EFFECT OF GUANYL NUCLEOTIDES ON FOLLITROPIN-DEPENDENT ADENYLATE CYCLASE IN THE TESTIS

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1. Introduction

Follitropin (FSH), like other polypeptide hormones, stimulates its target cells in the testis through binding to specific receptors and activation of membrane adenylate cyclase [1–5]. We have shown in [6] that a temporal correlation exists between binding of the hormone and activation of adenylate cyclase. Dose-dependence of hormone binding to testis membrane receptor and of adenylate cyclase activation fell in the same range of FSH concentrations. It has been demonstrated for other systems that adenylate cyclase is composed of at least two subunits: a catalytic one, which converts ATP into cyclic AMP; and a regulatory subunit which is GTP dependent [7].

We report here on the effect of a stable GTP analogue, guanosine 5′-(β,γ-imido) triphosphate (GppNHp), on the activation of the FSH-dependent adenylate cyclase. GppNHp stimulates the FSH-dependent adenylate cyclase of the testis synergistically with the hormone, by enhancing the V_{max} of the enzyme. The app. K_m of activation by FSH is not changed upon addition of the nucleotide. These observations contrast with those done on glucagon–hepatocyte [8] and catecholamine–turkey erythrocyte systems [9,10].

2. Materials and methods

Highly purified porcine FSH was prepared as in [11]. Its biological activity was 81-times that of NIH-FSH-P1 (NIH, Bethesda, MD). Nucleotide derivatives were obtained from Boehringer (Mannheim).

A crude membrane fraction was prepared from immature porcine testes as in [6] except that homogenization of the tissue and subsequent operations were performed in 10 mM Tris–Cl buffer (pH 7.4) containing 0.3 M sucrose, 5 mM β-mercaptoethanol, 1 mM EDTA and 2 mM $MgCl_2$.

Adenylate cyclase activity was determined by measurement of the amounts of cyclic AMP produced by the membranes during incubation at 34°C. Aliquots of the membrane suspension (25 μl) were added to a medium adjusted at pH 7.4 containing: 50 μl 50 mM Tris–Cl (pH 7.6), 10 mM $MgCl_2$, 2 mM EDTA, 5 mM ATP and 0.2 mM methylisobutylxanthine 25 μl 20 mM phosphocreatine and creatine kinase (1 mg/ml) with or without stimulators (FSH and GppNHp). The reaction was stopped by addition of 400 μl 25 mM Tris–Cl (pH 7.4), 4 mM EDTA. Proteins were denatured in boiling water during 3 min. The tubes were centrifuged at 1500 × g for 10 min and cyclic AMP was measured in the supernatants by radioimmunoassay [12] after acetylation. Proteins were assayed by the Lowry method [13] after solubilization of the particulate fraction in 0.075% sodium dodecylsulfate in 0.1 M sodium hydroxide.

3. Results

In the presence of 3 nM FSH, stimulation of adenylate cyclase in testicular membranes was maximal with GppNHp ranging from 10^{-5}–10^{-4} M (fig.1). GppNHp and FSH synergetically stimulate the enzyme as shown in fig.2 where the difference curve (C) between stimulation by FSH in the presence of

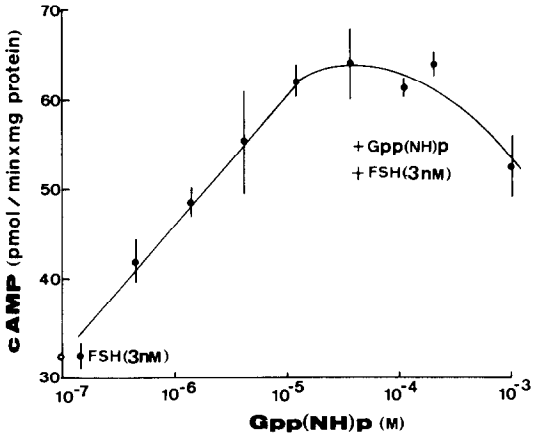


Fig.1. Adenylate cyclase activity (at 34°C) of testis membranes in the presence of 3 nM FSH as a function of GppNHp concentration. The membrane suspension was at 0.706 mg protein/ml incubation medium. Each point is the mean of quadruplicate determinations.

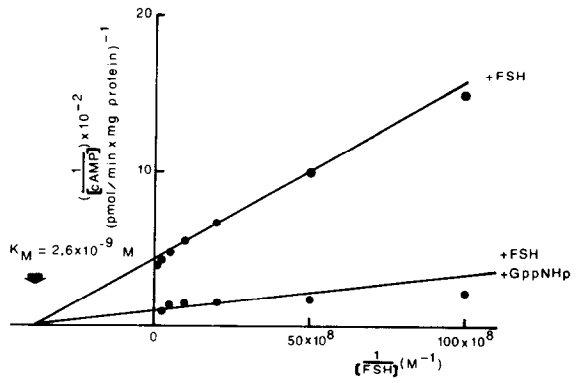
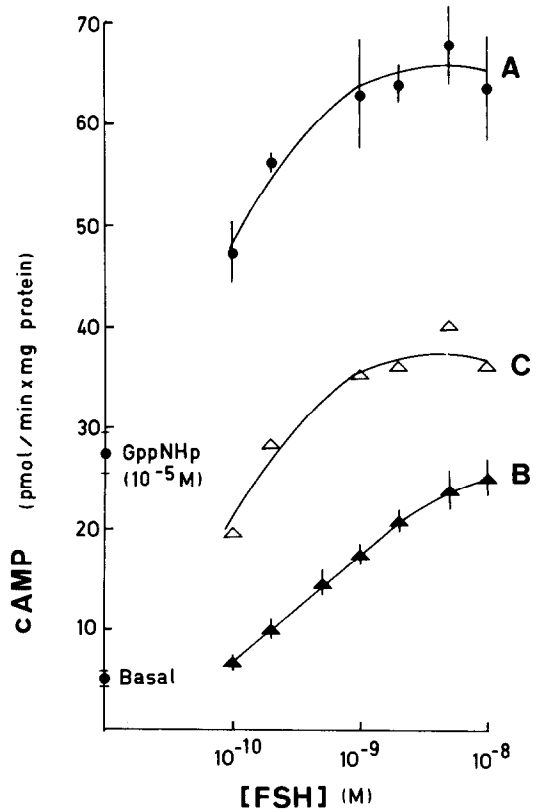


Fig.3. Lineweaver-Burk analysis of experimental data shown in fig.2.



10^{-5} M GppNHp (A) and the effect obtained with 10^{-5} M GppNHp alone is clearly different from that recorded when stimulation was performed in the presence of the hormone only (B). Lineweaver-Burk analysis (fig.3) of these data shows that the guanyl derivative activates the enzyme by changing V_{max} while the apparent K_m of activation (2.6×10^{-9} M) of the enzyme by FSH was not changed in the presence of GppNHp.

Kinetic studies of adenylate cyclase activity of testicular membranes indicate that the lag phase, observed at low membrane concentration (0.206 mg/ml) either in the presence of the hormone or in the presence of GppNHp alone, disappears when both stimulators are present in the incubation medium (fig.4). The lag phase was not observed at high membrane concentration (0.706 mg protein/ml) (data not shown).

4. Discussion

It has been well established [14,15] that GTP, the allosteric activator of hormone dependent adenylate

Fig.2. Adenylate cyclase activity at 34 °C of testis membranes in the presence of FSH (B) or in the presence of 10^{-5} M GppNHp plus FSH (A) as a function of FSH concentration. The difference curve (C) was computed from A minus B. Concentration of membrane suspension was as in fig.1.

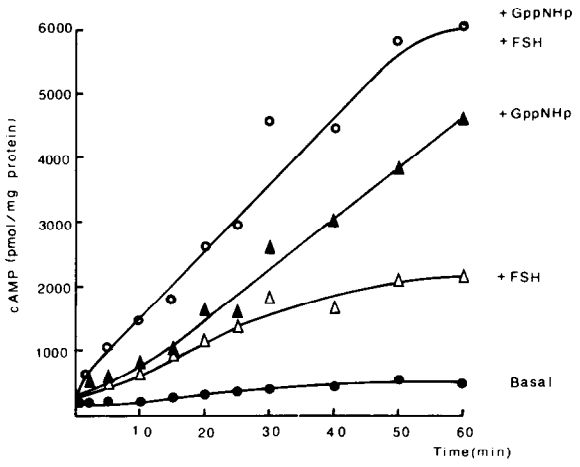


Fig.4. Time course at 34°C of adenylate cyclase activity of testis membranes. The concentrations of the reagents were as follows: 3 nM FSH; 10^{-5} M GppNHp; 3 nM FSH plus 10^{-5} M GppNHp. The membrane suspension was at 0.206 mg protein/ml incubation medium.

cyclases, plays a key role in the coupling between the hormonal receptor and the adenylate cyclase.

Studies of the adenylate cyclase performed on plasma membranes can be simplified by using a non-hydrolysable analogue of GTP such as GppNHp. Until now, only limited investigations have been conducted on the coupling between glycoprotein hormone receptors and adenylate cyclase in membranes isolated from target cells or organs.

The FSH-dependent adenylate cyclase of testis can be studied using a homologous porcine system, in which plasma membranes are prepared from immature porcine testes [6]. It is clear that in our testis material, several hormone-dependent adenylate cyclases are present (i.e., the lutropin (LH)-dependent adenylate cyclase), since no cellular purification had been performed.

Nevertheless, the effects of GppNHp on the FSH-dependent adenylate cyclase was easily recorded. Indeed, FSH receptor occupancy by the hormone was in good dose correlation with adenylate cyclase activation by FSH for 0.1–10 nM hormone [6]. The hormonal specificity of the FSH binding to testis receptors was shown [6] and thus the FSH-dependent adenylate cyclase can be specifically studied. We have shown that GppNHp and the hormone synergistically stimulate the FSH-dependent adenylate cyclase of the testis as it has been reported for numerous hormone-dependent adenylate cyclases. The addition of GppNHp to the medium does not affect the app. K_m of enzyme activation by the hormone; it only increases V_{max}. A reverse situation was described [8] for the hepatic adenylate cyclase stimulation by glucagon and [9,10] for the β-adrenergic receptor–adenylate cyclase system of turkey erythrocytes, where K_m values of cyclase activation by the hormones were affected by the presence of guanosine nucleotides, but not V_{max}. When binding of the hormone to its receptor is being considered, another important difference appears between these systems [15,16] and that of FSH testis receptor–adenylate cyclase. Indeed, guanosine nucleotides have no effect on the binding of FSH to the receptors; more precisely the addition of GppNHp or GTP does not induce the dissociation of labeled FSH from testis receptors [17].

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