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The Glucagon-sensitive Adenyl Cyclase System in Plasma Membranes of Rat Liver

V. AN OBLIGATORY ROLE OF GUANYL NUCLEOTIDES IN GLUCAGON ACTION

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SUMMARY

A method is described for the enzymatic synthesis of 5'-adenylyl imidodiphosphate labeled with ^{32}P at the α position (AMP-PNP-α-^{32}P), an analogue of ATP containing nitrogen substituted for oxygen between the terminal phosphates. The nucleotide is only slowly hydrolyzed during incubation with rat liver plasma membranes and is a substrate for adenyl cyclase in these membranes.

In the presence of 0.2 mM AMP-PNP, glucagon and fluoride ion stimulate adenyl cyclase activity; linear rates are maintained for at least 10 min of incubation at 30°. GTP enhanced the initial rate of basal and glucagon-stimulated adenyl cyclase activity. Reduction in concentration of Mg^{++} in the assay medium or incubation of liver membranes for 5 min at 30° prior to addition of glucagon results in loss of response of adenyl cyclase to glucagon and in reduction in the effects of GTP on basal activity. Under these conditions GTP, GDP, or GMP-PCP are required for glucagon stimulation of the enzyme even though the specific binding sites for glucagon are saturated with hormone; as little as 10 nM GTP or GDP is required. UTP and CTP exert smaller effects than the guanyl nucleotides and act only at concentrations higher than 0.1 mM.

The guanyl nucleotides inhibited the response of adenyl cyclase to fluoride ion (10 mM) over the same concentration range over which they stimulate the response of the enzyme to glucagon. This action of the nucleotides is observed in plasma membranes treated with phospholipase A under conditions that result in loss of glucagon binding and of hormonal response.

It is concluded that guanyl nucleotides play a specific and obligatory role in the activation of adenyl cyclase by glucagon. The nucleotides bind at sites, distinct from the glucagon binding sites, that appear to regulate both the response of adenyl cyclase to glucagon, and, possibly by a related mechanism, the actions of fluoride ion on this system.

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In the previous study (1), it was shown that guanyl nucleotides (GTP or GDP) equally and at concentrations as low as 0.05 μM, alter the properties of the specific binding sites for glucagon in rat liver plasma membranes. GMP-PCP,[1] a nonphosphorylating analogue of GTP, mimicked the actions of the natural nucleotides on glucagon binding, suggesting that the nucleotides act by binding to sites, as yet undefined, that influence the structure of the glucagon binding sites. ATP, ADP, UTP, and CTP similarly affected binding of glucagon but only at concentrations above 0.1 mM.

It was of obvious interest to determine whether the actions of guanyl nucleotides on the glucagon binding sites, which appear to be related to the adenyl cyclase system (2), have their correlate on the response of adenyl cyclase to glucagon. Studies reported elsewhere (3) in preliminary form, showed that guanyl nucleotides enhanced the response of adenyl cyclase to glucagon but only at concentrations considerably higher than those required for their actions on binding of glucagon. The preliminary studies were carried out with concentrations of ATP, the substrate for adenyl cyclase, that affected glucagon binding in the same manner as the guanyl nucleotides. Reduction of ATP concentration to low levels (0.2 mM or less) resulted in rapid hydrolysis of the nucleotide even in the presence of ATP-regenerating systems, creating difficulties in interpretation of kinetic data.

In this study, the enzymatic synthesis of ^{32}P-labeled AMP-PNP is described. This analogue of ATP contains nitrogen in place of oxygen between the terminal phosphates and was found to be resistant to hydrolysis by ATPases in liver membranes. It will be shown that it is a substrate for adenyl cyclase. It will also be shown that guanyl nucleotides, at concentrations as low as 10 nM, play an obligatory role in the activation of adenyl cyclase by glucagon but inhibit, possibly by a related mechanism, the response of the enzyme to fluoride ion.

EXPERIMENTAL PROCEDURE

Only those materials and methods not described in the pre-

[1] The abbreviations used are: GMP-PCP, 5'-guanylyl-diphosphonate; AMP-PNP, 5'-adenylyl-imidodiphosphate; PNP, diphosphoimide; ADP-NH_2, 5'-adenylyl-phosphoamide; cyclic AMP, cyclic-3',5'-AMP.

ceding papers (1, 2, 4, 5) are documented in the present communication.

Materials—AMP-PNP(Na_4) and PNP(Na_4) were kindly supplied by Dr. Ralph Yount (Washington State University, Pullman). AMP-PNP was contaminated with 15% ADP-NH_2.

Escherichia coli extract was prepared from *E. coli* (strain B) according to the procedure of Nirenberg (6) and represents the 105,000 × *g* supernatant (S-100 fraction) obtained in Step 4 of this procedure.

Preparation and Purification of AMP-PNP-α-^{32}P: Zamecnik and Stephenson have shown (7) that methylene diphosphonate is incorporated into ATP by purified *E. coli* lysyl-tRNA synthetase (EC 6.1.1.60) according to the following reactions:

$$ATP + Lys + enzyme_{Lys} \rightleftarrows (AMP \cdot Lys \cdot enzyme_{Lys}) + PP$$

$$PCP + (AMP \cdot Lys \cdot enzyme_{Lys}) \rightarrow AMP\text{-}PCP + enzyme_{Lys} + Lys$$

which in the presence of excess PCP essentially go to completion, particularly if pyrophosphatase is present during incubation. We were informed by Dr. Zamecnik that AMP-PNP can be prepared by the same reaction. Instead of *E. coli* lysyl-tRNA synthetase we used a crude *E. coli* extract containing a number of amino acid tRNA synthetases and which is rich in pyrophosphatase activity. A mixture of 19 naturally occurring amino acids (except leucine), each at 1 mM, was used as substrates for the reaction. The following procedure was used to obtain AMP-PNP-α-^{32}P of high specific activity. Solutions containing 0.15 to 0.3 μmole of ATP-α-^{32}P (3 to 5 Ci per mmole) were evaporated to dryness at room temperature under a stream of nitrogen. The following solutions were added to the residue: 15 μl of 1 M Tris-HCl, pH 7.6, 15 μl of 0.1 M $MgCl_2$, 30 μl of amino acid mixture (see above), 20 μl of 50 mM PNP, and 40 μl of *E. coli* extract. The reaction mixture was incubated for 1 hour at 30°, followed by the addition of 10 μl of a suspension of partially purified rat liver plasma membranes (4) containing 10 mg of membrane protein per ml and further incubation for 15 min at 30°. The latter step served to degrade residual labeled ATP by nucleotidases present in liver membranes (8); AMP-PNP is only slightly hydrolyzed in the presence of liver membranes (see "Results").

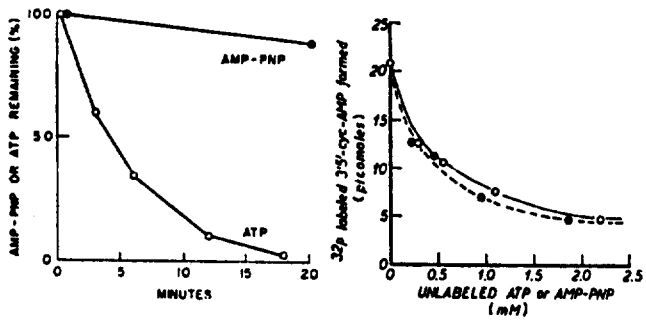

FIG. 1 (*left*). Effect of incubation of liver plasma membranes, under adenyl cyclase assay conditions, on levels of ATP and AMP-PNP. *a*, liver plasma membranes (0.7 mg of protein per ml) were incubated at 30° for varying periods of time in medium containing 0.2 mM ATP-α-^{32}P (140 cpm per pmole), 5 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.6, and an ATP-regenerating system consisting of 20 mM creatine phosphate and 1 mg per ml of creatine kinase. *b*, liver plasma membranes (0.4 mg of protein per ml) were incubated at 30° for 20 min in medium containing 0.25 mM AMP-PNP-α-^{32}P (205 cpm per pmole), 4.0 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, and 25 mM Tris-HCl, pH 7.6. Reactions were terminated by addition of 1 volume of 4 M formic acid. Zero times were prepared by addition of liver plasma membranes after formic acid. The percentage of ATP or AMP-PNP remaining was determined by chromatography on thin layer sheets of PEI-cellulose F (see "Experimental Procedures").

FIG. 2 (*right*). Effect of addition of unlabeled ATP or AMP-PNP on formation of cyclic AMP-^{32}P from ATP-α-^{32}P. Liver plasma membranes (0.4 mg of protein per ml) were incubated for 10 min at 30° in 0.05 ml of medium containing 0.53 mM ATP-α-^{32}P (81 cpm per pmole), 5.0 mM $MgCl_2$, 12 μM glucagon, 1.0 mM EDTA, 25 mM Tris-HCl, pH 7.6, an ATP-regenerating system consisting of 20 mM creatine phosphate and 1 mg per ml of creatine kinase, and the indicated concentrations of unlabeled ATP (○——○) or AMP-PNP (●- - -●). The reactions were terminated and the cyclic AMP-^{32}P formed was determined as described in Table I.

All subsequent procedures were carried out at 5°. The above reaction mixture was applied directly to a column (0.4 × 4.0 cm) of DEAE-cellulose (Whatman DE-52) that had been previously washed with 10 ml of 2 M ammonium formate and then washed with 20 ml of distilled water. The column was eluted with a linear gradient formed from 15 ml of water and 15 ml of 0.5 M formate, pH 7.4, at a flow rate of 0.5 ml per min; 0.5-ml fractions were collected. Labeled AMP-PNP appeared in Fractions 42 to 47 which were pooled and treated as follows to remove salt. Washed analytical grade Dowex 50 (H^+ form), 0.5 g (wet weight), was added to the pooled fractions. After stirring for 2 min in an ice bath, the mixture was filtered, the residue was washed twice with 10 ml of cold water, and the combined filtrates were lyophilized. Labeled AMP-PNP was dissolved in 0.5 ml of 25 mM Tris-HCl, pH 7.6, and stored at −10°. Radio purity of AMP-PNP-α-^{32}P was determined by thin layer chromatography (described below) and ranged from 96 to 98%; the major contaminant is ADP-NH_2. Based on the amount of radioactive ATP added to the incubation mixture, yields of labeled AMP-PNP ranged from 60 to 75%.

Separation of Nucleotides by Thin Layer Chromatography—Determinations of changes in concentration of labeled ATP or AMP-PNP during incubation with liver membranes were carried out by thin layer chromatography (ascending) on precoated, aluminum backed sheets of PEI-cellulose F (Brinkmann 6820310-4). A solution of 0.5 N LiCl-2 M formic acid was used as developing solvent. With this solvent system the R_F value for AMP-PNP is 0.68; ATP, 0.51; ADP-NH_2, 0.78. ADP does not separate from AMP-PNP in this system but readily separates on PEI-cellulose chromatography using 1 M Tris-HCl, pH 7.6. Labeled nucleotides were cochromatographed with unlabeled nucleotides which were detected under ultraviolet light. The areas containing the nucleotides were cut out and placed in counting vials containing 10 ml of Bray's scintillation fluid (9). Radioactivity was determined in a liquid scintillation counter.

RESULTS

In previous studies (4), measurements of adenyl cyclase activity in rat liver plasma membranes were carried out with 3.2 mM ATP as substrate. At this concentration, ATP affects binding of glucagon to about the same extent as 1.0 μM GTP (1). Reduction of ATP concentration to 0.2 mM as a means of minimizing its effects on glucagon binding resulted in rapid hydrolysis of the nucleotide despite the presence of an ATP-regenerating system, as illustrated in Fig. 1. AMP-PNP-α-^{32}P, at 0.2 mM, was hydrolyzed by liver membranes to a slight extent (about 15%) during 20 min of incubation with liver membranes in the absence of an ATP-regenerating system. The slight hydrolysis observed

TABLE I

Effect of glucagon and fluoride ion in absence and in presence of GTP on formation of cyclic AMP from AMP-PNP

Liver membranes (0.4 mg of protein per ml) were incubated for 10 min at 30° in medium containing 0.2 mM AMP-PNP-α-^{32}P (193 cpm per pmole), 4.0 mM $MgCl_2$, 1.0 mM EDTA, 1.0 mM cyclic AMP, 0.2% albumin, 25 mM Tris-HCl, pH 7.6, and the indicated additions. Final volume was 0.05 ml. The reactions were terminated by the addition of 0.10 ml of solution containing 40 mM ATP, 12.5 mM cyclic AMP-^{3}H (approximately 30,000 cpm), and 1% sodium dodecyl sulfate, followed by immediate boiling for 3½ min. Cyclic AMP-^{32}P formed was isolated according to Krishna, Weiss, and Brodie (11) as described previously (12, 13).

Addition	Cyclic AMP formed		
	Control	5 μM glucagon	10 mM NaF
	pmoles/mg protein		
None	125	248	890
GTP (0.2 mM)	207	835	565

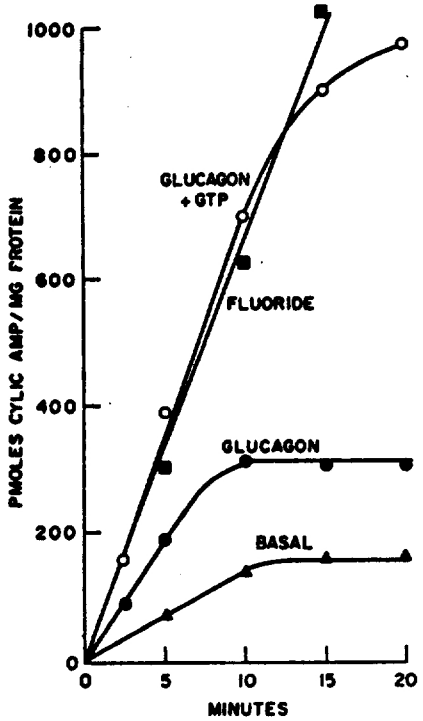

FIG. 3. Effect of fluoride ion, glucagon, and combination of glucagon and GTP on formation of cyclic AMP from AMP-PNP. Liver plasma membranes (0.38 mg of protein per ml) were incubated at 30° in 0.05 ml of medium containing 0.2 mM AMP-PNP-α-^{32}P (190 cpm per pmole), 4.0 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, 25 mM Tris-HCl, pH 7.6, and either 10 mM NaF plus 5 μM glucagon or 5 μM glucagon plus 0.01 mM GTP. The reactions were terminated and the cyclic AMP-^{32}P formed was determined as described in Table I.

may have been due to nucleotide triphosphate pyrophosphohydrolases reported in liver membranes (10).

Addition of varying concentrations of AMP-PNP and ATP to the assay medium containing ATP-α-^{32}P (0.5 mM) resulted in proportional reduction in the amount of labeled cyclic 3′,5′-AMP formed by adenyl cyclase (Fig. 2). These findings suggested

TABLE II

Specificity of action of nucleotides on glucagon-stimulated adenyl cyclase activity

Liver plasma membranes (0.36 mg of protein per ml) were incubated in 0.05 ml of medium containing 0.24 mM AMP-PNP-α-^{32}P (230 cpm per pmole), 2.5 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, 25 mM Tris-HCl, pH 7.6, 5 μM glucagon, and the indicated additions. Incubations were for 10 min at 30°. The reactions were terminated and cyclic AMP-^{32}P formed was determined as described in Table I.

Addition	Cyclic AMP formed
	pmoles/mg protein
None	21
GTP (0.01 mM)	235
GDP (0.01 mM)	237
GMP-PCP (0.1 mM)	201
UTP (0.1 mM)	75
CTP (0.1 mM)	53

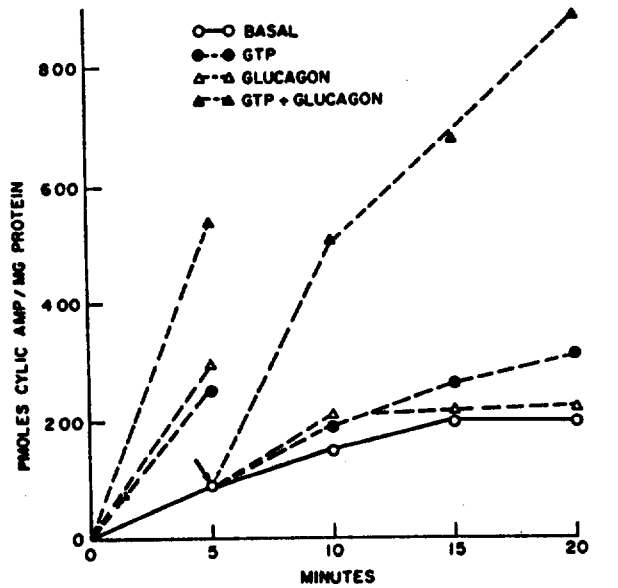

FIG. 4. Effect of time of addition of glucagon and GTP on formation of cyclic AMP from AMP-PNP. Liver plasma membranes (0.4 mg of protein per ml) were incubated at 30° in 0.05 ml of medium containing 0.24 mM AMP-PNP-α-^{32}P (180 cpm per pmole), 4.0 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, and 25 mM Tris-HCl, pH 7.6. The following additions were made at either zero time or after 5 min of incubation: 5 μM glucagon, 0.01 mM GTP, and 5 μM glucagon plus 0.01 mM GTP. The reactions were terminated at the indicated times and the cyclic AMP-^{32}P formed was determined as described in Table I.

that ATP and AMP-PNP behaved identically as substrate for adenyl cyclase.

Incubation of AMP-PNP-α-^{32}P with liver membranes resulted in the formation of labeled cyclic 3′,5′-AMP which was stimulated by glucagon and fluoride ion (Table I). This provided direct evidence that AMP-PNP serves as substrate for adenyl cyclase. It will be noted also that GTP (0.2 mM) stimulated basal activity by 65% and caused a 3.5-fold stimulation of the response of the enzyme system to glucagon. The guanyl nucleotide inhibited the fluoride response by 37%.

Glucagon (5.0 μM) and fluoride ion (10 mM) stimulated the

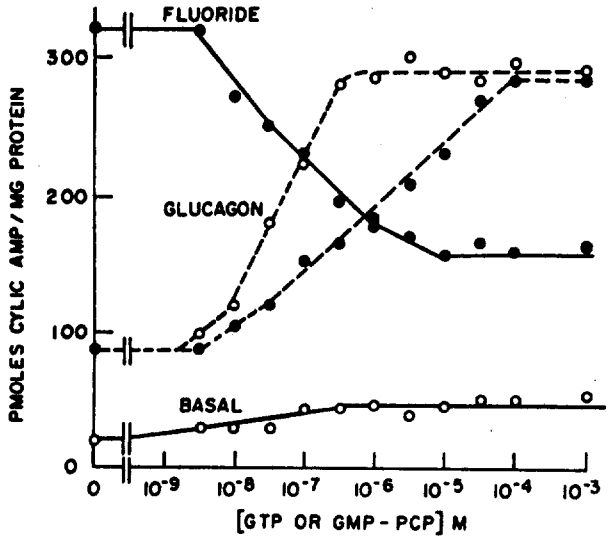

FIG. 5. Effect of varying concentrations of GTP and GMP-PCP on adenyl cyclase activity determined in the absence and in the presence of glucagon and fluoride ion. Liver membranes were incubated in the presence of 5 μM glucagon (*broken lines*) with GTP (*open circles*) or GMP-PCP (*closed circles*). Adenyl cyclase activity in response to NaF (10 mM) was investigated only in the presence of GMP-PCP. Basal activity was studied in the presence of GTP or GMP-PCP, both of which gave identical activities. Other incubation conditions consisted of 0.25 mM AMP-PNP-α-^{32}P, 2.0 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, and 25 mM Tris-HCl, pH 7.6.

production of labeled cyclic 3',5'-AMP at a linear rate for at least 10 min at 30° (Fig. 3); GTP enhanced the initial rate of glucagon-stimulated activity.

GTP or GDP, equally at 100 μM, stimulated the response of adenyl cyclase to glucagon by at least 10-fold when the Mg^{++} concentration was reduced to 2.5 mM. This is shown in Table II (*cf.* Table I where the Mg^{++} concentration was 4.0 mM). The role of magnesium ion in the process of activation of the enzyme by glucagon and guanyl nucleotides is under current investigation. We have shown in a previous study (4) that the Mg^{++} concentration is critical for the response of the enzyme to glucagon. UTP and CTP, at 100 μM exerted smaller stimulatory effects on the response of adenyl cyclase to glucagon.

As shown in Fig. 4, GTP (or not shown, the other guanyl nucleotides) also stimulated basal adenyl cyclase activity (absence of glucagon or fluoride ion) in the presence of 4.0 mM Mg^{++}; under these conditions basal activity in the presence of GTP was about equal to that observed with glucagon (5 μM) alone. As shown, incubation of the membranes for 5 min at 30° prior to addition of GTP or glucagon resulted in a marked decrease in the effect of GTP on basal activity and in essentially no effect of glucagon alone; addition of GTP was required to obtain a glucagon response. The loss of response of adenyl cyclase to glucagon after the 5-min incubation period was not due to a change in binding of glucagon; uptake of ^{125}I-glucagon (4.5 nM) was found to be identical before and after incubation of the membranes under the conditions described in Fig. 4.

The basis of the loss of response of adenyl cyclase to glucagon or the diminished effect of GTP on basal activity after incubation is not known but may be related, respectively, to destruction of endogenous, membrane-bound guanyl nucleotides by nucleotidases and of membrane-bound glucagon by glucagon-inactivating process or processes; both destructive activities are present in liver membranes (2).

TABLE III

Effect of treatment of liver plasma membranes with phospholipase A on inhibitory action of GMP-PCP on fluoride-stimulated adenyl cyclase activity

Liver plasma membranes (1.2 mg of protein per ml) were incubated in the absence and presence of 150 units per ml of phospholipase A in medium containing 1 mM $CaCl_2$ and 25 mM Tris-HCl, pH 7.6. Incubation was for 5 min at 30°. Treatment was terminated by addition of 0.1 volume of 25 mM EDTA and immediate cooling to 0°. Adenyl cyclase activity was subsequently determined on 10-μl aliquots in the absence or in the presence of 0.1 mM GMP-PCP in 0.05 ml of medium containing 0.24 mM AMP-PNP-α-^{32}P (195 cpm per pmole), 2.5 mM $MgCl_2$, 1 mM EDTA, 1 mM cyclic AMP, 0.2% albumin, 25 mM Tris-HCl, pH 7.6, and 10 mM NaF. Incubations were for 10 min at 30°. The reactions were terminated and cyclic AMP-^{32}P formed was determined as described in Table I.

Addition during treatment	Cyclic AMP formed		Inhibition due to GMP-PCP
	Control	GMP-PCP	
	pmoles/mg protein		%
None	173	113	35
Phospholipase A	86	58	33

In the previous study (1), it was shown that GTP and GDP alter binding and dissociation of bound glucagon over a concentration range of 0.05 μM to 50 μM; GMP-PCP also altered these processes but at higher concentrations. Maximal effects of the analogue were observed at 100 μM. Investigations of the response of the adenyl cyclase system to glucagon, illustrated in Fig. 5, revealed that guanyl nucleotides exert their effects on this system at somewhat lower concentrations than were observed in the binding studies. Significant effects of GTP and GMP-PCP were observed at 0.01 μM and 0.05 μM, respectively. As was observed in the binding studies, maximal effects of GMP-PCP on glucagon response were seen at 100 μM.

Since GTP inhibits the response of the enzyme system to fluoride ion (Table I), it was of interest to determine the effects of GMP-PCP on this process. As shown in Fig. 5, the analogue of GTP inhibited the fluoride response at concentrations similar to those at which it stimulated the response of the enzyme to glucagon.

Treatment of liver membranes with phospholipase A results in complete loss of glucagon binding or activation of adenyl cyclase but in retention of the fluoride response (2, 5). Under conditions in which the glucagon response of adenyl cyclase was abolished and the fluoride response was reduced by 50%, phospholipase A treatment did not alter the inhibitory effects of GMP-PCP on the response of adenyl cyclase to fluoride ion (Table III).

Studies were also carried out to determine whether guanyl nucleotides alter the concentration of glucagon required for half-maximal response of adenyl cyclase, which was previously shown to be 0.004 μM (4). In the presence of 0.1 mM GTP, adenyl cyclase activity in response to 0.005 μM glucagon was 66% of that given by glucagon at 5 μM, indicating that GTP does not influence the apparent affinity of adenyl cyclase system for the hormone. It can be concluded from these findings that guanyl nucleotides and glucagon interact with the adenyl cyclase system in a non-competitive fashion.

DISCUSSION

The purpose of the present study was to ascertain the effects of guanyl nucleotides on the response of adenyl cyclase to glucagon. The use of AMP-PNP as substrate for adenyl cyclase facilitated this study. Compared to ATP, AMP-PNP was only slowly hydrolyzed during incubation with liver membranes, thus circumventing the usual problems encountered in maintaining substrate concentration during kinetic analysis of adenyl cyclase activity. For this reason, addition of ATP-regenerating systems, which may complicate analysis because of their possible influence on the adenyl cyclase system, can be avoided. Initial rates of adenyl cyclase activity were maintained for at least 10 min; changes in rate that occurred can be attributed to factors other than changes in substrate concentration. Indeed, the main advantages of using AMP-PNP in this study was that effects of nucleotides on adenyl cyclase activity could be assessed, at low concentrations of substrate, with certainty that the effects were not due to changes in substrate concentration.

A major finding was that guanyl nucleotides (GTP, GDP, and GMP-PCP) stimulated, by an immediate action, the response of adenyl cyclase to glucagon. Hormonal response was dependent upon the presence of guanyl nucleotides either when the magnesium ion concentration was lowered from 4.0 to 2.0 mM or after incubation of the liver membranes. The basis for these conditions leading to dependence of hormone response on guanyl nucleotides is under current investigation. An important point is that the response of adenyl cyclase to glucagon required as little as 10 nM GTP. Only the guanyl nucleotides stimulated the hormonal response of adenyl cyclase at concentrations less than 0.1 mM, indicating that the hormone-activation process is relatively specific for guanyl nucleotides. The low concentrations required, the equivalent effects of GTP and GDP, and the similar action of GMP-PCP, a nonphosphorylating analogue of GTP, on the response of adenyl cyclase to glucagon suggest that the guanyl nucleotides regulate this process through binding but not through phosphorylation.

The present studies were initiated because of the previous findings (1) that guanyl nucleotides affected the binding sites for glucagon in a manner that results in enhanced dissociation of bound hormone, and in decreases in both the apparent affinity of the binding sites for glucagon and in the amount of glucagon bound. The guanyl nucleotides stimulated release of the total quantity of glucagon bound, indicating that all of the binding sites were affected by nucleotides. It was also shown that the binding sites were specific for glucagon and that these sites have the same apparent affinity for glucagon as does the process involved in activation of adenyl cyclase (2). It was also found that inhibition of binding by such agents as urea, detergents, and phospholipase A leads to inhibition of activation of adenyl cyclase by glucagon (2). Taken *in toto*, the previous studies indicate that all of the binding sites share characteristics with the initial process through which glucagon activates adenyl cyclase and can be appropriately termed "discriminator," as defined previously to be that material which is involved in the primary action of glucagon (2). It remains unknown how binding of glucagon to its discriminator, which appears to be a separate entity from adenyl cyclase (5), is potentially translated into activation of adenyl cyclase.

The findings that guanyl nucleotides alter both the binding of glucagon and the response of the enzyme to the hormone offer another means of relating binding to the hormone-activation process. There appears to be some relationship between the effects of guanyl nucleotides on glucagon binding and hormone activation as evidenced by the following correlations. (*a*) Both processes are relatively specific for guanyl nucleotides; UTP, CTP, and ATP act only at concentrations greater than 0.1 mM; (*b*) GTP and GDP are equally effective, GMP-PCP is relatively less effective on hormone binding and activation; and (*c*) the nucleotides exert their effects on both processes independently of the concentration of glucagon in the medium. Such correlates do not establish how these effects of the guanyl nucleotides are interrelated, but indicate that both processes are affected specifically by guanyl nucleotides through sites that are independent of the sites reacting with glucagon. Since, under appropriate incubation conditions, both glucagon and guanyl nucleotides are required for activation of adenyl cyclase, it appears that there are two regulatory sites, requiring the binding, respectively, of glucagon and guanyl nucleotides for activation of the enzyme.

In a previous study (5), it was shown that glucagon and fluoride ion activate adenyl cyclase in liver membranes through processes that have different characteristics, indicating that fluoride ion does not operate through the discriminator. It was of interest, therefore, to find that guanyl nucleotides, specifically and at low concentrations, inhibited the response of adenyl cyclase to fluoride ion. GMP-PCP inhibited the fluoride response over the same range of concentrations as it stimulated the response of adenyl cyclase to glucagon. Furthermore, GMP-PCP inhibited the fluoride response in liver membranes treated with phospholipase A under conditions that abolished both glucagon binding and activation of adenyl cyclase by the hormone. The specificity of action of guanyl nucleotides and the similar concentration ranges over which the nucleotides alter glucagon binding, hormonal activation of adenyl cyclase, and inhibition of fluoride response suggest that the effects are related, possibly by a common action. However, the site and mode of action of guanyl nucleotides on this complex adenyl cyclase system remains obscure.

These studies were initiated on the premise, widely held, that hormone receptors receive and transmit information imparted by the hormone to its target cell depending only upon the circulating levels of the hormone. This premise seems untenable for glucagon in view of the finding that guanyl nucleotides play an obligatory role in regulating the response of liver adenyl cyclase to glucagon. Further studies of the actions of the guanyl nucleotides on the liver and other adenyl cyclase systems may provide new insights into not only how hormones regulate target cell metabolism at the receptor level but also how a target cell metabolite regulates the initial response to a hormone.

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