The role of a guanine nucleotide-binding protein in the activation of rat liver plasma-membrane adenylate cyclase by forskolin

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1. The effects of the photoreactive GTP analogue GTP- γ -azidoanilide on rat liver plasma-membrane adenylate cyclase are described. U.v. irradiation in the presence of the analogue abolished activation by any effector or combination of effectors that function via the activatory G protein. Partial protection against this inhibition was given by F⁻ and guanosine 5'-[γ -thio]triphosphate. It is concluded that GTP- γ -azidoanilide acts by a light-induced covalent reaction with the G protein. In the dark the effects of the analogue were similar to those of GTP. 2. Irradiation in the presence of GTP- γ -azidoanilide was found to reduce but not to abolish activation of rat liver plasma membrane adenylate cyclase by forskolin. The activation by forskolin and GTP together were greater than the sum of the individual activations. Forskolin doubled adenylate cyclase activity in the presence of glucagon and guanosine 5'-[β , γ -imido]triphosphate, which might be expected to activate to the maximum possible extent via the G protein. 3. It is concluded that there are two components to the forskolin activation, a guanine nucleotide-dependent and a guanine nucleotide-independent component.

It is well established that both activation and inhibition of adenylate cyclase by hormones require guanine nucleotides. Under physiological conditions this requirement is fulfilled by GTP. The effects of guanine nucleotides have been shown to be mediated via a binding protein that is distinct from both the catalytic component of adenylate cyclase and the hormone receptor. It appears likely that activatory and inhibitory hormone responses make use of different classes of guanine nucleotide binding proteins or G proteins (Rodbell, 1980; Hildebrandt *et al.*, 1982; Stiles & Lefkowitz, 1982; Cooper, 1983).

During the last 2 or 3 years there has been considerable interest in the mechanism of action of the diterpene forskolin, which activates adenylate cyclase from a number of sources (Seaman & Daly, 1981*a*). Forskolin is able to activate adenylate cyclase in *cyc*⁻ S49 lymphoma cell plasma membranes. This preparation lacks a functional activatory G protein (Ross & Gilman, 1977). The ability of forskolin to activate implies that it acts directly upon the catalytic component with no requirement

for guanine nucleotides or a G protein (Seaman & Daly, 1981*b*). Forskolin has also been reported to activate a preparation of rabbit liver adenylate cyclase catalytic unit that was free of G protein (Ross, 1982). However, the extent of activation by forskolin in these preparations that lack G protein is less than the activation in plasma membranes where the activatory G protein function is intact. Seaman *et al.* (1981) also reported that forskolin activation of adenylate cyclase in cerebral cortex plasma membranes was potentiated by GTP, although there was substantial activation in the absence of added GTP.

The photoreactive GTP analogue GTP- γ -azidoanilide [guanosine(5')triphospho(5')-4-azidoanilide] has been used to label GTP-binding proteins in pigeon erythrocyte plasma membranes (Pfeuffer, 1977). In the dark this analogue was able to substitute for GTP in mediating hormonal activation of adenylate cyclase. U.v. irradiation in the presence of the analogue led to a covalent reaction with the G protein and results in a marked inhibition of all guanine nucleotide-mediated effects. This presumably reflects the blocking of the guanine nucleotide binding site on the activatory G protein. In the present study we have used this analogue as a means of irreversibly inhibiting the func-

Abbreviations used: GTP[S], guanosine 5'-[γ -thio]triphosphate; p[NH]ppG, guanosine 5'-[β , γ -imido]triphosphate.

tion of the G protein. This allowed us to examine the extent to which guanine nucleotides acting through a G protein are required for the forskolin activation of adenylate cyclase.

Experimental

Materials

ATP, cyclic AMP, GTP, p[NH]ppG, GTP[S], phosphocreatine and creatine kinase were obtained from Boehringer (London), Lewes, East Sussex, U.K. Cyclic [^3H]AMP and [^{32}P]P, were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. [$\alpha\text{-}^{32}\text{P}$]ATP was prepared by the method of Martin & Voorheis (1977). 4-Aminoacetanilide was obtained from Hopkins and Williams. *N'*-(3-Dimethylaminopropyl)-*N*-ethylcarbodi-imide hydrochloride was obtained from BDH. GTP- γ -azidoanilide was prepared as described by Pfeuffer (1977) from 4-azidoaniline, which was prepared by the method of Silberrad & Smart (1906). The purity of GTP- γ -azidoanilide was checked by paper chromatography (Girshovich *et al.*, 1976) and by t.l.c. on PEI plates (Pfeuffer, 1977).

Methods

Rat liver plasma membranes were prepared by the method of Pilkis *et al.* (1974).

Adenylate cyclase activity was determined by the method of Salomon *et al.* (1974). Assay incubations were at 30°C for 15 min in a total volume of 0.1 ml containing 25 mM-Tris/HCl, pH 7.4, 10 mM-MgCl₂, 1 mM-dithiothreitol, 0.5 mM-cyclic AMP, 1.0 mM-ATP, [$\alpha\text{-}^{32}\text{P}$]ATP (1 μCi /assay), 5 mM-phosphocreatine and 50 units of creatine kinase/ml.

Photoreaction with GTP- γ -azidoanilide was performed as follows. Rat liver plasma membranes were incubated at 4°C at a final concentration of 1 mg of protein/ml. The medium contained 25 mM-Tris/HCl, pH 7.4, 0.5% bovine serum albumin and when present 250 μM -GTP- γ -azidoanilide. Suspensions were irradiated at 4°C with a Hanovia mercury lamp equipped with a cut-off filter at 366 nm.

Photoreaction was terminated by removing the sample from the u.v. light source. Assays were then performed in the dark. In many of the experiments a photoreaction of 1 h was employed. The effects observed in the absence of protecting agents were always complete by 30 min. After 1 h all the GTP- γ -azidoanilide was photolysed. At the end of the photoreaction period 40 μl of the suspension containing approx. 40 μg of membrane protein was taken into a final volume of 100 μl of cyclase assay mix. Guanine nucleotides when used were present at a concentration of 250 μM in pre-incubation sus-

pensions giving a concentration of 100 μM in the subsequent assay. F⁻ was used at a concentration of 25 mM in pre-incubations giving a final concentration of 10 mM in the adenylate cyclase assay.

Results and discussion

Effect of GTP- γ -azidoanilide on adenylate cyclase activity

Fig. 1 shows the effect of GTP- γ -azidoanilide on the activation of rat liver plasma membrane adenylate cyclase in the presence of 100 nM-glucagon. Half-maximal activation required a slightly higher concentration of GTP- γ -azidoanilide (300 nM) than GTP (50 nM). The extent of activation was very similar, GTP- γ -azidoanilide being slightly more effective than GTP. In the same experiment, basal activity was increased from 14.5 pmol/min per mg of protein to 43.7 pmol/min per mg by 100 μM -GTP and to 46.6 pmol/min per mg by 100 μM -GTP- γ -azidoanilide. The results

Fig. 1. Effect of GTP- γ -azidoanilide on the activity of adenylate cyclase in rat liver plasma membranes in the dark. Rat liver plasma membranes (30 μg /assay) were incubated in the dark as described in the Experimental section. Glucagon (100 nM) was present in all incubations. GTP (○) or GTP- γ -azidoanilide (●) were added as shown in the Figure. Results are means $\pm$ S.E.M. of three separate incubations.

Table 1. *Effect of GTP[S] on the photoinactivation of liver plasma-membrane adenylate cyclase by GTP- γ -azidoanilide*
Liver plasma membranes were incubated as described in the Experimental section for 1 h at 0°C in the dark or with u.v. irradiation. Additions were as shown. Adenylate cyclase activity was then determined as described in the legend to Fig. 1 with additions as shown. Results are means $\pm$ S.E.M. of three separate incubations.

Additions to photoreaction medium	Additions to assay	Adenylate cyclase (pmol/min per mg of protein)	
		No irradiation	U.v.-irradiated
None	None	11.8 $\pm$ 1.1	12.9 $\pm$ 0.05
GTP- γ -azidoanilide (250 μ M)	Glucagon (100 nM)	133.8 $\pm$ 3.9	3.1 $\pm$ 0.36
GTP- γ -azidoanilide (250 μ M)	Glucagon (100 nM) + GTP[S] (100 μ M)	279.7 $\pm$ 11.2	10.9 $\pm$ 0.82
GTP- γ -azidoanilide (250 μ M)	Glucagon (100 nM) + GTP[S] (100 μ M)	306.6 $\pm$ 31.6	182.4 $\pm$ 10.4
GTP[S] (250 μ M)			

show that in the dark GTP- γ -azidoanilide is an effective substitute for GTP.

The effects of u.v. irradiation in the presence of GTP- γ -azidoanilide are shown in Table 1. Irradiation, in the presence of the analogue alone, led to a complete loss of activation by GTP[S] and the activity was reduced below the basal level in untreated membranes. Irradiation in the presence of GTP[S] as well as GTP- γ -azidoanilide resulted in the retention of about half the activity. This protection suggests that the loss of activation by GTP[S] results from a covalent reaction of GTP- γ -azidoanilide with the guanine nucleotide binding site on the G protein. Pfeuffer (1977) reported a much less extensive inactivation of adenylate cyclase in pigeon erythrocyte plasma membranes after irradiation in the presence of GTP- γ -azidoanilide. However, he used a 100-fold lower concentration of the analogue and the incubation medium contained dithiothreitol, which might be expected to react with GTP- γ -azidoanilide (Czarnecki *et al.*, 1979). In the present study we found that the inhibitory effect of photoreaction with GTP- γ -azidoanilide upon the response to GTP[S] was markedly reduced by the presence of dithiothreitol in the reaction medium.

Irradiation in the presence of GTP- γ -azidoanilide reduced the activity of adenylate cyclase to below the basal activity in untreated membranes and the protective effect of GTP[S] was incomplete (Table 1). The reduction in basal activity may reflect the presence of GTP contaminating the assay system so that the basal activity was partly dependent upon such endogenous GTP. The lack of complete protection by GTP[S] may reflect competition by the two analogues for a common binding site. However, both effects could result from the interaction of GTP- γ -azidoanilide with a different site such as the catalytic centre of adenylate cyclase. To test the possibility of an interaction with the catalytic site we examined the effects of adding the substrate, ATP, during the photoreaction.

Fig. 2. *Effects of ATP on photoinactivation of adenylate cyclase by GTP- γ -azidoanilide*

Rat liver plasma membranes were incubated in the presence of GTP- γ -azidoanilide and u.v. light for 1 h at 4°C as described in the Experimental section. Various concentrations of ATP were present. Adenylate cyclase activity was determined as described in the Experimental section except that the ATP concentration was 4 mM. GTP[S] (100 μ M; ○) or forskolin (100 μ M; ●) was present in the adenylate cyclase assay medium. The activity of adenylate cyclase in untreated membranes with no additions is given by □. Results are means $\pm$ S.E.M. of three separate incubations.

Effects of ATP on photoinactivation of adenylate cyclase by GTP- γ -azidoanilide

Fig. 2 shows the effects of adding various concentrations of ATP during u.v. irradiation in the presence of GTP- γ -azidoanilide on the ability of GTP[S] and forskolin to activate adenylate cyclase. As the ATP concentration was increased, adenylate cyclase activity in the presence of GTP[S] increased until it was comparable with, but did not

Table 2. *Effect of GTP, GTP[S] and forskolin on the photoinactivation of rat liver adenylate cyclase by GTP- γ -azidoanilide in the presence of ATP*

Rat liver plasma membranes were irradiated for 1 h at 4°C in the presence of ATP (3.0 mM) and GTP- γ -azidoanilide (250 μ M) as described in the Experimental section. Further additions to the pre-incubation and assay media were as shown in the Table. Results are means $\pm$ S.E.M. of three separate incubations.

Additions during u.v. irradiation	Additions to assay	Adenylate cyclase (pmol/min per mg of protein)
None	None	9.03 $\pm$ 0.29
GTP- γ -azidoanilide (250 μ M)	None	9.22 $\pm$ 0.44
None	GTP (100 μ M)	30.09 $\pm$ 2.24
GTP- γ -azidoanilide (250 μ M)	GTP (100 μ M)	12.0 $\pm$ 1.4
GTP (250 μ M)	GTP (100 μ M)	30.9 $\pm$ 0.125
GTP- γ -azidoanilide (250 μ M), GTP (250 μ M)	GTP (100 μ M)	16.2 $\pm$ 1.25
None	F ⁻ (10 mM)	98.1 $\pm$ 3.9
GTP- γ -azidoanilide (250 μ M)	F ⁻ (10 mM)	23.0 $\pm$ 1.7
F ⁻ (25 mM)	F ⁻ (10 mM)	117.4 $\pm$ 4.2
GTP- γ -azidoanilide (250 μ M), F ⁻ (25 mM)	F ⁻ (10 mM)	38.2 $\pm$ 1.5
None	GTP[S] (100 μ M)	65.7 $\pm$ 5.2
GTP- γ -azidoanilide (250 μ M)	GTP[S] (100 μ M)	15.7 $\pm$ 0.51
GTP[S] (250 μ M)	GTP[S] (100 μ M)	66.1 $\pm$ 0.78
GTP- γ -azidoanilide (250 μ M), GTP[S] (250 μ M)	GTP[S] (100 μ M)	36.8 $\pm$ 1.45
None	Forskolin (100 μ M)	106.6 $\pm$ 3.9
GTP- γ -azidoanilide (250 μ M)	Forskolin (100 μ M)	52.6 $\pm$ 1.3
Forskolin (100 μ M)	Forskolin (100 μ M)	179.9 $\pm$ 8.3
GTP- γ -azidoanilide (250 μ M), forskolin (100 μ M)	Forskolin (100 μ M)	91.2 $\pm$ 3.9

exceed, the basal activity in untreated membranes. However, activation by forskolin was increased from about 2-fold when no ATP was present in the photoreaction incubation to 10-fold when 3 mM-ATP was added. This suggests that GTP- γ -azidoanilide is able to react with the catalytic centre of adenylate cyclase and that this can be prevented by the addition of ATP. It also suggests that forskolin does not require a functional G protein to activate adenylate cyclase.

In view of these observations all further experiments were performed with the addition of 2.5 mM-ATP to the photoreaction medium. Table 2 shows the effects of adding GTP, F⁻, GTP[S] and forskolin after incubation in the presence of GTP- γ -azidoanilide together with ATP. The activation by GTP, GTP[S] and F⁻ was reduced to between 12 and 17% of the activation seen in membranes that were treated identically except that GTP- γ -azidoanilide was omitted. The activation by forskolin was reduced to 45% of the control. In this experiment basal activity was not affected by photoreaction in the presence of GTP- γ -azidoanilide. The effects of pre-incubating liver plasma membranes in the presence of GTP, F⁻, GTP[S] or forskolin before u.v. irradiation in the presence of GTP- γ -azidoanilide and ATP were also tested. Pre-incubation with GTP led to the retention of about 30% of the activation, pre-incubation with F⁻ to the retention of about 28% of the activation, pre-incubation with GTP[S] to the retention of 50% and

pre-incubation with forskolin to the retention of 63% of the activation. The results suggest that ATP was able to protect the catalytic site of adenylate cyclase from reaction with GTP- γ -azidoanilide since basal activity was unaffected. GTP[S] protected substantially against inactivation and GTP and F⁻ also gave some protection. The ability of F⁻ to protect is interesting since it implies that the interaction of F⁻ with the G protein affects the binding of guanine nucleotides. Activation by forskolin was much more resistant to the effects of photoreaction with GTP- γ -azidoanilide than activation by the other three agents known to act via the activatory G protein. However, the forskolin activation was still substantially reduced and pre-incubation with forskolin protected against this inactivation to some extent. This suggests that the action of forskolin may require, at least in part, a functional activatory G protein.

Effects of temperature

All the incubations in the presence of GTP- γ -azidoanilide described above were performed at 0°C. At this temperature there is little activation of rat liver plasma membrane adenylate cyclase by guanine nucleotides (Martin *et al.*, 1979). Krall *et al.* (1982) have shown that the binding of p[NH]-ppG is reversible at 0°C but irreversible at higher temperatures. We might expect GTP- γ -azidoanilide therefore to react more effectively with adenylate cyclase at 30°C than at 0°C. This is difficult to

Table 3. *Effect of temperature on the protection against photoinactivation of adenylate cyclase by GTP[S] and p[NH]ppG*

Rat liver plasma membranes were pre-incubated at 0°C or 30°C for 10 min in a medium containing 25 mM-Tris/HCl, pH 7.4, and 0.5% bovine serum albumin. GTP[S] or p[NH]ppG were added as shown at 500 µM. The membrane suspensions were diluted to give a final concentration of 1 mg of protein/ml, 250 µM-GTP[S] or -p[NH]ppG. GTP-γ-azidoanilide (250 µM) was added as shown and the suspension was irradiated for 1 hr at 4°C as described in the Experimental section and assayed for adenylate cyclase activity as described in the Experimental section with a guanine nucleotide concentration of 100 µM when present. Results are means ± s.e.m. of three separate incubations.

Pre-incubation temperature (°C)	Additions to pre-incubation	Additions during u.v. irradiation	Additions to assay	Adenylate cyclase (pmol/min per mg of protein)
0	None	None	None	28.8 ± 2.4
0	None	GTP-γ-azidoanilide (250 µM)	None	17.9 ± 1.3
0	None	None	GTP[S] (100 µM)	98.1 ± 5.5
0	None	GTP-γ-azidoanilide (250 µM)	GTP[S] (100 µM)	20 ± 2.5
0	GTP[S] (500 µM)	GTP[S] (250 µM)	GTP[S] (100 µM)	119.7 ± 2.3
0	GTP[S] (500 µM)	GTP[S] (250 µM), GTP-γ-azidoanilide (250 µM)	GTP[S] (100 µM)	58.1 ± 3.2
0	None	None	p[NH]ppG (100 µM)	111.9 ± 6.0
0	None	GTP-γ-azidoanilide (250 µM)	p[NH]ppG (100 µM)	13.7 ± 2.7
0	p[NH]ppG (500 µM)	p[NH]ppG (250 µM)	p[NH]ppG (100 µM)	127.7 ± 7.0
0	p[NH]ppG (500 µM)	p[NH]ppG (250 µM), GTP-γ-azidoanilide (250 µM)	p[NH]ppG (100 µM)	21.1 ± 6.3
30	None	None	None	19.85 ± 1.1
30	None	GTP-γ-azidoanilide (250 µM)	None	21.6 ± 1.7
30	None	None	GTP[S] (100 µM)	80.5 ± 4.9
30	None	GTP-γ-azidoanilide (250 µM)	GTP[S] (100 µM)	18.1 ± 1.3
30	GTP[S] (500 µM)	GTP[S] (250 µM)	GTP[S] (100 µM)	114.9 ± 9.2
30	GTP[S] (500 µM)	GTP[S] (250 µM), GTP-γ-azidoanilide (250 µM)	GTP[S] (100 µM)	71.4 ± 4.1
30	None	None	p[NH]ppG (100 µM)	71.1 ± 6.9
30	None	GTP-γ-azidoanilide (250 µM)	p[NH]ppG (100 µM)	21.9 ± 1.3
30	p[NH]ppG (500 µM)	p[NH]ppG (250 µM)	p[NH]ppG (100 µM)	104.6 ± 3.8
30	p[NH]ppG (500 µM)	p[NH]ppG (250 µM), GTP-γ-azidoanilide (250 µM)	p[NH]ppG (100 µM)	38.9 ± 3.2

test, however, since the analogue is rapidly hydrolysed under the conditions of photoreaction with liver plasma membrane at 30°C. We might also expect that the effectiveness of p[NH]ppG as a protecting agent should be greater at 30°C. Table 3 shows the effect of pre-incubating liver plasma membranes with p[NH]ppG and GTP[S] at 0°C and 30°C before photoreaction with GTP-γ-azidoanilide at 0°C in the presence of ATP. Protection by GTP[S] was increased and protection by p[NH]ppG increased markedly by pre-incubation at 30°C rather than at 0°C. These results reinforce the view that the effects of GTP-γ-azidoanilide result from a specific interaction with the G protein.

Protection by GTP

The protection against photoreaction with GTP-γ-azidoanilide by the presence of GTP was small (Table 2). At first sight this is surprising since GTP appears to have higher affinity for the system than GTP-γ-azidoanilide (Fig. 1). A likely explanation lies in the nature of the two interactions. GTP-γ-

azidoanilide reacts covalently and irreversibly with the G site, whereas GTP can turn over on the site to GDP and P_i and at 0°C probably binds reversibly. It is to be expected therefore that, given sufficient time, reaction with GTP-γ-azidoanilide should completely block the GTP binding site whether or not GTP is present, but in the presence of GTP the inactivation should be slower. Fig. 3 shows the effect of GTP on the rate of inactivation by photoreaction with GTP-γ-azidoanilide. In both cases inactivation was complete after 30 min but the rate of inactivation was slower in the presence of GTP. The tight-binding analogue GTP[S] was even more effective in slowing down the rate of inactivation and its effectiveness was improved by pre-incubation at 30°C.

Effects of forskolin

The results presented in Table 2 suggest that there may be two components to the activation of adenylate cyclase by forskolin, one dependent upon the G protein and one independent of the G protein. Two further experiments were done to

Fig. 3. Effect of GTP and GTP[S] on the time course of photoinactivation by GTP- γ -azidoanilide

Liver plasma membranes were inactivated in the presence of ATP (2.5 mM) and GTP- γ -azidoanilide (330 μ M) at 4°C as described in the Experimental section. ○, No additions during photoreaction and GTP (100 μ M) present in adenylate cyclase assay; □, no additions during photoreaction and GTP[S] (100 μ M) present in the adenylate cyclase assay; ●, pre-incubation with GTP (1.67 mM) at 0°C for 10 min before photoreaction, and dilution into photoreactive medium to give GTP (250 μ M) and finally GTP (100 μ M) in the adenylate cyclase assay; ■, pre-incubation with GTP[S] (0.55 mM) at 0°C, for 10 min dilution to give GTP[S] (250 μ M) during photoreaction, and GTP[S] (100 μ M) in the adenylate cyclase assay; △, pre-incubation with GTP[S] as above but at 30°C, cooled to 4°C for photoreaction. Results are means $\pm$ S.E.M. of three separate incubations.

investigate this possibility. Table 4 shows the effect of forskolin on liver plasma-membrane adenylate cyclase in the presence and in the absence of glucagon and guanine nucleotides. In the absence of any other effectors forskolin gave a 15-fold activation. The activation was not significantly different in the presence of glucagon, implying that the glucagon receptor does not directly influence the forskolin response. In the presence of GTP the activation by forskolin was increased synergistically, suggesting that an activated G protein is able to potentiate the forskolin response. In

Fig. 4. Time course of photoinactivation by GTP- γ -azidoanilide

Rat liver plasma membranes were irradiated in the presence of ATP (3.0 mM) and GTP- γ -azidoanilide (330 μ M) for various periods of time as described in the Experimental section. Adenylate cyclase activity was determined in the presence of GTP[S] (100 μ M; ●), forskolin (100 μ M; ○) or no additions (■). The adenylate cyclase of untreated membrane with no additions is given by □. Results are means $\pm$ S.E.M. of three separate incubations.

the presence of p[NH]ppG, together with glucagon, forskolin doubled the adenylate cyclase activity. However, the increase in activity owing to forskolin was little greater than the increase in activity in the absence of other effectors.

Fig. 4 shows the effect of irradiation in the presence of GTP- γ -azidoanilide on the ability of adenylate cyclase to respond to forskolin and GTP[S]. As the length of the irradiation was increased the activation by GTP[S] was abolished. The ability of forskolin to activate declined with a similar time course. The activation was not, however, abolished but reduced from about 26-fold to 8-fold.

General discussion

The results suggest two main conclusions. In the dark GTP- γ -azidoanilide was able to substitute for GTP in allowing the activation of adenylate

Table 4. *Effect of glucagon and guanine nucleotides on the activation of liver adenylate cyclase by forskolin*
Adenylate cyclase activity was determined as described in the Experimental section with further additions as shown. Results are means $\pm$ S.E.M. of three separate incubations.

Additions to assay	Adenylate cyclase activity (pmol/min per mg of protein)	
	No additions	Forskolin (100 μ M)
None	12.3 $\pm$ 1.2	179.0 $\pm$ 9.8
GTP (100 μ M)	32.8 $\pm$ 0.92	265.0 $\pm$ 8.1
p[NH]ppG (100 μ M)	110.0 $\pm$ 4.8	309.0 $\pm$ 14.6
Glucagon (100 nM)	27.1 $\pm$ 0.77	190 $\pm$ 3.6
Glucagon (100 nM), GTP (100 μ M)	104.0 $\pm$ 3.0	328 $\pm$ 21.0
Glucagon (100 nM), p[NH]ppG (100 μ M)	196.0 $\pm$ 13.0	402 $\pm$ 13.0

cyclase via the activatory G protein (Fig. 1). U.v. irradiation in the presence of GTP- γ -azidoanilide blocked the ability of guanine nucleotides to activate adenylate cyclase presumably by a covalent interaction with the guanine nucleotide-binding protein (Tables 1, 2 and 3; Figs. 2 and 3).

Inhibition of the guanine nucleotide response by irradiation in the presence of GTP- γ -azidoanilide reduced but did not abolish the activation of adenylate cyclase by forskolin (Table 2; Fig. 4) and activation of adenylate cyclase by forskolin was potentiated by GTP (Table 4).

In the presence of glucagon together with p[NH]ppG liver plasma-membrane adenylate cyclase displays the maximum activity that can be achieved by activation via the activatory G protein (Rodbell *et al.*, 1975). Forskolin was able to double this activity (Table 2).

The results suggest that there are two components to the action of forskolin on adenylate cyclase. One component of the effect requires that the activatory G protein be occupied by GTP. The other component of the effect does not require the G protein or at least does not require that the G protein contain bound guanine nucleotide.

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