Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 121–127 (1979)

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Different Inhibitory Effect of Adrenaline on Platelet Adenylate Cyclase in the Presence of GTP Plus Cholera Toxin and of Stable GTP Analogues*

Karl H. Jakobs and Günter Schultz

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, Federal Republic of Germany

Summary. GTP is generally required for hormonal stimulation of adenylate cyclase. On the other hand, the presence of GTP is essential for hormone-induced inhibition of adenylate cyclase in cell-free preparations of human platelets and other cells. In order to differentiate the dual roles of GTP in hormonal stimulation and inhibition of adenylate cyclase, we have studied the effect of adrenaline on the platelet enzyme under conditions where guanine mucleotides caused marked stimulation. In the presence of GTP ($\geqq 1\,\mu M$), which by itself had no or only a small stimulatory effect on adenylate cyclase, adrenaline inhibited the basal and prostaglandin E_1-stimulated forms of the enzyme. In contrast, the stable GTP analogues, GMP-P(NH)P and GTP-γ-S, which caused a time-dependent, persistent activation of the enzyme, reversed or prevented the inhibitory effect of adrenaline. Cholera toxin, which activates adenylate cyclase presumably by inhibition of a specific GTPase, increased cyclase activity in platelet membranes up to 4-fold, and GTP addition (0.1–30 μM) augmented this activation about 2-fold. The α-adrenergic component of adrenaline (0.1–100 μM), in a concentration-dependent manner, prevented the GTP-induced increase in cholera toxin-stimulated activity. The inhibition was also observed in enzyme preparations that had been fully activated by pretreatment with cholera toxin. These data suggest that α-adrenergic agonists may inhibit platelet adenylate cyclase through increased inactivation of the enzyme, possibly involving a stimulation of the GTPase connected to the adenylate cyclase system.

Key words: α-Adrenergic effects – GTP effects on adenylate cyclase – Adenylate cyclase inhibition – GTPase.

Introduction

Stimulation of adenylate cyclase [ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1] from various tissues by hormones is a GTP-dependent process (for recent reviews see Helmreich et al., 1976; Birnbaumer, 1977; Rodbell, 1978; Levitzki and Helmreich, 1979), in which a distinct guanine nucleotide binding protein is involved (Pfeuffer and Helmreich, 1975; Pfeuffer, 1977). As shown in avian erythrocytes, hormone-induced activation of adenylate cyclase appears to be mediated by displacement of bound GDP by GTP (Cassel and Selinger, 1978) and to be terminated by a specific GTPase that hydrolyzes the bound GTP to GDP and P_i (Cassel and Selinger, 1976). The cholera toxin-induced inhibition of the GTPase recently observed in turkey erythrocytes (Cassel and Selinger, 1977) appears to be an excellent explanation for the persistent activation of the adenylate cyclase by the toxin, which has been shown to activate the cyclase in vitro in a GTP-dependent manner in all hormone-responsive systems thus far examined (for reviews see Finkelstein, 1973; Gill, 1977; Vaughan and Moss, 1978). An accumulation of the adenylate cyclase in the active guanine nucleoside triphosphate-bound configuration can also be obtained by using stable GTP analogues such as guanylyl 5′-imidodiphosphate [GMP-P(NH)P] and guanosine 5′-(γ-thio)triphosphate (GTP-γ-S), which are slowly hydrolyzed by GTP-degrading enzymes and which can cause a persistent increased adenylate cyclase activity (Helmreich et al., 1976; Birnbaumer, 1977; Rodbell, 1978; Levitzki and Helmreich, 1979).

Send offprint requests to K. H. Jakobs at the above address

* Parts of the data were presented in preliminary form (Jakobs and Schultz, 1978)

Abbreviations. GMP-P(NH)P, guanylyl 5′-imidodiphosphate; GTP-γ-S, guanosine 5′-(γ-thio)triphosphate; GTPase, guanosine 5′-triphosphatase; P_i, inorganic phosphate.

0028-1298/79/0310/0121/$01.40

We have recently shown (Jakobs et al., 1976) that adrenaline and noradrenaline, by means of their α-adrenergic component, cause inhibition of adenylate cyclase in human platelet lysates. The α-adrenergic agonists decreased the basal as well as the prostaglandin- or fluoride-stimulated activities. In studies on the mechanism underlying this inhibition, we found that the presence of GTP is absolutely necessary for the inhibitory effect on adenylate cyclase (Jakobs et al., 1978a). In washed platelet particles, basal and prostaglandin E_1-activated forms of the enzyme were either not affected or were only slightly activated by added GTP, whereas GTP added at increasing concentrations revealed the inhibitory effect of adrenaline.

In order to differentiate the dual effects of GTP, which appears to be involved both in the hormonal stimulation and inhibition of the adenylate cyclase, we have studied the effect of α-adrenergic agonists on the platelet adenylate cyclase stimulated by GTP in the presence of cholera toxin and by stable GTP analogues. In this paper we present data indicating that adrenaline, by its α-adrenergic component, inhibited the enzyme activated by cholera toxin plus GTP by abolishing the stimulatory effect of GTP and that stimulation by GTP analogues reversed or prevented the adenylate cyclase inhibition. The data suggest that the adenylate cyclase inhibition caused by α-adrenergic agonists involves an increased inactivation of the enzyme.

Materials and Methods

Materials and methods used were as previously described (Jakobs et al., 1976; 1978a). Cholera toxin was obtained from Schwarz-Mann, Heidelberg, FRG, or was a gift of the Behring-Werke, Marburg, FRG. GMP-P(NH)P, GTP-γ-S and NAD were purchased from Boehringer Mannheim, FRG. [α-^{32}P]ATP was prepared by a chemical procedure (Symons, 1974) or by the enzymatic method described by Walseth and Johnson (1979).

Preparations of Platelet Lysates and Particles. Platelets obtained from blood of healthy volunteers were lysed by rapid freezing and thawing as previously described (Jakobs et al., 1976). Platelet particles were prepared by centrifugation of the lysates for 20 min at 30,000 × g and resuspension of the pellets in 10 mM triethanolamine-HCl buffer, pH 7.4, containing 145 mM NaCl, followed by two identical centrifugation steps and resuspension in the above buffer.

Adenylate Cyclase Assay. The standard reaction mixture for the determination of adenylate cyclase activity contained 0.1 mM [α-^{32}P]ATP (0.3 – 0.8 μCi per tube, substantially freed from GTP contamination), 5 mM $MgCl_2$, 5 mM creatine phosphate, 0.4 mg/ml of creatine kinase (25 U/mg), 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM ethyleneglycol-bis-(β-aminoethylether)N, N'tetraacetic acid (EGTA) and 50 mM triethanolamine-HCl, pH 7.4, in a final volume of 0.1 ml. The final concentration of NaCl, which was mainly added with the enzyme preparation, was about 70 mM. The β-adrenergic blocking agent, pindolol (10 μM), was present under each condition in order to block the β-adrenergic, stimulatory component of adrenaline (Jakobs et al., 1978b). The reactions were initiated by the addition of platelet lysate (about 100 – 150 μg of protein) or particles (about 20 – 50 μg of protein) to reaction mixtures that had been preincubated for 5 min at 37° C. Reactions were for 10 min or as indicated and were terminated by the addition of 0.5 ml of 0.12 M zinc acetate. Cyclic AMP formed was isolated by coprecipitation of related 5'-nucleotides with $ZnCO_3$ and subsequent column chromatography on neutral alumina as previously described (Jakobs et al., 1976). Under these conditions, cyclic AMP formation was linear as a function of time for at least 20 min. Standard deviations from triplicate reactions were generally less than 5% of the means. Results comparable to those presented were obtained in at least three separate experiments in each case.

For the studies of the effects of cholera toxin, the toxin was preincubated with 20 mM dithiothreitol for 10 min at 37° C, and 2 mM NAD was included in the adenylate cyclase assay, which addition was absolutely necessary to show any effect of cholera toxin, as was also found by others (Gill, 1977). In order to overcome the initial lag phase of the cholera toxin-induced adenylate cyclase activation (see Fig. 1), cholera toxin and platelet particles were generally preincubated for 3 min with the whole adenylate cyclase reaction mixture with the exception of the labelled ATP.

Protein was determined as described by Lowry et al. (1951), using bovine serum albumin as standard.

Results

Treatment of human platelet particles with cholera toxin increased the basal adenylate cyclase activity up to 4-fold. After a short time-lag period of about 2 – 3 min, cyclic AMP accumulation was linear for up to 40 min when assayed in the presence of the toxin (Fig. 1). The toxin-induced activation was half-maximal at about 1 μg/ml, and maximal activation occurred at about 10 – 30 μg/ml (not shown). In the presence of prostaglandin E_1 (0.01 – 10 μM), which increased platelet adenylate cyclase activity up to 10-fold, the effect of cholera toxin was not more than additive. Enzyme activated by sodium fluoride (10 mM) was not affected by cholera toxin at concentrations up to 30 μg/ml. In contrast, addition of GTP (0.1 – 100 μM), which had no effect on basal activity, increased the extent of toxin-induced activation in platelet particles by about 2-fold (Fig. 2). The effect was halfmaximal at about 0.3 – 1 μM and maximal at about 30 μM GTP. The addition of GTP did not change the time kinetics of the cholera toxin-induced adenylate cyclase activation (see Fig. 1). In the presence of cholera toxin, GTP more effectively activated the enzyme than did its hydrolysis-resistant analogue, GMP-P(NH)P (see Fig. 2).

We have recently reported (Jakobs et al., 1978a) that inhibition of platelet adenylate cyclase by the α-adrenergic component of adrenaline becomes evident with increasing concentrations of GTP. The effect of GTP was half-maximal at about 1 μM and maximal at about 30 μM. This effect of GTP occurred without an apparent lag phase. In the lysates and washed particulate preparations used, addition of GTP did not substantially increase the basal activity and was apparently not necessary to allow stimulation by prostaglandin E_1. Since a stimulatory effect of GTP was