EPINEPHRINE INHIBITS ADENYLATE CYCLASE AND STIMULATES A GTPase IN HUMAN PLATELET MEMBRANES VIA α-ADRENOCEPTORS

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1. Introduction

Studies on the β-adrenergic agonist-stimulated turkey erythrocyte adenylate cyclase indicated that a GTPase activity associated with the guanine nucleotide-binding regulatory subunit (N) leads to the inactivation of the hormone-stimulated adenylate cyclase by hydrolysis of N-bound GTP to GDP and P_i [1,2]. This model was validated by the finding that treatment of the membranes with cholera toxin, which almost abolishes the difference between GTP and its poorly hydrolyzable analogs in adenylate cyclase activation, causes a concomitant inhibition of the hormone-stimulated GTPase activity [3]. In various tissues and with various hormonal factors that inhibit adenylate cyclase, it has been shown that the negative hormonal control of adenylate cyclase activity in cell-free systems requires the presence of GTP [4,5], as is known for hormonal stimulation of the enzyme [6]. However, in contrast to hormonal stimulation, which is usually potentiated by stable GTP analogs [6], these analogs prevented or reversed hormonal inhibition [4,5,7,8]. Therefore, it has been suggested [4,7] that inhibitory hormones may decrease adenylate cyclase activity by accelerating the off-rate of the active adenylate cyclase via an increase in GTPase activity. In membrane preparations of human platelets, whose adenylate cyclase is inhibited by α-adrenergic agonists in a GTP-dependent manner [9], we studied the influence of epinephrine on GTPase activity and we report here that epinephrine inhibits adenylate cyclase and stimulates a low K_m GTPase via α-adrenoceptors in this system.

2. Materials and methods

Materials used were essentially as in [9]. [α-^{32}P]-ATP and [γ-^{32}P]GTP were prepared according to [10]. Lysates of human platelets, obtained by rapid freezing and prior to assay thawing in 10 mM triethanolamine–HCl, pH 7.4, containing 5 mM EDTA, were centrifuged for 20 min at 30 000 × *g* at 4°C. The pellet was washed twice in the same medium and finally suspended in 10 mM triethanolamine–HCl, pH 7.4, without EDTA. Adenylate cyclase activity was determined with 0.1 mM [α-^{32}P]ATP (essentially GTP-free; 0.3–0.6 μCi per tube), 2 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM cyclic AMP, 5 mM creatine phosphate, 1.2 mg ml^{-1} creatine kinase, 0.2% bovine serum albumin, 1 mM dithiothreitol, 0.1 mM EDTA and 50 mM triethanolamine–HCl, pH 7.4, in a total volume of 0.1 ml. GTP (0.3 μM) and *d,l*-propranolol (30 μM) were included under each condition. Reactions were initiated by the addition of platelet particles (40–70 μg protein) and allowed to proceed for 10 min at 25°C. The cyclic AMP formed was purified as in [11]. GTPase activity was determined according to [1], with the same reaction mixture as described above for the determination of adenylate cyclase activity, with the exception that unlabeled ATP (0.1 mM) and [γ-^{32}P]GTP (0.08–0.2 μCi per tube; 0.3 μM or at the indicated concentration) were present. Reactions were initiated by the addition of platelet particles (4–10 μg protein), and continued for 10 min or as indicated at 25°C, and terminated by the addition of 0.5 ml ice-cold sodium phosphate buffer (20 mM), pH 7.0, containing 5% (w/v) activated charcoal. The reaction tubes were centrifuged for 10 min at 10 000 × *g* at 4°C, and 0.4 ml of the supernatant was transferred into scintillation vials. Radioactivity was counted using Cerenkov radiation. Release of $^{32}P_i$ from [γ-^{32}P]GTP in the absence of membranes was 0.5–3% of added [γ-^{32}P]-GTP. All reactions were performed in triplicate with

an intraassay variation of less than 5%. Results similar to those shown here were obtained in repeated experiments.

3. Results

Hydrolysis of [γ-^{32}P]GTP was potently reduced by low concentrations of unlabeled GTP, as shown by the isotope dilution curve (fig.1, left). This finding indicated that a low K_m GTPase was mainly responsible for GTP hydrolysis in human platelet membrane preparations. However, the curvilinearity of the Lineweaver-Burk diagram (fig.1, right) suggests that at least two enzymes with different affinities for the substrate, GTP, were involved in [γ-^{32}P]GTP hydrolysis. From the linear portion of the Lineweaver-Burk graph an apparent K_m value of 0.3–0.4 μM GTP was extrapolated for a GTPase with high affinity for GTP. Addition of the ATP analog, adenylyl-5′-imidodiphosphate, up to 1 mM, to the GTPase assay medium did not decrease the activity of the high K_m GTPase(s) as has been reported in turkey erythrocytes [1].

In the presence of 0.3 μM GTP, epinephrine inhibited adenylate cyclase in human platelet membranes by a maximum of 60–70% at 30 μM; half-maximal

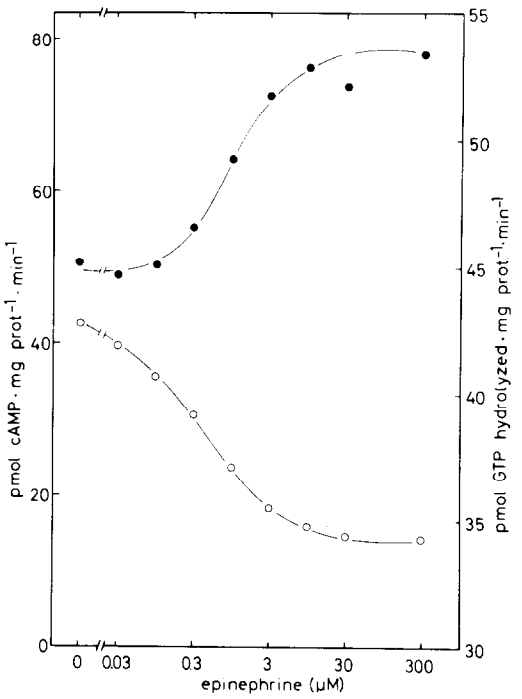

Fig.2. Influence of epinephrine on human platelet adenylate cyclase and GTPase activities. Activity of adenylate cyclase (○), indicated on the left ordinate, and activity of the GTPase (●), indicated on the right ordinate, were determined at increasing concentrations of epinephrine. GTP concentration was 0.3 μM.

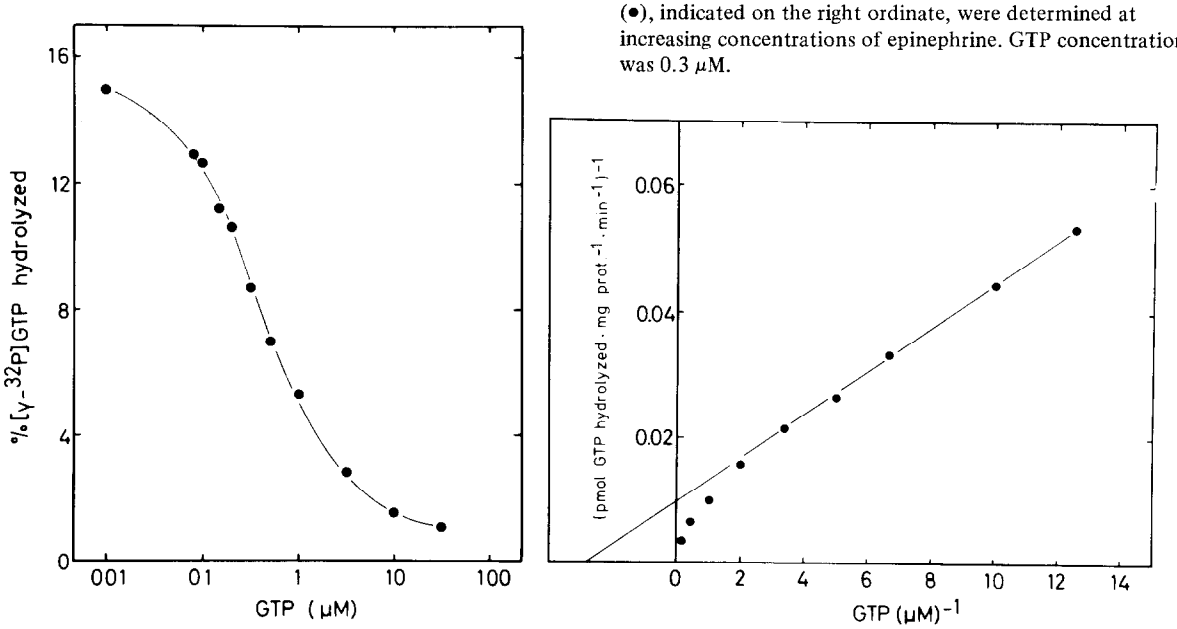

Fig.1. Hydrolysis of [γ-^{32}P]GTP by human platelet particles at various concentrations of GTP. Increasing concentrations of unlabeled GTP were added to a constant amount of labeled [γ-^{32}P]GTP to give the final concentration indicated on the abscissa. Left panel: Isotope dilution curve of GTP hydrolysis with percent of [γ-^{32}P]GTP hydrolyzed indicated on the ordinate. Right panel: Lineweaver-Burk plot of GTP hydrolysis. The apparent K_m value (0.3–0.4 μM) of a GTPase with a high affinity for GTP was extrapolated from the linear part of the curve.

inhibition was observed at 0.5–1 μM epinephrine (fig.2). Under the same assay conditions, epinephrine increased the activity of the GTPase in a concentration-dependent manner. Maximal stimulation of the GTPase was observed at 10–30 μM and half-maximal stimulation at about 1 μM epinephrine. Shown in fig.3 is the influence of epinephrine (300 μM) on GTPase activity at increasing concentrations of GTP. The stimulation of the GTPase by epinephrine was half-maximal and maximal at about 0.3 and 1–3 μM GTP.

The increase in GTP hydrolysis induced by epinephrine occurred without an apparent lag phase and was linear for at least up to 10 min (fig.4, left). The α-adrenergic antagonist, phentolamine (30 μM), added together with epinephrine (100 μM), or 6 min after the reaction had been started, abolished the epinephrine effect on the GTPase activity without an apparent lag period. Prostaglandin (PG) E_1, which causes maximal stimulation of platelet adenylate cyclase (about 20-fold) [9,11], also increased the hydrolysis of [γ-^{32}P]GTP. Maximal activation of GTPase induced by PGE_1 (1 μM) was always less than that induced by epinephrine at maximally effective concentrations. In the presence of PGE_1 (1 μM), epinephrine (100 μM) caused a further increase in GTP hydrolysis (fig.4,

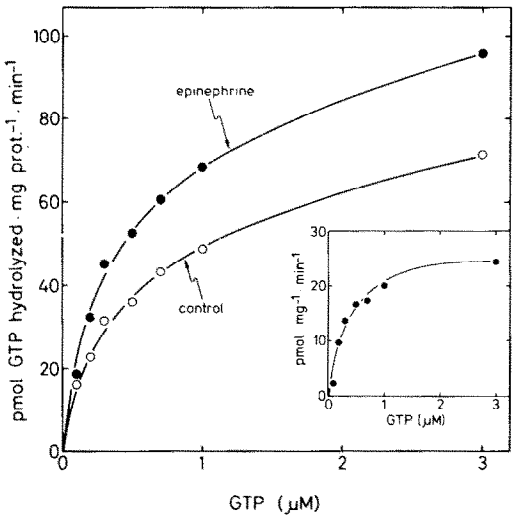

Fig.3. Influence of epinephrine on the rate of GTP hydrolysis at various GTP concentrations. GTP hydrolysis was determined with varying concentrations of GTP in the absence (○) and presence (●) of 300 μM epinephrine as indicated. The inset shows the increment in GTPase activity caused by epinephrine.

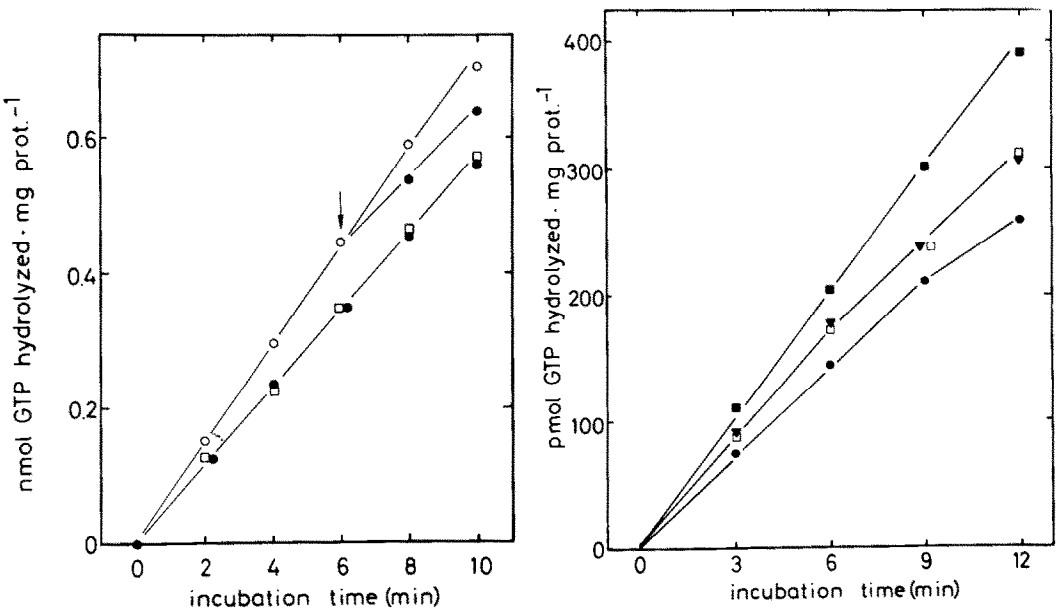

Fig.4. Time course of GTP hydrolysis in the absence and presence of epinephrine, phentolamine and PGE_1. GTP concentration was 0.3 μM. Left panel: □, control; ○, 100 μM epinephrine; ●, 100 μM epinephrine plus 30 μM phentolamine, added at zero time or 6 min after the reaction has been started, as indicated by the arrow. Right panel: ●, control; ▼, 1 μM PGE_1; ■, 1 μM PGE_1 plus 100 μM epinephrine; □, 1 μM PGE_1 plus 100 μM epinephrine plus 30 μM phentolamine.

right). Phentolamine (30 μM) abolished this epinephrine-induced increase in GTP breakdown, but did not affect the stimulation of GTPase by PGE_1.

4. Discussion

In human platelet membrane preparations, epinephrine, via α-adrenoceptors, inhibits adenylate cyclase by a GTP-dependent process, which is prevented or reversed by stable GTP analogs [7,9], as has been shown for various inhibitory hormones in different tissues [4,5]. Based on these opposite actions of GTP and its stable analogs in hormone-induced adenylate cyclase inhibition, we previously suggested [4,7] that inhibitory hormones may decrease adenylate cyclase activity by increasing the off-rate of the system, which appears to be controlled by a GTPase activity associated to the guanine nucleotide-binding regulatory subunit [1–3,6]. The data presented indicate that epinephrine increases the activity of a low K_m GTPase in human platelet membrane preparations. The apparent K_m of about 0.3 μM GTP is about 3-fold higher than that obtained for β-adrenergic agonist-stimulated GTPase in turkey erythrocyte membranes [1]. This difference may be based on the different tissues studied, but may also reflect the finding in adenylate cyclase studies that in some tissues higher GTP concentrations were required for hormonal inhibition than for stimulation of adenylate cyclase [4,5]. The maximal increase in GTPase activity induced by epinephrine in the platelet system (20–30 pmol GTP hydrolyzed per mg protein and per min) is far higher than that reported for the isoproterenol-induced stimulation in turkey erythrocyte membranes (3–7 pmol per mg and per min) [1]. PGE_1, which stimulates platelet adenylate cyclase, also increased the GTPase activity, but not to the same extent as epinephrine, which inhibits the cyclase.

There was a good correlation between the data obtained in adenylate cyclase and in GTPase activity studies. The concentrations of epinephrine required for half-maximal and maximal inhibition of the cyclase were almost identical to those required for half-maximal and maximal stimulation of the low K_m GTPase. Additionally, half-maximal stimulation of the GTPase and inhibition of the adenylate cyclase [9] occurred at about 0.3 μM GTP. Furthermore, the epinephrine-induced stimulation of the GTPase was antagonized by the α-adrenergic blocking agent, phentolamine, which also antagonizes epinephrine-induced inhibition of the platelet adenylate cyclase [11]. This finding indicates that, similar to adenylate cyclase inhibition, stimulation of the GTPase by epinephrine is mediated by α-adrenoceptors.

Recently, we have found in studies on hamster adipocyte adenylate cyclase that inhibitory hormonal factors increase the off-rate of this enzyme and stimulate a low-K_m GTPase in the same membrane system [12]. Therefore, we suggest that in human platelet membranes stimulation of a GTPase is involved in adenylate cyclase inhibition by α-adrenergic agonists and that an acceleration of the turn-off reaction of the adenylate cyclase mediated by an increase in GTPase activity may be a feature common to hormone-induced adenylate cyclase inhibition. Further studies are required to elucidate which mechanism inhibitory hormones increase the GTPase activity, and whether the GTPase activated by stimulatory hormones is identical to that activated by inhibitory hormones.

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