Islet-activating protein discriminates the antilipolytic mechanism of insulin from that of other antilipolytic compounds

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In vivo administration of islet-activating protein to rats resulted in an increase in fat cell lipolysis in vitro, which was associated with almost complete resistance of adipocytes towards the antilipolytic effects of N^6-phenylisopropyladenosine, prostaglandin E_2 and nicotinic acid. Concomitantly, the inhibitory effects of these compounds on adenylate cyclase activity in membranes were impaired. In contrast, the antilipolytic action of insulin was not only preserved, but even augmented in cells from rats treated with islet-activating protein. The data suggest that insulin exerts its antilipolytic effects via mechanisms which are different from those involved in the effects of prostaglandin E_2, N^6-phenylisopropyladenosine and nicotinic acid.

Insulin *Islet-activating protein* *Rat adipocyte* *Lipolysis* *Adenylate cyclase*

1. INTRODUCTION

Lipolysis is bidirectionally regulated [1,2]. Hormones and drugs which increase lipolysis usually also increase cAMP levels via stimulation of the membrane-bound adenylate cyclase. Conversely, most of the inhibitory hormones and drugs have been demonstrated to reduce cAMP levels and adenylate cyclase activity. The compounds causing inhibition of the rat fat cell adenylate cyclase include adenosine and adenosine analogues, prostaglandins of the E-series and nicotinic acid [3–5].

Insulin is the antilipolytic hormone par excellence. No hormone appears to be more important in the regulation of adipocyte metabolism than insulin [1]. Insulin lowers the hormonally stimulated rise of cAMP levels in isolated adipocytes [6–8]. This activity of insulin may be a result of an increase in the insulin-sensitive low K_m cAMP phosphodiesterase activity [6] or could be related to an inhibitory effect of insulin on adenylate cyclase activity [7,8].

Recently, it has been shown that islet-activating protein (IAP), one of the toxins of *Bordetella pertussis*, impairs or abolishes the inhibitory effects of alpha-adrenergic agonists and muscarinic cholinergic agonists on adenylate cyclase in pancreatic and myocardial membranes, respectively [9,10]. It is now clear that IAP inactivates a regulatory subunit of the adenylate cyclase system (N_i) which is involved in hormone-induced inhibition of the enzyme [11,12]. The possibility that administration of IAP to rats may alter the sensitivity of their adipocytes to antilipolytic agents including insulin was, therefore, studied.

2. MATERIALS AND METHODS

Islet-activating protein was purified as in [13] from the supernatant of *Bordetella pertussis* suspensions kindly donated by Drs L. Robbel and F. Backkolb (Behringwerke Marburg). N^6-phenylisopropyladenosine (PIA) was from Boehringer Mannheim, and prostaglandin E_2 (PG E_2) and in-

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sulin were from Serva AG (Heidelberg).

Male rats (180–220 g) were treated once with IAP (4 μg/kg i.v.). Three days later, the rats were killed by decapitation, and adipocytes were isolated by collagenase digestion [14].

For metabolic studies, the isolated cells were suspended in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 40 g/l human serum albumin, 5 mM of glucose and, when present, 1 μg/ml of adenosine deaminase either alone or in combination with theophylline (1 mM) and hormones as indicated in the legends to fig. 1 and table 1. Incubations were carried out under gas phase of O_2 and CO_2 (95 + 5) for 180 min at 37°C. Glycerol release was determined by a newly-developed bioluminescent method in [15].

Adenylate cyclase activity was determined essentially as in [16]. The assay medium contained 50 μM [^{32}P]ATP (0.2–0.4 μCi/tube), 2 mM $MgCl_2$, 1 μM GTP, 0.1 mM cAMP, 100 mM NaCl, 4 mM 3-isobutyl-1-methyl-xanthine, 2 mg/ml bovine serum albumin, 0.4 mg/ml creatine kinase, 5 mM creatine phosphate as its tris (hydroxymethyl)aminomethane salt, and 50 mM triethanolamine–HCl (pH 7.4) in a total volume of 0.1 ml. Reactions were started by addition of fat cell membranes (20–40 μg/tube) to the prewarmed reaction mixture and were performed for 10 min at 25°C.

3. RESULTS

Table 1 shows the effects of IAP administration in vivo on basal glycerol release of isolated fat cells in the presence and absence of adenosine deaminase and theophylline. In cells from non-treated animals, basal lipolysis was hardly measurable (0.3 μmol.10^6 cells^{-1}.180 min^{-1}). Inclusion of adenosine deaminase (1 μg/ml) resulted in a marked increase in glycerol release (1.2 μmol.10^6 cells^{-1}.180 min^{-1}), which was further increased by simultaneous addition of theophylline (1 mM). IAP treatment caused a marked increase in lipolysis. In contrast to controls, adenosine deaminase had no stimulatory effect under these conditions, indicating that the antilipolytic effect of endogenously produced adenosine had been abolished by previous administration of IAP. Simultaneous addition of theophylline caused a further increment in glycerol release in cells from IAP-treated animals.

Table 1

Effects of IAP on basal glycerol release in the absence or presence of adenosine deaminase (1 μg/ml) alone or in combination with theophylline (1 mM)

Additions	Glycerol release (μmol.10^6 cells^{-1}.180 min^{-1})	
	Control	IAP
None	0.03	2.5
Adenosine deaminase	1.2	2.3
Adenosine deaminase + theophylline	3.75	4.0

Values are means of duplicate determinations. Glycerol release was determined in adipocytes from rats pretreated with and without IAP as described in section 2

Illustrated in fig. 1 are the effect of various antilipolytic agents on lipolysis in the presence of either adenosine deaminase alone (insulin, PIA) or in combination with theophylline (PGE_2). All compounds tested caused a dose-dependent inhibition of lipolysis in fat cells obtained from non-treated animals. In contrast to controls, the inhibitory effects of those agents known to affect lipolysis via inhibition of adenylate cyclase (PIA, PGE_2) was impaired or abolished in fat cells from IAP-treated animals. The same qualitative pattern of effects was obtained with nicotinic acid (not shown).

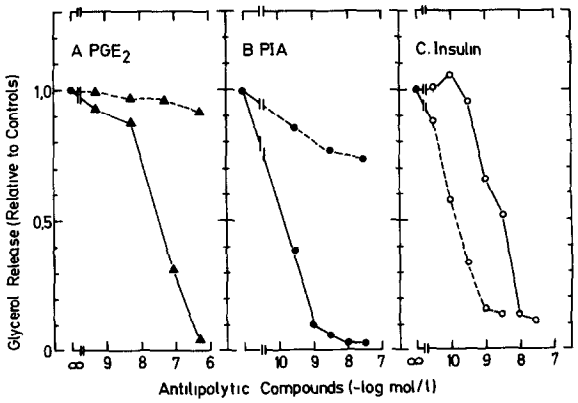

Fig. 1. Influence of IAP treatment on the antilipolytic effects of PGE_2, PIA and insulin. The medium contained adenosine deaminase (1 μg/ml) either alone (PIA, insulin) or in combination with theophylline (1 mM, PGE_2). Solid lines: controls; dotted lines: cells from IAP-treated rats.

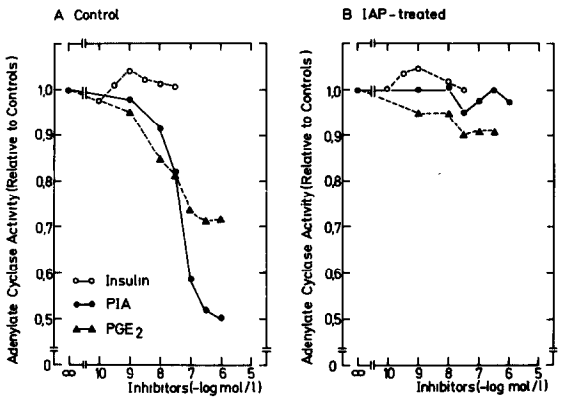

Fig. 2. Effects of various antilipolytic compounds on adenylate cyclase activity in adipocyte membranes obtained from control and IAP-treated animals.

Among the agents tested, only insulin was capable of inhibiting lipolysis in cells of IAP-treated animals. This latter hormone was even more potent in cells from IAP-rats than in adipocytes from non-treated controls.

Fig. 2 shows the effects of insulin, PIA and PGE_2 on adenylate cyclase activity in adipocyte membranes. Similar to lipolysis in intact cells, adenylate cyclase activity was dose-dependently decreased by PIA and PGE_2. As in intact cells, the inhibitory effects of PIA, PGE_2 and also of nicotinic acid (not shown) were almost completely abolished in adipocyte membranes of IAP-treated animals. Insulin failed to decrease cAMP formation in adipocyte membranes of controls and IAP-treated rats.

4. DISCUSSION

These results show that pretreatment of rats with IAP abolishes the antilipolytic effects of PGE_2, PIA and nicotinic acid. Concomitantly, the inhibitory effects of these compounds on adenylate cyclase are almost completely lost (fig.2), indicating that the antilipolytic effects of PIA, PGE_2 and nicotinic acid are mediated via inhibition of adenylate cyclase.

The mechanisms of insulin's antilipolytic action appear to be different. Like others, we failed to obtain an inhibitory effect of the peptide hormone on adenylate cyclase under conditions where other antilipolytic compounds caused marked inhibition (fig.2). In addition, in intact cells the antilipolytic action of insulin was not only preserved, but even augmented after IAP treatment, which abolished the inhibitory effects of other antilipolytic agents such as PIA, PGE_2 and nicotinic acid.

Our results are in agreement with preliminary studies showing that pretreatment of hamsters with pertussis vaccine can reduce the inhibitory effects of various antilipolytic agents on cAMP levels and lipolysis in vitro [17]. They also explain results obtained with histamine-sensitizing factor (HSF), a pertussis toxin similar, or identical to IAP [9]. This toxin has been reported to reveal an antilipolytic effect of insulin under conditions where no such effect was seen in the absence of the toxin [18]. In the light of our findings, this permissive effect of HSF might in part be due to an increased insulin sensitivity induced by the toxin treatment.

The actions of insulin on adipocyte metabolism are diverse and may be mediated via different mechanisms [19]. From the present results, we cannot exclude that the antilipolytic effect of insulin is mediated via inhibition of adenylate cyclase, either directly by insulin receptor-mediated processes or indirectly via formation of low -M_r mediators [20]. However, if insulin affects adenylate cyclase activity, the mechanisms of action are clearly different from those responsible for inhibition of glycerol release by other antilipolytic compounds such as prostaglandin E_2, N^6-phenylisopropyladenosine or nicotinic acid.

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