# INHIBITION OF HAMSTER FAT CELL ADENYLATE CYCLASE BY PROSTAGLANDIN $E_1$ AND EPINEPHRINE: REQUIREMENT FOR GTP AND SODIUM IONS

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## 1. Introduction

Various hormonal factors such as catecholamines by their β-adrenergic component, ACTH and glucagon increase the rate of lipolysis by increasing adenylate cyclase (EC 4.6.1.1) activity and cyclic AMP levels [1]. On the other hand, catecholamines by their α-adrenergic component and prostaglandin $E_1$ ($PGE_1$) have been shown to lower stimulated cyclic AMP levels and to decrease the lipolysis in hamster fat cells [2,3]. Except for a preliminary communication [4], inhibition of fat cell adenylate cyclase by catecholamines or $PGE_1$ has not been demonstrated in cell-free preparations.

The essential role of guanyl nucleotides in hormonal stimulation of adenylate cyclase is well established (reviewed [5]). On the other hand, a requirement for GTP has been shown for the inhibitory effect of α-adrenergic agonists on platelet adenylate cyclase [6] and subsequently also for adenylate cyclase inhibition by adenosine in rat fat cells [7], by muscarinic cholinergic agonists in dog and rabbit myocardium [8,9] and by α-adrenergic agonists and opiates in neuroblastoma × glioma hybrid cells [10]. Guanyl nucleotides have been shown to decrease the apparent affinity of hormone receptors to various agonists that stimulate adenylate cyclase [5,11]. Similar effects have been described for opiate [12] and α-adrenergic [13] receptors, which appear to be involved in hormone-induced adenylate cyclase inhibition. In studies on these receptors, it has been found that besides guanyl nucleotides monovalent cations can modulate the receptor affinity with similar but not identical patterns [12–15].

In hamster fat cells, where α-adrenergic receptors have been demonstrated [16], we have studied the influence of the α-adrenergic component of epinephrine and of $PGE_1$ on adenylate cyclase and report here that GTP and $Na^+$ are required for the inhibition of the enzyme by these hormonal factors.

## 2. Materials and methods

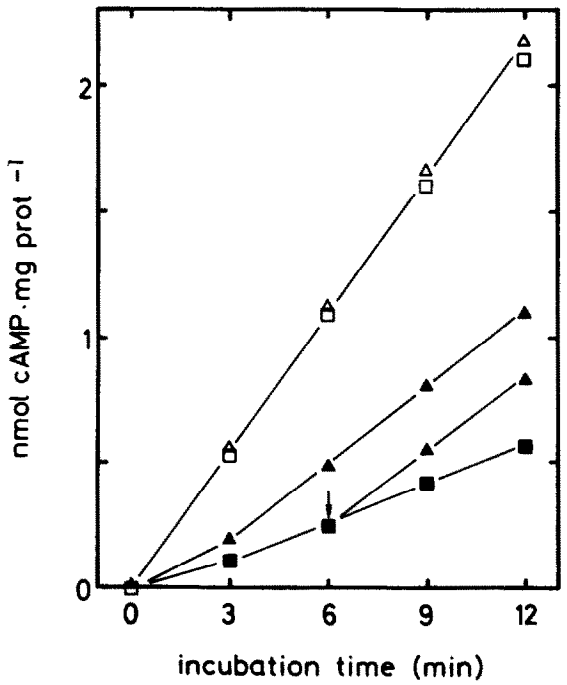
These were essentially as in [17]. Male golden hamsters (60–100 g) were fed ad libitum. After decapitation, epididymal fat pads were removed, and isolated fat cells were prepared following the methods in [18]. Usually, 2 g adipose tissue were incubated for 5–10 min at 37°C in 3 ml 130 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11 mM glucose, 23.8 mM Hepes (pH 7.4) containing 50 mg/ml bovine serum albumin and 1.67 mg/ml collagenase. The isolated, washed cells were lysed in a medium containing 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, 1 mM ATP, 2 mM tris(hydroxymethyl)-aminomethane (pH 7.6) and the resulting fat cell ghosts were finally suspended in 1 mM $KHCO_3$ and frozen in liquid nitrogen, by the method in [19].

Adenylate cyclase activity was determined as in [17], with usually 0.02 mM [α-$^{32}$P]ATP, 10 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM cAMP, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase and 0.2% bovine serum albumin in 50 mM triethanolamine–HCl buffer (pH 7.4) at 25°C in 0.1 ml total vol. Reactions were initiated by the addition of hamster fat cell ghosts (5–20 μg protein) and conducted for 10 min or as indicated. Cyclic AMP formed

was isolated as in [17]. For sodium-free conditions, creatine phosphate was used as its ammonium salt, which was prepared by ion-exchange chromatography on QAE-Sephadex A-25, formate form, and elution by ammonium formate. Comparable results to those shown in the figures and table were obtained in ⩾2 separate experiments in each case. Standard deviations of triplicate determinations were <5% of the means. Protein was determined according to [20], using bovine serum albumin as standard.

## 3. Results

In rat fat cells, GTP exerts an inhibitory effect on basal adenylate cyclase activity, with pronounced inhibition at <37°C [21]. When adenylate cyclase activity was determined in hamster fat cell ghosts at 25°C, GTP caused a marked reduction of the activity, which was most pronounced during the first 3 min incubation (fig. 1). This effect of GTP was half-maximal at ~0.1 μM and a maximal reduction by ~80% occurred at 10–30 μM GTP (not shown). NaCl, when added at 120 mM, had no effect on cyclic AMP formation in the absence of GTP, but increased the GTP-



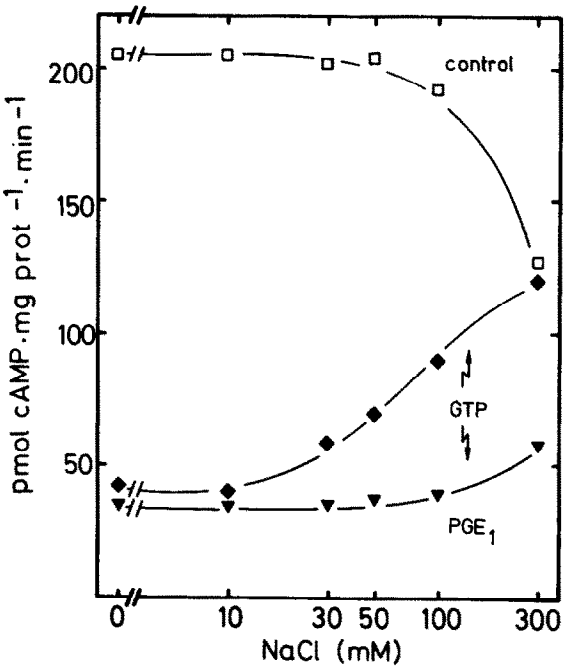




Fig. 2. Influence of NaCl on GTP- and GTP plus $PGE_1$-induced adenylate cyclase inhibition. The effect of NaCl on adenylate cyclase activity was studied in the absence (open squares) and presence of GTP (10 μM; filled symbols), which was added without (squares) and with $PGE_1$ (0.1 μM; triangles). Creatine phosphate was used as its ammonium salt.

reduced activity by ~2-fold. The effect of NaCl appeared to be immediate and was constant over ⩾12 min incubation. The activation of the GTP-inhibited form of the enzyme by NaCl was half-maximal at 50–100 mM NaCl and maximal at ~300 mM (fig. 2). NaCl, at higher concentrations, reduced the activity as it did in the absence of GTP.

$PGE_1$ (0.01–10 μM) had no effect on adenylate cyclase activity in the absence of GTP. In the presence of GTP (10 μM), $PGE_1$ caused only slight inhibition, when added in the absence of NaCl, but a strong

Fig. 1. Effects of GTP and NaCl on adenylate cyclase activity. In the absence (open symbols) and presence of GTP (30 μM; filled symbols), cyclic AMP accumulation was studied without (squares) and with NaCl (120 mM; triangles), which was added at zero time point and at 6 min as indicated by the arrow.

inhibitory effect of $PGE_1$ (0.1 μM) revealed with increasing concentrations of NaCl (see fig. 2). The activation of the GTP-inhibited enzyme by NaCl was apparently counteracted by $PGE_1$. The $PGE_1$-induced inhibition was half-maximal at ~10–30 mM NaCl, and maximal inhibition by 50–60% occurred at 100 mM NaCl. Similar findings were obtained with epinephrine, which inhibited the enzyme by its α-adrenergic component.

Figure 3 shows the requirement for GTP besides that for NaCl for the inhibition of hamster fat cell adenylate cyclase by $PGE_1$ and the α-adrenergic component of epinephrine, which was added in combina-

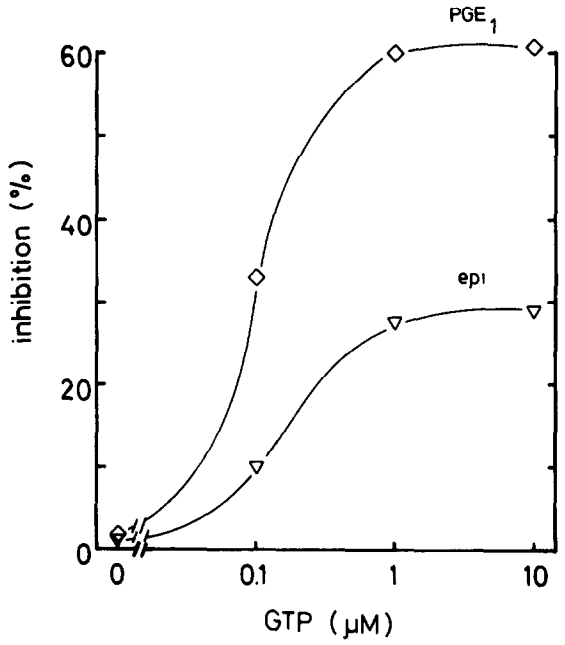



Fig. 3. Influence of GTP on $PGE_1$- and epinephrine-induced adenylate cyclase inhibition. In the presence of NaCl (120 mM), the influence of GTP was studied on adenylate cyclase activity without and with $PGE_1$ (0.1 μM) or l-epinephrine (300 μM). Inhibitions (%) caused by $PGE_1$ (squares) or epinephrine (triangles) are given at the indicated concentrations of GTP.

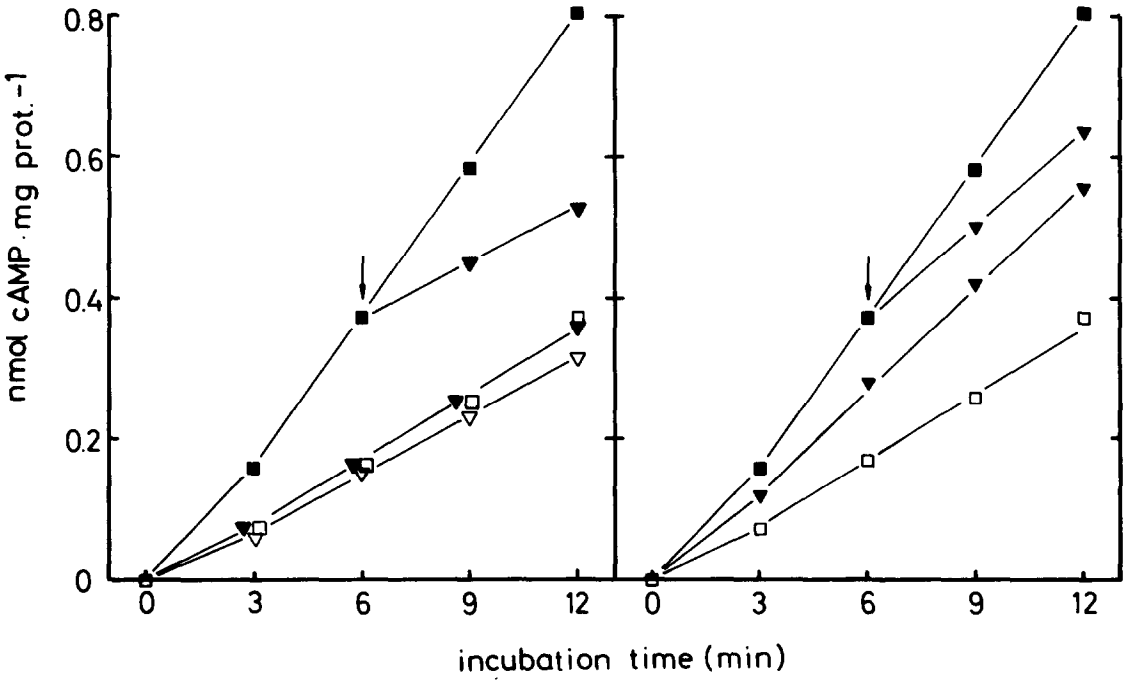



Fig. 4. Time-course of $PGE_1$- and epinephrine-induced adenylate cyclase inhibition. In the presence of GTP (10 μM), the effects of $PGE_1$ (0.1 μM; left panel) and l-epinephrine (100 μM; right panel) were studied on cyclic AMP accumulation without (open symbols) and with NaCl (120 mM; closed symbols). Squares represent control values and triangles the presence of $PGE_1$ or epinephrine, which were added at zero time point and at 6 min as indicated by the arrows.

tion with the β-adrenergic blocking agent, propranolol (30 μM). Inhibition by either agonist was half-maximal at ~0.1 μM GTP, and maximal inhibition by $PGE_1$ and epinephrine by 60% and 30%, respectively, occurred at 1 μM GTP. At higher concentrations (≤1 mM), GTP did not reverse the hormone-induced adenylate cyclase inhibition. The inhibitory effects of $PGE_1$ and epinephrine observed in the presence of GTP and NaCl were apparently immediate, independently whether the agents were added with the enzyme or 6 min after having started the assay reaction (fig. 4). The effects were also independent of the ATP concentration used (≤0.2 mM; not shown). The enzyme inhibition by epinephrine was completely reversed by the α-adrenergic blocking agent, phentolamine (30 μM), which finding supports the assumption that α-adrenergic receptors are involved in this catecholamine effect.

To determine the specificity of NaCl and to find out whether the effects of NaCl were primarily due to the cation or the associated anion or were due to the increased osmolarity of the assay medium, we have evaluated the influences of a variety of monovalent cations as their respective chloride salts and have compared the effects of $Na_2SO_4$, NaCl and sucrose on adenylate cyclase activity in the absence and presence of $PGE_1$ and epinephrine (table 1). $Na_2SO_4$ and LiCl, similar to NaCl, increased enzyme activity in the presence of GTP by ~2-fold. However, only with NaCl and $Na_2SO_4$ epinephrine and $PGE_1$ caused maximal inhibition of adenylate cyclase. KCl, CsCl and RbCl increased enzyme activity less effectively than NaCl in the presence of GTP, and these salts like LiCl did not allow maximal hormonal inhibition. Sucrose (200 mM) was completely ineffective. These data suggest that $Na^+$ besides GTP may be specifically required for the hormonal inhibitions of adenylate cyclase.

Table 1
Influence of various salts of monovalent cations on adenylate cyclase inhibition

| Addition | | Activation (-fold) | Inhibition (%) by | |
|---|---|---|---|---|
| | | | Epinephrine | $PGE_1$ |
| None | (100 mM) | 1.0 | 0 | 13 |
| NaCl | (100 mM) | 2.2 | 21 | 47 |
| KCl | (100 mM) | 1.5 | 3 | 25 |
| CsCl | (100 mM) | 1.3 | 0 | 23 |
| RbCl | (100 mM) | 1.5 | 9 | 28 |
| LiCl | (100 mM) | 2.4 | 7 | 26 |
| $Na_2SO_4$ | ( 50 mM) | 1.9 | 28 | 62 |
| Sucrose | (200 mM) | 1.0 | 0 | 18 |

In the presence of GTP (10 μM), the effects of the additions shown in column 1 were studied on adenylate cyclase activity without and with l-epinephrine (100 μM) or $PGE_1$ (0.1 μM). Column 2 shows activation (-fold) of the enzyme relative to control activity, which was 21.2 pmol cAMP . mg $protein^{-1}$ . $min^{-1}$. Columns 3 and 4 indicate inhibitions (in % of the respective control activities) observed with the additions of epinephrine and $PGE_1$, respectively. Creatine phosphate was used as its ammonium salt

## 4. Discussion

Guanyl nucleotides and $Na^+$ independently of each other can reduce binding of agonists to α-adrenergic receptors [13,15], very similar as described for opiate receptors [12,14]. In rabbit platelets, a correlation between the NaCl-induced reduction of agonist-binding to α-adrenergic receptors and their inhibitory potency on adenylate cyclase has been demonstrated [15]. Similarly to the general guanyl nucleotide requirement for hormonal stimulation of adenylate cyclase by hormones [5], GTP has recently been shown in various tissues to be absolutely necessary for the inhibition of adenylate cyclase by α-adrenergic [6,10] and cholinergic agonists [8,9], by opiates [10] and by adenosine [7].

The present data indicate that besides GTP, $Na^+$ are required for the inhibition of hamster fat cell adenylate cyclase by $PGE_1$ and the α-adrenergic component of epinephrine. Similar GTP and $Na^+$ requirements have been found for α-adrenergic inhibition of liver adenylate cyclase (K.H.J., R. A. Johnson, unpublished observations) and for the inhibition of the fat cell and neuroblastoma × glioma hybrid cell enzyme by nicotinic acid (K.A., unpublished observations) and opiates [22], respectively.

The data so far available suggest that $Na^+$ besides their effects on the hormone binding may also be generally involved in the transduction of the inhibitory signal to the adenylate cyclase. Further studies on different systems and with isolated components of the adenylate cyclase system are required to elucidate the exact nature of actions of $Na^+$ and

guanyl nucleotides in the hormone-induced adenylate cyclase inhibition.

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