Islet-activating protein prevents nicotinic acid-induced GTPase stimulation and GTP but not GTPγS-induced adenylate cyclase inhibition in rat adipocytes

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The influence of islet-activating protein (IAP), a *Bordetella pertussis* toxin, was studied on adenylate cyclase and GTPase activities in rat adipocyte membranes. Pretreatment of rats or intact rat adipocytes with IAP did not affect adenylate cyclase inhibition by the stable GTP analog, GTPγS, whereas inhibition by GTP was abolished. Concomitantly, activation of the adipocyte enzyme by sodium and its inhibition by nicotinic acid were prevented. Furthermore, IAP treatment of adipocyte membranes prevented nicotinic acid-induced stimulation of a high affinity GTPase. The data suggest that a GTP-hydrolyzing system involved in the inhibitory regulation of adenylate cyclase is the target of IAP's action.

Islet-activating protein Bordetella pertussis *toxin* *Rat adipocyte* *Adenylate cyclase* *GTPase* *Nicotinic acid*

1. INTRODUCTION

Islet-activating protein (IAP), a *Bordetella pertussis* toxin, modulates adenylate cyclase activity probably by an ADP-ribosylation of a regulatory component of the adenylate cyclase system. The target of the ADP-ribosylation reaction caused by IAP is distinct from the guanine nucleotide-binding regulatory site, that is ADP-ribosylated by cholera toxin and that is involved in adenylate cyclase stimulation by hormones [1,2]. Since hormone-induced inhibition of adenylate cyclase (e.g., by α-adrenergic agonists in pancreatic cells [3] or by muscarinic cholinergic agonists in heart cells [4]) is impaired or abolished by IAP, it is feasible that the site affected by IAP is functionally involved in the negative control of adenylate cyclase activity.

Adipocyte adenylate cyclase is inhibited by various hormonal factors such as prostaglandin E_1, adenosine or nicotinic acid by a GTP-dependent process [5–7]. Additionally, the adipocyte enzyme can be inhibited by GTP itself and by stable GTP analogs such as guanyl 5′-yl-imidodiphosphate and guanosine 5′-*O*-(3-thiotriphosphate) (GTP-S) [8,9]. Hormonal factors that inhibit adenylate cyclase stimulate a high affinity GTPase in adipocyte membranes, which is not affected by cholera toxin [10,11]. There appeared to be a close connection between adenylate cyclase inhibition and GTPase stimulation. To further elucidate the role of IAP in the inhibitory regulation of adenylate cyclase, the influence of IAP was studied on guanine nucleotide and nicotinic acid-induced rat adipocyte adenylate cyclase inhibition and on nicotinic acid-induced GTPase stimulation. We report here that IAP treatment impairs or abolishes inhibition of adenylate cyclase by GTP and nicotinic acid, whereas the inhibitory effect of GTPγS is not affected by the toxin, and that IAP can prevent the nicotinic acid-induced GTPase stimulation.

2. MATERIALS AND METHODS

2.1. *Materials*

The IAP used was partially purified on hydroxyapatite columns or purified to apparent

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homogeneity by subsequent chromatography on haptoglobin–Sepharose as in [12] from the supernatant of *B. pertussis* suspensions kindly provided by Drs L. Robbel and F. Blackkolb, Behringwerke (Marburg). Forskolin was donated by Dr H. Metzger, Hoechst AG (Frankfurt). Other materials used were as in [7,9].

2.2. *IAP treatment*

Male rats (180–220 g) were treated once with 30–100 μl (i.v.) of partially purified IAP (1 mg/ml). Three days later, the rats were killed by decapitation, and adipocyte ghosts were prepared as in [7]. For pretreatment of adipocytes with IAP, intact rat adipocytes (1 ml of packed cell suspension) were incubated in the medium used for adipocyte isolation [7] for 1–3 h at 30°C with and without 100 μl partially purified IAP. Thereafter, the cells were lysed and adipocyte ghosts were prepared [7]. For pretreatment of rat adipocyte ghosts with IAP, the incubation medium contained in 200 μl final vol. 5 mM $MgCl_2$, 10 mM thymidine, 0.5 mM NAD, 0.5 mM ATP, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase, 2 mg/ml bovine serum albumin, 50 mM triethanolamine–HCl (pH 7.4) and purified IAP at the indicated concentrations. The pretreatment was for 10 min at 37°C. Thereafter, the membranes were pelleted by centrifugation for 4 min at 10000 × *g* and 4°C, once washed with 1 ml of 10 mM triethanolamine–HCl (pH 7.4) and resuspended in 1 mM $KHCO_3$ for GTPase assay.

2.3. *Adenylate cyclase and GTPase assays*

Adenylate cyclase activity was determined as in [9] with 50 μM [α-^{32}P]ATP (~0.4 μCi/tube), 2 mM $MgCl_2$, 0.1 mM cyclic AMP, 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase, 2 mg/ml bovine serum albumin and 50 mM triethanolamine–HCl (pH 7.4) in 100 μl total vol. Reactions were initiated by the addition of adipocyte ghosts (5–30 μg protein/tube) and conducted for 10 min at 25°C. Cyclic AMP formed was isolated as in [13]. GTPase activity was determined as in [10] with essentially the same reaction mixture as used for adenylate cyclase assay, with the exception that 0.5 μM [γ-^{32}P]GTP (~0.1 μCi/tube), 0.1 mM unlabeled ATP, 3 mM adenyl 5′-yl-imidodiphosphate, 0.1 mM EDTA and 100 mM NaCl were present. Incubation with adipocyte ghosts (2–10 μg protein/tube) was for 10 min at 25°C. Specific, low K_m GTPase activity was determined as in [10].

3. RESULTS

In adipocyte ghosts from control rats, GTP inhibited the forskolin (50 μM)-stimulated adenylate cyclase by maximally about 50%, with half-maximal inhibition occurring at about 0.2 μM GTP (fig.1). Similar to that described for hamster adipocytes [9] and cyc$^-$ variants of S49 lymphoma cells [14,15], the rat adipocyte adenylate cyclase stimulated by forskolin was inhibited by the stable GTP analog, GTPγS, much more potently than by GTP. Whereas GTPγS caused the same maximal degree of inhibition as observed with GTP, half-maximal inhibition was obtained with only 5 nM GTPγS. In adipocyte ghosts from IAP-pretreated rats, GTPγS caused a similar adenylate cyclase inhibition as observed in control adipocytes, both with regard to the degree of enzyme inhibition and to the concentration required for half-maximal effect. In contrast, inhibition of the adenylate cyclase by GTP was almost completely abolished in adipocyte ghosts of IAP-pretreated rats. Similar results were obtained when intact rat adipocytes were pretreated with IAP (fig.2). After incubation of intact rat adipocytes for 1–3 h in the absence of

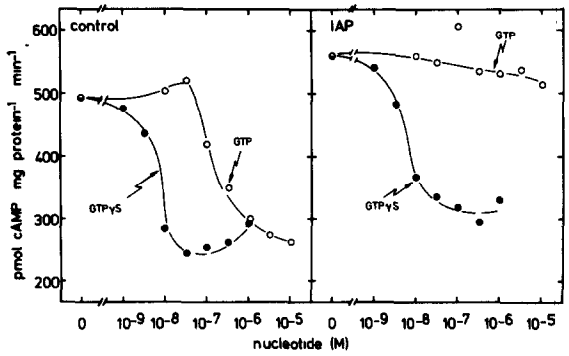

Fig.1. Influence of in vivo treatment of rats with IAP on guanine nucleotide-induced adipocyte adenylate cyclase inhibition. Adipocyte ghosts were prepared from rats pretreated without (control, left panel) and with partially purified IAP (right panel) as described in section 2. Adenylate cyclase activity was determined in the presence of 50 μM forskolin at increasing concentrations of GTP or GTPγS as indicated.

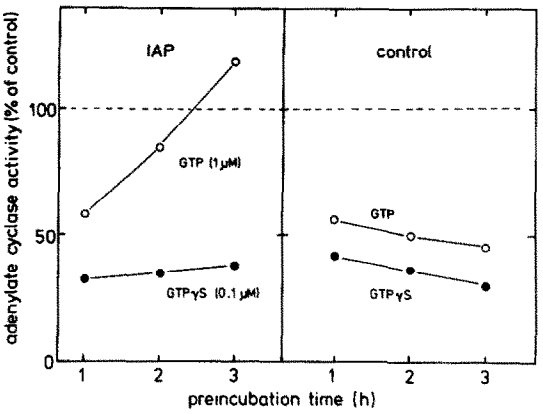

Fig.2. Influence of IAP treatment of intact rat adipocytes on guanine nucleotide-induced adenylate cyclase inhibition. Intact rat adipocytes were pretreated for 1, 2 or 3 h without (control, right panel) and with partially purified IAP (left panel) as in section 2. In adipocyte ghosts prepared thereof, adenylate cyclase activity was determined in the presence of 50 μM forskolin without and with 1 μM GTP or 0.1 μM GTPγS as indicated. Enzyme activity is given as % of control activity measured without GTP or GTPγS.

Table 1

Influence of IAP on rat adipocyte adenylate cyclase regulation by GTP, NaCl and nicotinic acid

Additions	Adenylate cyclase activity (pmol cAMP.min^{-1}.mg protein^{-1})	
	Control	IAP
None	636	466
GTP (3 μM)	220	466
GTP + NaCl (150 mM)	551	477
GTP + NaCl + nicotinic acid (30 μM)	269	477

Adenylate cyclase activity was determined in adipocyte ghosts from rats pretreated with and without IAP as described in section 2. The incubation was for 15 min at 25°C in the presence of 50 μM forskolin with and without the additions indicated

IAP, adenylate cyclase inhibition by GTP (1 μM) and GTPγS (0.1 μM) was even slightly increased. The presence of IAP in the preincubation medium did not affect the GTPγS-induced inhibition of the forskolin (50 μM)-stimulated enzyme. In contrast, the IAP pretreatment reversed the GTP-induced inhibition in a time-dependent manner. After incubation for 2 h, the inhibition was largely reduced, and after 3 h GTP even caused a small increase in enzyme activity.

Pretreatment of rats with IAP did not only impair GTP-induced adenylate cyclase inhibition but also the inhibition of the adipocyte enzyme by nicotinic acid, which has been shown to inhibit the adipocyte adenylate cyclase in a hormone-like manner [7]. In control adipocyte ghosts, GTP (3 μM) decreased the forskolin (50 μM)-stimulated activity, and addition of NaCl (150 mM) largely reversed this GTP-induced inhibition (table 1). In the presence of both GTP and NaCl, nicotinic acid (30 μM) decreased the enzyme activity by ~50%, apparently by counteracting the NaCl-induced increase in activity [7,11]. In adipocyte ghosts from IAP-pretreated rats, however, the inhibitory effect of GTP was lost and NaCl no longer increased adenylate cyclase activity. Accordingly, nicotinic acid, at a concentration maximally effective in control membranes [7], had no inhibitory effect on the adipocyte adenylate cyclase from IAP-pretreated rats.

The data obtained with GTP and GTPγS after IAP treatment of whole animals and of intact rat adipocytes suggested that IAP may alter GTP hydrolysis in adipocyte membranes. Therefore, we studied the influence of IAP, purified to apparent homogeneity [12], on nicotinic acid-induced GTPase stimulation. Rat adipocyte ghosts were pretreated with and without purified IAP for 10 min at 37°C. After a washing step, GTP hydrolysis was determined in these membranes. In the absence of IAP in the pretreatment medium, nicotinic acid (30 μM) increased rat adipocyte high affinity GTPase activity by about 80% (fig.3) as in [10]. With increasing concentrations of IAP in the pretreatment medium, the nicotinic acid-induced GTPase stimulation was decreased, whereas the 'basal', unstimulated GTPase activity was only slightly affected. With 7 μg IAP in the preincubation medium, the stimulatory effect of nicotinic acid was almost abolished. After finishing this study, inhibition of enkephaline-induced GTPase stimulation in neuroblastoma × glioma hybrid

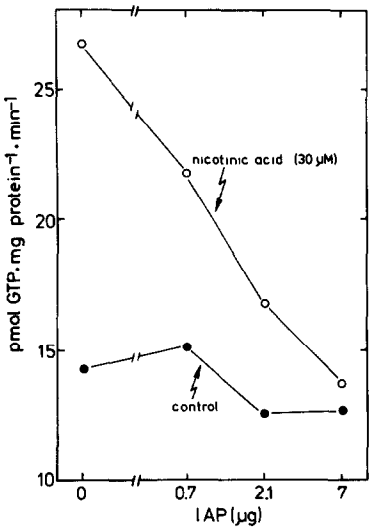

Fig.3. Influence of IAP on nicotinic acid-induced GTPase stimulation. Rat adipocyte ghosts were pretreated without and with purified IAP at the indicated concentrations (μg/tube) as in section 2. After a washing step, GTPase activity was determined in these membranes without (control) and with 30 μM nicotinic acid as indicated. High affinity GTPase activity estimated as in [10] is given on the ordinate.

cells by IAP was reported [16]. In this regard, the data are in accordance with those shown herein for the effect of IAP in rat adipocytes.

4. DISCUSSION

There is increasing evidence that inhibition of adenylate cyclase by hormones and neurotransmitters is mediated by a guanine nucleotide-binding, regulatory component (N_i) that is distinct from that (N_s) mediating adenylate cyclase stimulation by hormones and stable GTP analogs [17], N_i apparently also mediates adenylate cyclase inhibition by stable GTP analogs [9,18]. This assumption has been corroborated by findings in cyc$^-$ variants of S49 lymphoma cells. In these N_s-deficient cells, the adenylate cyclase can be inhibited by stable GTP analogs [14,15]. In addition, the peptide hormone, somatostatin, inhibits the cyc$^-$ adenylate cyclase in a GTP-dependent manner [19]. Similar to N_s-mediated adenylate cyclase stimulation, N_i-mediated adenylate cyclase inhibition induced by hormones is accompanied by an increased GTP hydrolysis as observed in membranes of human platelets [20], neuroblastoma × glioma hybrid cells [21], hamster and rat adipocytes [10,11] and S49 lymphoma cells [22].

These data suggest that the *B. pertussis* toxin, IAP, prevents N_i activation by GTP and hormonal factors, whereas N_i activation by the stable GTP analog, GTPγS, is not impaired. Concomitantly, GTP and nicotinic acid-induced adenylate cyclase inhibition and nicotinic acid-induced GTPase stimulation are abolished, whereas GTPγS still inhibits the adipocyte adenylate cyclase. Some data presented may also suggest that GTP hydrolysis is required for N_i-mediated adenylate cyclase inhibition and IAP prevents GTP-dependent inhibition of adenylate cyclase by inhibition of N_i-associated GTPase stimulation. However, the data reported with stable GTP analogs in various cellular systems [8,9,14,15,18] and those shown herein with GTPγS in rat adipocytes are in apparent contradiction to this assumption. It may be speculated that stable GTP analogs not only act in a manner similar to GTP but that these analogs may have additional unknown effects on the adenylate cyclase system; e.g., causing conformational changes of the guanine nucleotide-binding coupling component(s).

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