INHIBITION OF PLATELET ADENYLATE CYCLASE BY EPINEPHRINE REQUIRES GTP

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1. Introduction

Epinephrine and norepinephrine cause aggregation of human platelets. This effect is blocked by α-adrenergic blocking agents such as phentolamine and dihydroergotamine and, therefore, is assumed to involve α-adrenergic receptors [1–3]. Epinephrine is capable of lowering prostaglandin E_1-stimulated cyclic AMP levels, an effect that involves α-adrenergic receptors [4–8].

We have recently demonstrated an inhibitory effect of epinephrine and norepinephrine on adenylate cyclase (EC 4.6.1.1) in cell-free preparations of human platelets [9]. This effect was enhanced by β-adrenergic blocking agents such as propranolol and pindolol and was reversed by α-adrenergic blocking agents such as phentolamine and dihydroergotamine. Inhibition by α-adrenergic agonists was seen with the unstimulated, 'basal' form of the enzyme and with the enzyme activated by sodium fluoride, prostaglandins [9] and adenosine (K. H. J., W. S. and R. A. Johnson, unpublished observations) but not after stimulation by guanylylimidodiphosphate. The above studies were performed in whole lysates of platelets. We report here that GTP as a constituent of the cytosol is required for epinephrine-induced inhibition of membrane-bound adenylate cyclase.

2. Materials and methods

Materials and methods used were essentially as described [9]. Isolated human platelets were lysed by rapid freezing in liquid nitrogen and subsequent thawing in about 50 vol. 10 mM triethanolamine–HCl buffer, pH 7.4, containing 150 mM NaCl. Platelet particles were obtained by 20 min centrifugation of the lysates at 30 000 × *g* and 0°C and were resuspended in the above buffer. Adenylate cyclase activity was determined as described [9] with 0.1 mM [α-^{32}P]ATP (purified, essentially free of GTP [10], 0.3–0.8 μCi/tube), 5 mM $MgCl_2$, 0.1 mM ethyleneglycol-bis(β-aminoethylether) *N,N'* tetraacetic acid, 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate and 0.4 mg/ml creatine kinase in 50 mM triethanolamine–HCl buffer, pH 7.4, at 37°C in total vol. 0.1 ml. Reactions were initiated by the addition of platelet particles (20–100 μg protein) and conducted for 10 min or as indicated. Cyclic AMP formed was purified as described [9]. Under these conditions cyclic AMP formation was linear as a function of time for at least 20 min and as function of platelet protein. The β-adrenergic blocking agent, pindolol (10 μM), was present under each condition. When L-epinephrine was added, its concentration was 30 μM. Comparable results to those shown in the figures were obtained in at least two separate experiments in each case. Standard deviations of triplicate determinations were less than 5% of the means. Protein was determined according to [11] using bovine serum albumin as standard.

3. Results

Epineprhine by its α-adrenergic component reduced adenylate cyclase activity by maximally 50–60% when added to lysates of human platelets [9]. The epinephrine effect was largely reduced (0–10% inhibition) in particulate preparations. Re-addition of supernatant fluid restored the inhibitory effect of epinephrine. Treatment of the supernatant fluid with heat,

charcoal or alkaline phosphatase reduced or abolished its capability of restoring epinephrine-induced inhibition (data not shown).

Therefore, we considered the possibility that a nucleotide other than ATP was a constituent of the supernatant fluid that was required for the epinephrine effect. Of various nucleoside triphosphates tested, GTP was most effective in restoring the effect of epinephrine on adenylate cyclase in particulate preparations (fig.1). Maximal inhibition (about 45%) was seen with 10^{-5}–10^{-4} M GTP; 10^{-6} M GTP was half-maximally effective. Deoxy-GTP was slightly less effective, and ITP was about one-tenth as potent as GTP. UTP, CTP and XTP were far less effective. With GDP added at 10^{-5} and 10^{-4} M in the absence of a nucleoside triphosphate-regenerating system, epinephrine-induced inhibition was only 10% and 20%, respectively. Adenylate cyclase activity measured in the absence of epinephrine was not significantly changed by these nucleotides except for a small increase observed with 10^{-7}–10^{-4} M GTP (fig.2). Similarly, prostaglandin E_1-stimulated activity, which was about 10-fold above basal activity with 1 μM prostaglandin E_1, was slightly increased by GTP. The effect of GTP to support epinephrine-induced inhibition of prostaglandin E_1-stimulated adenylate cyclase was very similar to that seen with the unstimulated enzyme (fig.2). Both prostaglandin E_1-stimulated and basal activities were inhibited by epinephrine in the presence of GTP; maximal inhibition was seen with about 10^{-5} M GTP and was 50% for the basal and 30% for the prostaglandin E_1-stimulated activity, very similar to the inhibition observed in platelet lysates [9]. Half-maximal inhibition was observed at the same GTP concentration

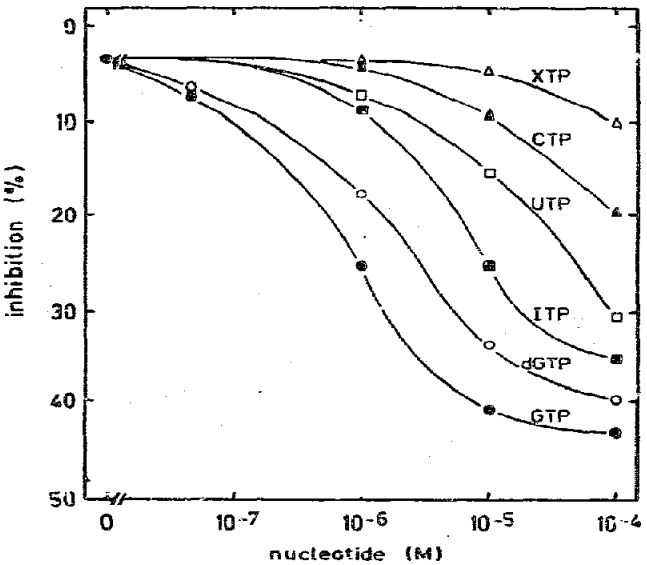

Fig.1. Influences of various nucleoside triphosphates on the inhibitory effect of epinephrine on adenylate cyclase. Nucleoside triphosphates were added at the concentrations indicated to a particulate preparation of human platelets in the absence and presence of 30 μM epinephrine. Adenylate cyclase activity measured with epinephrine relative to the corresponding control activity is indicated on the ordinate. Activity determined in the absence of epinephrine was not changed by the nucleotides except for a slight increase caused by GTP.

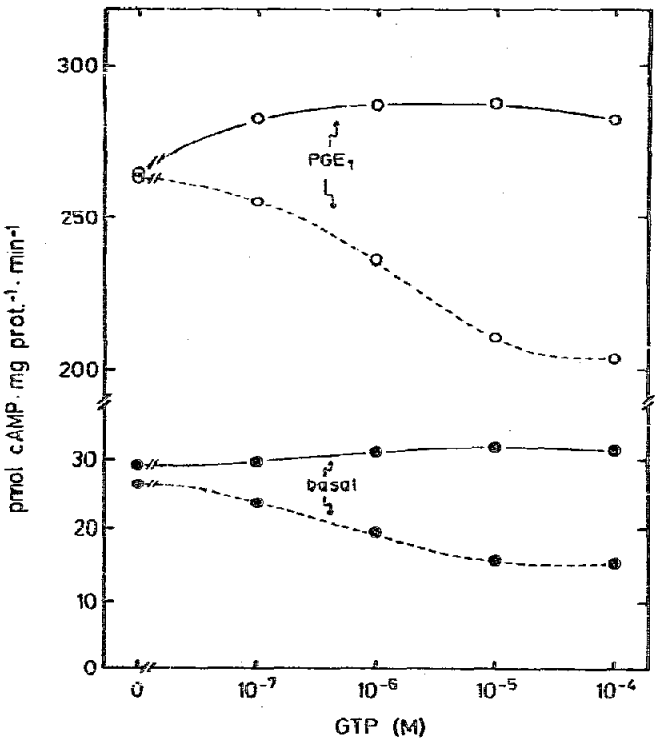

Fig.2. Effect of GTP on the inhibition of basal and prostaglandin E_1 (PGE_1)-stimulated adenylate cyclase by epinephrine. GTP was added at concentrations indicated on the abscissa to a particulate preparation from human platelets. Basal (•) and PGE_1 (1 μM)-stimulated (○) adenylate cyclase activities determined without (——) or with (– – –) 30 μM epinephrine added are indicated on the ordinate.

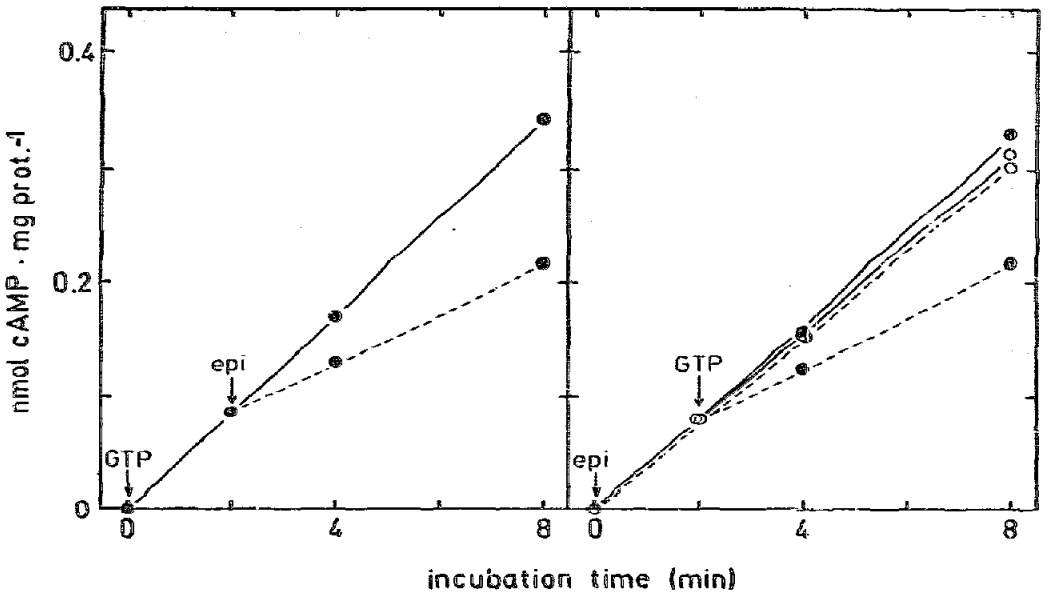

Fig.3. Time course of the effect of GTP on epinephrine-induced inhibition of cyclic AMP accumulation in platelet particles. Left panel: After 2 min incubation with GTP (10 μM), epinephrine (epi, 30 μM) was added, and the reaction was continued for 6 min. Right panel: After 2 min incubation with epinephrine, GTP was added. (——) Indicates the absence of epinephrine, (– – –) indicates the presence of epinephrine; (○) indicates the absence, (●) the presence of GTP.

(10^{-6} M) with the basal and the stimulated enzyme.

We have recently shown that the inhibitory effect of epinephrine in platelet lysates is immediate, with no apparent lag phase [9]. Similar observations were made with the effect of GTP in the presence of epinephrine. When epinephrine was added to a particulate preparation that had been preincubated for 2 min with 10^{-5} M GTP, epinephrine caused an apparently immediate reduction of the enzyme activity (fig.3). Similarly, GTP induced immediate inhibition of adenylate cyclase when added to particles that had been preincubated with epinephrine for 2 min. The rate of cyclic AMP accumulation was constant for at least 8 min under each condition.

4. Discussion

The data presented indicate that GTP is one, and possibly the most important, factor of the cytosol involved in the inhibitory effect of α-adrenergic agonists on platelet adenylate cyclase. It cannot be ruled out, however, that factors other than GTP are involved. Guanyl nucleotides have been shown to play a central role in the regulation of adenylate cyclase activity [12–14]. In several systems, GTP is required for stimulatory hormone effects on adenylate cyclase, whereas in other cell types including renal medulla [15] and human platelets (K. H. J. and W. S., unpublished observations) GTP does apparently not facilitate responses of adenylate cyclase to hormone stimulation. Inhibitory effects of GTP on basal activity have been demonstrated in ghosts of rat fat cells [16]. In human platelet lysates, guanylylimidodiphosphate caused a concentration-dependent initial inhibition by 50% or more of adenylate cyclase before stimulation became apparent with prolonged incubation [17]. These data indicate that guanyl nucleotides can cause inhibition of adenylate cyclase under some conditions. Therefore, it is conceivable that α-adrenergic agonists make an inhibitory effect of GTP more pronounced, possibly by favoring the formation of a form of the enzyme that is more susceptible to an inhibitory effect of GTP.

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