insulin upon adenyl cyclase activity in lipocyte ghosts submaximally stimulated by norepinephrine, glucagon, and ACTH.

Additions	No. of experiments	Adenyl cyclase activity (nmoles × g protein $^{-1}$ × min^{-1})	Δ	ρ
None	10	108.7 ± 13.3		
Norepinephrine 5 × 10^{-7}M	10	167.5 ± 21.1	50.5	< 0.005
+ Insulin, 50 μU/ml	10	117.0 ± 14.8	± 7.5	
Glucagon, 0.2 μg/ml	7	181.3 ± 19.1	56.2	< 0.01
+ Insulin, 50 μU/ml	7	125.1 ± 10.9	± 15.8	
ACTH, 0.03 μg/ml	2	297.9	101.3	
+ Insulin, 50 μU/ml	2	196.6		

The stimulation was almost compeltely reversed in the case of norepinephrine and glucagon.

Table 4
Absence of insulin inhibition of basal and fluoride-stimulated adenyl cyclase activity in lipocyte ghosts.

Additions	Adenyl cyclase activity (nmoles × g protein^{-1} × min^{-1})
Experiment I	
Basal	128.4
+ insulin, 50 μU/ml	125.9
+ insulin, 500 μU/ml	157.9
Experiment II	
NaF, 1 mM	131.9
+ insulin, 50 μU/ml	131.4
NaF, 2 mM	261.7
+ insulin, 50 μU/ml	262.2
NaF, 10 mM	958.3
+ insulin, 50 μU/ml	917.5

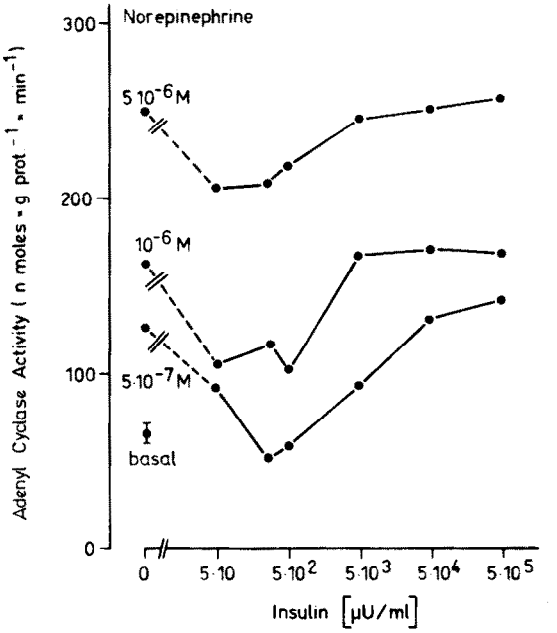

Fig. 2. Antagonism of norepinephrine and insulin in activation of adenyl cyclase activity of fat cell ghosts.

activity (table 4). In accordance with our findings on the liver preparation, the effect was reversed by increasing the concentration of insulin (fig. 2). This phenomenon may explain the earlier observations by Lavis et al. [21] who showed a paradoxical failure of high concentrations of insulin to block epinephrine-induced lipolysis in isolated fat cells. In all insulin preparations used glucagon contamination was far too low to explain these effects.

From the present data it appears that several experimental conditions are essential for the demonstration of an insulin effect upon the hormone stimulated adenyl cyclase system: (1) the use of submaximal concentrations of stimulant and low concentrations of insulin, (2) low concentration of substrate, (3) fresh membrane preparations, and (4) possibly the omission of methylxanthines from the assay system. Our findings suggest that the earlier observed decrease of the levels of cyclic AMP in liver and adipose tissue is a consequence of the interaction of insulin and its antagonists at some step in the activation system of adenyl

cyclase. The primary event of insulin action in the course of its effect upon metabolic functions such as glycogen metabolism, gluconeogenesis, ketogenesis, urea production in liver and lipolysis in adipose tissue could thus be inhibition of the adenyl cyclase system in the plasma membrane. For this the hormone would not have to enter the cells. Although an additional action of insulin upon the cyclic nucleotide phosphodiesterase seems possible, convincing effects have thus far only been shown after treatment of whole cells with the hormone [8, 9]. In this case an indirect activation could still have been caused by metabolite changes secondary to insulin action upon the adenyl cyclase system.

Although there is some indication that in the liver preparations insulin, like NSILA-S, inhibits the basal as well as the hormone-stimulated cyclase activity, it is not clear whether adhering stimulatory hormones may have still been active under "basal" conditions. In view of the longer preparative procedure this seems less likely in the ghost preparation where no effect could be observed upon the basal activity. It is, however, evident that under most physiological conditions *in vivo*, agonistic as well as antagonistic hormones will be present.

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