GLUCAGON-STIMULATED GTP HYDROLYSIS IN RAT LIVER PLASMA MEMBRANES

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1. Introduction

Guanine nucleotide plays an important role in the hormonal regulation of adenylate cyclase from various tissues [1,2]. Guanine nucleotide, which is absolutely essential for the enzyme to be stimulated by hormones [3], is now considered a primary regulator of the adenylate cyclase; GDP acts as an inhibitor and GTP as a stimulator of the enzyme by interacting with a specific regulatory site, a GTP binding protein [4]. Thus it is assumed that hormones may exert their effects through making a GTP binding protein more accessible to GTP in a manner which is still unknown.

A catecholamine stimulated GTPase in turkey erythrocyte membranes was reported [5]. They postulated that hormones activated the adenylate cyclase by displacing GDP by GTP at the regulatory site [6] at which active GTP was readily hydrolyzed to inactive GDP by a specific GTPase, a turn-off mechanism. Furthermore, they suggested that cholera toxin activated the adenylate cyclase by inhibiting GTP hydrolysis at the regulatory site [7]. However, in systems other than avian erythrocyte there have been only two reports [8,9] on the hormone stimulated GTPase. These studies may be hampered by the presence of extremely high non-specific nucleoside triphosphatase activity and high basal GTPase activity in the other membrane preparations. It is still unclear whether the existence of a hormone stimulated GTPase is a common feature in hormone stimulated adenylate cyclase systems. The difference in the properties of the turkey and other adenylate cyclase systems is described in [10] and the question is posed as to whether a GTPase turn-off mechanism can adequately explain the dynamic properties of all adenylate cyclase systems.

Here we demonstrate the presence of glucagon stimulated GTPase activity in purified rat liver plasma membranes and discuss the possible role of GTPase in terms of a turn-off mechanism in the well known adenylate cyclase system.

2. Materials and methods

2.1. *Materials*

[γ-^{32}P]GTP (34 Ci/mmol) was purchased from New England Nuclear. PGE_1 was generously donated by Ono Pharmaceutical Co. Other chemicals used were described in [11].

2.2. *Preparation of liver plasma membranes*

Purified rat liver plasma membranes were obtained as in [3].

2.3. *GTPase assay*

GTPase activity was determined by following the release of $^{32}P_i$ from [γ-^{32}P]GTP as in [5] with some modifications. The reaction mixture contained, in 100 μl final vol., 0.35 μM [γ-^{32}P]GTP, 5 mM $MgCl_2$, 1 mM ATP, 10 mM theophylline, 1 mg/ml bovine serum albumin, 50 mM Tris–HCl (pH 7.4) and appropriate amounts of liver plasma membranes. The reaction was started by the addition of the membranes, carried out for 5 min at 37°C and terminated by adding 0.5 ml of 5% acid-activated charcoal (Norit-A) suspension in an ice-cold 4 mM NaH_2PO_4. After the tubes were centrifuged for 5 min at 3000 rev./min the radioactivity was determined from aliquots of the supernatant. To determine the specific low K_m GTPase activity, the activity due to high K_m GTPase was calculated by including 50 μM GTP in incubation medium and was subtracted from the total activity obtained at low substrate concentration.

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2.4. *Adenylate cyclase assay*

Adenylate cyclase activity was determined as in [11]. Protein contents were determined as in [12] using bovine serum albumin as a standard.

3. Results and discussion

Fig.1 shows double-reciprocal plots of GTP hydrolysis by hepatic membranes tested over 19 nM–2 mM GTP in the presence of 1 mM ATP. The presence of two different enzymes is clearly indicated by the obvious break in this plot. The corrected K_m value [13] of the low K_m enzyme estimated by extrapolation of the linear portion of the plot was 0.2–0.5 μM and the approximate K_m value of the high K_m enzyme was 0.15–0.35 mM. ATP appeared to behave as a non-competitive inhibitor for the low K_m enzyme with K_i 0.09 mM and as a competitive inhibitor for the high K_m enzyme with a K_i 0.06 mM.

GTP hydrolysis was time- and membrane protein-dependent (not shown) and was stimulated by the presence of 1 μM glucagon by 30–100% above the basal value without a lag phase (fig.2). The stimulation of P_i release from GTP by glucagon was only apparent at low substrate concentrations (20 nM–2 μM) and could not be detected at higher concentrations (0.01–2 mM). The stimulation of the hydrolysis of GTP was maximal at 1–10 μM of glucagon being half-maximal at 50 nM (fig.3). This dose–response relationship is similar to that obtained for the stimulation of adenylate cyclase by the hormone [3] although

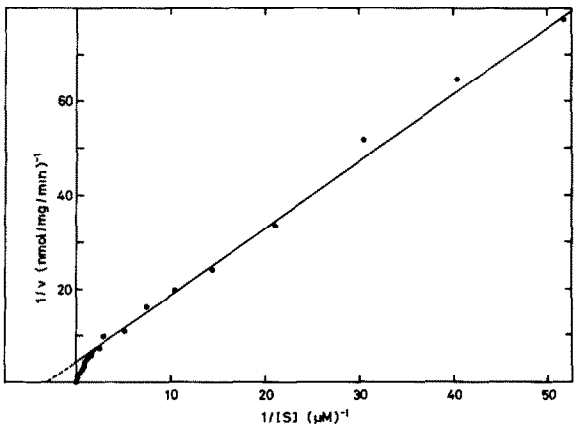

Fig.1. Lineweaver-Burk plots of GTP hydrolysis by purified rat liver plasma membranes in the presence of 1 mM ATP. Assay methods were as given in the text.

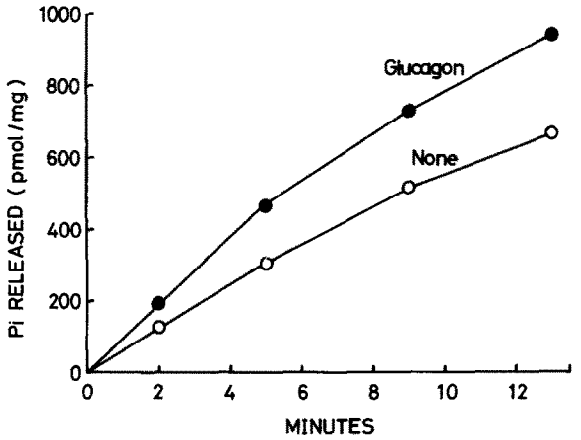

Fig.2. Effects of glucagon on GTP hydrolysis as a function of time. Specific low K_m GTPase activities were calculated as in section 2. Glucagon was 1 μM.

the stimulatory effect of glucagon on low K_m GTPase was much smaller than that on adenylate cyclase.

Among the reagents which are known to affect the adenylate cyclase activity, only glucagon stimulated the low K_m GTPase activity and isoproterenol, PGE_1 and NaF had no effect (table 1). Gpp(NH)p markedly reduced the low K_m enzyme activity, presumably in a competitive manner with respect to the substrate. On the other hand, glucagon had no effect on the high K_m GTPase activity, while PGE_1 and NaF significantly inhibited its activity. The absence of the effects of isoproterenol and PGE_1 on the low K_m GTPase activity may be related to their small stimu-

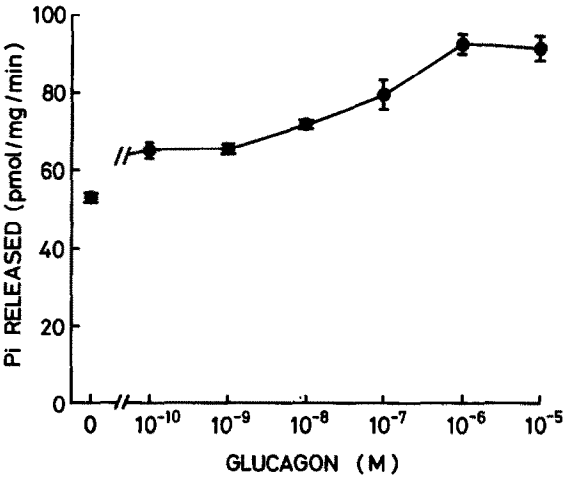

Fig.3. Effects of the concentration of glucagon on GTP hydrolysis by hepatic membranes. Specific low K_m GTPase activities were calculated as in section 2.

Table 1
Effects of various reagents on GTP hydrolysis and adenylate cyclase activity in rat hepatic membranes

	GTPase act. (nmol . mg^{-1} . min^{-1})		Adenylate cyclase act. (pmol . mg^{-1} . min^{-1})		
	Low K_m (0.36 μM)	High K_m (50 μM)	0 μM GTP	0.35 μM GTP	50 μM GTP
None	0.081 ± 0.005	9.2 ± 1.0	61 ± 9	59 ± 12	93 ± 7
Glucagon (1 μM)	0.114 ± 0.007	8.7 ± 0.8	90 ± 1	591 ± 15	871 ± 54
Isoproterenol (0.2 mM)	0.086 ± 0.006	8.8 ± 0.7	56 ± 8	79 ± 6	95 ± 7
PGE_1 (10 μg/ml)	0.086 ± 0.003	7.4 ± 0.4	64 ± 13	89 ± 13	361 ± 30
NaF (10 mM)	0.081 ± 0.000	4.9 ± 0.4	1701 ± 92	1335 ± 205	1309 ± 39
Gpp(NH)p (10 μM)	0.018 ± 0.002	8.4 ± 0.7	196 ± 6	202 ± 11	101 ± 14

Specific low K_m GTPase activities were calculated as in section 2. Values are means ± SE (n = 3)

latory effect on the adenylate cyclase activity.

According to a GTPase turn-off mechanism, GDP acts as an inhibitor of the adenylate cyclase and it binds tightly to the regulatory site [5–7]. However, some doubts have been cast on the role of the GTPase in hormone-sensitive adenylate cyclase systems because GDP has been shown to stimulate adenylate cyclase from several tissues in the presence of hormones [10,14,15]. To clarify this problem, we explored the mechanism of glucagon stimulation of adenylate cyclase in the presence of GDP [11] and showed that the stimulatory effect of the hormone observed in the presence of GDP was absolutely dependent on the formation of GTP from GDP by transphosphorylation. Results showing stimulatory effects of GDP in the presence of hormone were well explained by the fact that the assay mixture contained a nucleoside diphosphokinase as well as small amounts of impurities which donated a phosphoryl group to GDP to form GTP [11]. These observations show that GDP acts only as an inhibitor and favour the possible role of GTPase in the turn-off mechanism in adenylate cyclase systems. Our observation that glucagon stimulated the low K_m GTPase in hepatic plasma membranes has further confirmed this hypothesis.

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