ELSEVIER

FEBS Letters 343 (1994) 208–212

FEBS 13940

FEBS LETTERS

Activated $G_{s\alpha}$ but not $G_{i\alpha}$ prevents the thermal inactivation of adenylyl cyclase in plasma membranes derived from S49 lymphoma cells

Petr Kvapil, Jiri Novotny, Lennart A. Ransnäs*

Wallenberg Laboratory for Cardiovascular Research, University of Gothenburg, S-41336 Gothenburg, Sweden

Received 23 November 1993; revised version received 12 March 1994

Abstract

The thermal inactivation of adenylyl cyclase was studied in plasma membranes isolated from wild-type and the mutant cell strain cyc$^-$ of S49 lymphoma. The half-life of adenylyl cyclase activity at 30°C was decreased from 14.2 min to 3.4 min by the presence of detergents. ATP as well as forskolin prevented the adenylyl cyclase inactivation in a dose–response manner independent of the utilized type of cell membranes. Activation of G-proteins by GTPγS or by AlF_4^- in wild-type membranes but not in cyc$^-$ membranes partially prevented adenylyl cyclase inactivation. Adenylyl cyclase activity in cyc$^-$ membranes was preserved in the presence of GTPγS or AlF_4^- from the observed detergent-induced inactivation by complementation of these membranes with an extract from wild-type membranes. ADP-ribosylation of $G_{i\alpha}$ in cyc$^-$ membranes did not influence the kinetics of the inactivation process of adenylyl cyclase, whereas ADP-ribosylated $G_{s\alpha}$ protein protected adenylyl cyclase more effectively than non-ribosylated $G_{s\alpha}$ in wild-type plasma membranes when GTP was used as an activator.

Key words: Adenylyl cyclase; Thermal inactivation; Forskolin; G_s and G_i proteins

1. Introduction

Adenylyl cyclases (EC 4.6.1.1.) detected in S49 lymphoma cells (types VI and VII) belong to a family of high eucaryotic cyclases which consist of an amino-terminal cytoplasmatic region and two cytoplasmatic domains punctuated by two transmembrane stretches [1–3]. All cytoplasmic domains are of high importance for production of cAMP. The activity of mammalian adenylyl cyclases can be modulated by the subunits of G proteins, by Ca^{2+}-calmodulin complex, and by forskolin [4–6].

G proteins composed of α and $\beta\gamma$ subunits are central components of the majority of the transmembrane signalling pathways [7–9]. G proteins are classified according to the type of their α subunit [10]. Different α as well as $\beta\gamma$ subunit types have been shown to be involved in the transduction of distinctly different signals in the same cell. Binding of a receptor agonist to a receptor catalyses activation of the coupled G-protein by increasing the rate of GDP-release followed by binding of GTP to the α subunit. Binding of GTP is thought to cause dissociation of the heterotrimeric G-protein into its components and the liberated subunits then modulate activities of related effector systems [10,11]. This process is terminated by hydrolysis of the G-protein-bound GTP to GDP by the intrinsic GTPase activity in the α subunit [10] and by reassociation of the α-GDP complex and $\beta\gamma$ subunits. G-stimulatory (G_s) and G-inhibitory (G_i) proteins are designated according to their roles in modulation of the activity of adenylyl cyclase [10]. Whereas the stimulatory effect of G_s has been well characterized and is thought to be due to direct interaction of adenylyl cyclase and $G_{s\alpha}$, the mechanism of the inhibition of adenylyl cyclase mediated by G_i remains largely unresolved [6,12–14].

Adenylyl cyclase activity declines over time during exposure to an elevated temperature irrespective of the presence or absence of detergent [15]. In this study, we describe the time course of the thermal inactivation of adenylyl cyclase and the effect of various agents as ATP (substrate), forskolin (direct activator) and activated G_α proteins ('coenzyme'). ATP as well as forskolin protected adenylyl cyclase against the thermal inactivation in a dose–response manner. Activated $G_{s\alpha}$ prevented the adenylyl cyclase inactivation, whereas neither G_s protein in the GDP-liganded form nor $G_{i\alpha}$ in GDP- or GTP-liganded forms influenced this process.

* Corresponding author.

Abbreviations: adenylyl cyclase, ATP pyrophosphate-lyase (cyclizing) (EC 4.6.1.1.); G protein, GTP-binding regulatory protein; G_i and $G_{i\alpha}$, the inhibitory G protein and its α subunit, respectively; G_s and $G_{s\alpha}$, the stimulatory G protein and its α subunit, respectively; GDPβS, guanosine-5′-*O*-(2-thiodiphosphate); GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); wt and cyc$^-$ cells, S49 lymphoma wild-type and cyc$^-$cells, respectively.

SSDI 0014-5793(94)00325-P

2. Materials and methods

2.1. Preparation of S49 lymphoma wt and cyc⁻ plasma membranes

Plasma membranes were isolated as described by Ross et al. [16], with some modifications. The cells were harvested and washed in PBS buffer at room temperature. All subsequent steps were performed at 4°C. The cells were resuspended in homogenization buffer (20 mM HEPES pH 8.0, 2 mM $MgCl_2$, 1 mM EDTA and 150 mM NaCl) and equilibrated with N_2 at 500 psi in a Parr cell disruption chamber for 30 min. Rapid decompression produced a cell lysate, which was centrifuged at $1,200 \times g$ for 10 min. Plasma membranes were prepared by sucrose-gradient ultracentrifugation. Eight ml of low speed centrifugation supernatant was layered on the top of a discontinuous sucrose density gradient (20%–33%–40% (w/w) sucrose in HME buffer (20 mM HEPES pH 8.0, 2 mM $MgCl_2$ and 1 mM EDTA) and spun at $100,000 \times g$ in a swing-out rotor for 1 h. The bands occurring at 20 to 33.3% and 33.3 to 40% sucrose interfaces (purified plasma membranes) were collected, diluted in HME buffer, and pelleted at $100,000 \times g$ for 45 min. Plasma membranes were resuspended by re-pipetting in HME buffer, frozen in liquid nitrogen, and stored at −80°C.

2.2. Extraction of membranes with sodium cholate

Plasma membranes were extracted for 1 h at 4°C in 1% sodium cholate. The extracts were spun down at $100,000 \times g$ at 4°C for 30 min and resulted cyc⁻ or wt membrane pellets were resuspended by repipetting in HME buffer. The supernatants were used as membrane extracts. The extraction procedure was performed separately in each experiment.

2.3. Protein concentration

Protein concentration was determinated as described by Lowry et al. [17].

2.4. Adenylyl cyclase activity

The activity of adenylyl cyclase was measured as described previously [18], with some modifications. Briefly, plasma membranes (35 μg) were diluted with HME buffer containing lubrol PX and sodium cholate or sodium cholate extracts were added. Preservatives (ATP, forskolin, GTPγS, AlF_4^-, GDPβS, 1,9-dideoxyforskolin and cAMP, respectively) dissolved in H_2O were added in appropriate concentrations and the mixtures (30 μl) were preincubated at 30°C (or at indicated temperature) for 10 min (or for indicated time period). The concentration of detergents under this 'inactivation procedure' was 0.033% lubrol PX and 0.3% sodium cholate. Twenty μl of buffer A (1.65 mM ATP, 165 μM forskolin, 50 μg/ml pyruvate kinase, 33 mM potassiumphosphoenolpyruvate, 2 mM $MgCl_2$, 500 μg/ml BSA, and 90 mM HEPES pH 8.0) and aqueous solution (10 μl) of preservatives (to equilibrate their respective levels) were added, and the adenylyl cyclase assay was started by addition of 40 μl of buffer B ([α-^{32}P]ATP (about $1–2 \times 10^6$ cpm), 0.025 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidion, 4 mM EDTA and 2 mM $MgCl_2$). The assay was performed at 30°C for 30 min. The reaction was stopped by the addition of 100 μl of stop buffer (0.3% SDS, 4 mM ATP and 0.14 mM [^{3}H]cAMP (about 2×10^5 cpm)) and boiling at 95°C for 3 min. cAMP was separated using the Dowex-Alumina chromatographic system.

2.5. ADP-ribosylation of $G_{i\alpha}$ by pertussis toxin

ADP-ribosylation was performed as described previously [19], with some modifications. Plasma membranes (1 mg/ml) were incubated in 1.2 ml of buffer C (10 mM thymidine, 400 μM ATP, 1 mM GTP, 150 μM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 67 μM AMP-PNP, 0.05% BSA, 10 μM β-NAD and 7.5 mM Tris-HCl pH 7.5) at 30°C for 45 min in the presence or absence of activated pertussis toxin (0.0035 mg/ml). The reaction was stopped by addition of 1 ml of ice-cold HME buffer and the membranes were spun down ($100,000 \times g$, 4°C, 30 min), washed and resuspended in HME buffer.

2.6. ADP-ribosylation of $G_{s\alpha}$ by cholera toxin

ADP-ribosylation was performed as described previously [20], with some modifications. Plasma membranes (1 mg/ml) were incubated in 1.2 ml of buffer D (10 mM thymidine, 1 mM ATP, 1 mM GTP, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.05% BSA, 10 μM β-NAD and 300 mM Na/PO_4 buffer pH 7.0) at 30°C for 60 min in the presence or absence of activated cholera toxin (0.033 mg/ml). The reaction was stopped by addition of 1 ml of ice-cold HME buffer and the membranes were spun down ($100,000 \times g$, 4°C, 30 min), washed and resuspended in HME buffer.

2.7. Reagents

All chemicals were purchased from Sigma and of the highest analytical grade commercially available. [α-^{32}P]ATP and [^{3}H]cAMP were purchased from Amersham.

3. Results

3.1. The effect of detergents on the adenylyl cyclase inactivation

The time course of the thermal inactivation of adenylyl cyclase was investigated under various conditions. Adenylyl cyclase was completely inactivated within 25 min at physiological temperature (37°C) in the absence of lubrol PX and sodium cholate but as early as within 10 minutes in the presence of these detergents (Fig. 1A and B). The presence of detergents during the inactivating step increased the sensitivity of adenylyl cyclase to inactivation and had an additional effect on the temperature-dependent inactivation. The half-life of adenylyl cyclase in plasma membranes (35 μg) at 30°C (14.2 min) decreased markedly in the presence of 0.033% lubrol PX and 0.33% sodium cholate (3.4 min). The role of protein concentration in the inactivation kinetics of adenylyl cyclase was significant at lower temperatures (up to 20°C), but only a week protective effect was observed at higher temperatures (over 20°C) (Fig. 1C). No significant difference was found between the inactivation process of adenylyl cyclase in wt and cyc⁻ plasma membranes.

3.2. The effect of ATP and forskolin on the adenylyl cyclase inactivation

We examined the role of ATP as well as forskolin in the process of thermal inactivation of adenylyl cyclase accelerated by detergents (Fig. 2). ATP (0.05–2.5 mmol/l) and forskolin (10^{-4}–10^{-8} mol/l), respectively, protected adenylyl cyclase against thermal inactivation in a dose-response manner similarly in both wt and cyc⁻ plasma membranes (data not shown). The adenylyl cyclase activity measured after the inactivation procedure (9 ± 4%) was increased in the presence of 0.5 mM ATP (59 ± 4%) or 50 μM forskolin (55 ± 7%) during the inactivating treatment. Adenylyl cyclase activity decreased only by 14 ± 5% when both 0.5 mM ATP and 50 μM forskolin were present during the inactivation procedure.

Other preservatives such as cAMP (product of adenylyl cyclase activity, secondary messenger), 1,9-dideoxyforskolin (analogue of forskolin) or GDPβS failed to change the kinetics of the inactivation process (Fig. 2).

3.3. The role of G-protein subunits in the inactivation process

When GTPγS (10^{-4}–10^{-8} mol/l) was present during the inactivation procedure adenylyl cyclase was protected

against thermal inactivation in a dose-response manner in wt plasma membranes (Fig. 3) but not in cyc$^-$ ones (Fig. 2B). Similar results were measured when AlF_4^- was used as an activator of G-proteins instead of GTPγS (data not shown). Reconstitution of sodium cholate extract from wt plasma membranes with cyc$^-$ membranes in the presence of GTPγS but not in the absence of GTPγS during the inactivation procedure preserved adenylyl cyclase in cyc$^-$ plasma membranes (Fig. 4). This protective effect was dose-dependent on both GTPγS (10^{-4}–10^{-8} mol/l) as well as the wt extract (0–35 μg/sample). Adenylyl cyclase was preserved to the same degree by GTP and GTPγS in wt plasma membranes when $G_{s\alpha}$ had been ADP-ribosylated by cholera toxin (Table 1) prior to the inactivation procedure, whereas in an analogues set of experiments GTPγS but hardly at all GTP induced protection against the effect of detergents in nonribosylated wt plasma membranes (Fig. 2). The cholera toxin catalysed ADP-ribosylation of wt plasma membranes decreased the protective effect of AlF_4^- (Table 1).

The pertussis toxin catalysed ADP-ribosylation of wt as well as cyc$^-$ plasma membranes did not affect the inactivation process of adenylyl cyclase regardless of the used G protein activators (GTP, GTPγS, and AlF_4^-). The results obtained with GTP in cyc$^-$ plasma membranes are shown in Table 1.

Table 1
Effect of ADP-ribosylation of G proteins on the thermal inactivation of adenylyl cyclase

Preservative	Sample	Adenylyl cyclase activity (pmol cAMP × mg^{-1} × min^{-1})	
		cyc$^-$ membranes	
		Non-ribosylated	Ribosylated by PT
None	Control	137 ± 15 (100%)	175 ± 18 (100%)
	Inactivated	56 ± 8 (41%)	71 ± 8 (41%)
GTP	Control	146 ± 7 (100%)	159 ± 12 (100%)
	Inactivated	70 ± 5 (48%)	78 ± 7 (50%)
		wt membranes	
		Non-ribosylated	Ribosylated by CT
GTP	Control	156 ± 7 (100%)	212 ± 14 (100%)
	Inactivated	37 ± 4 (24%)	114 ± 4 (54%)
GTPγS	Control	264 ± 10 (100%)	294 ± 7 (100%)
	Inactivated	156 ± 9 (59%)	178 ± 12 (61%)
ALF_4^-	Control	384 ± 14 (100%)	265 ± 5 (100%)
	Inactivated	168 ± 13 (44%)	64 ± 3 (24%)

cyc$^-$ or wt plasma membranes were treated in the presence or absence of respective toxin as described in section 2. The plasma membranes were then incubated for 5 min (cyc$^-$) or 10 min (wt) at 0° (control) or at 30° (Inactivated) in the presence of detergents and indicated preservative. Afterwards, buffer A and solutions of preservatives (to equilibrate their concentrations) were added and adenylyl cyclase assay was performed. The data represent the means ± S.D. of three independent experiments performed in duplicate.

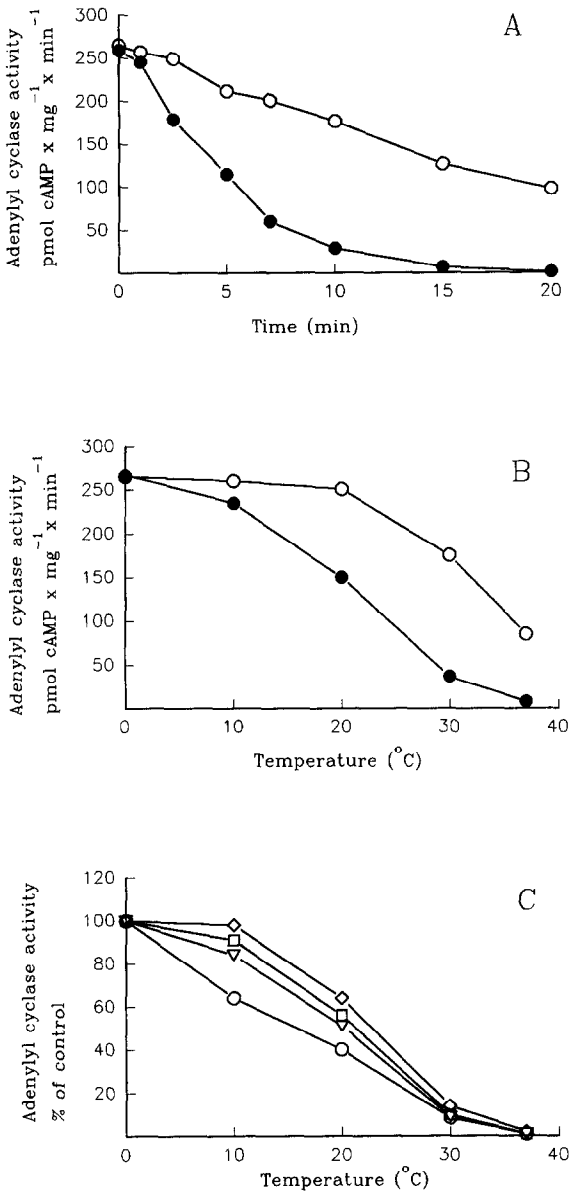

Fig. 1. Time course (A) and effect of temperature (B) and protein concentration (C) on the adenylyl cyclase inactivation. (A) wt plasma membranes were incubated (35 μg) for indicated time periods at 30°C in the absence (○) or presence (●) of detergents. Consequently buffer A and solution of detergents to equilibrate their levels were added and the adenylyl cyclase assay was performed. One representative experiment of four independent ones run in duplicate is shown. (B) wt plasma membranes (35 μg) were incubated for 10 min at indicated temperatures in the absence (○) or presence (●) of detergents and then the adenylyl cyclase assay was performed at 30°C. One representative out of four independent experiments run in duplicate is shown. (C) Before running the adenylyl cyclase assay wt plasma membranes (9 μg, ○; 18 μg, ▽; 27 μg, □; and 35 μg, ◇) in HME buffer (30 μl) were preincubated for 10 min at the indicated temperatures in the presence of detergents. Activities are expressed as percentage of the activities determined in the corresponding control samples (9 μg control: 242 ± 15 pmol cAMP × mg^{-1} × min^{-1}, 18 μg control: 253 ± 7 pmol cAMP × mg^{-1} × min^{-1}, 27 μg control: 282 ± 19 pmol cAMP × mg^{-1} × min^{-1} and 36 μg control: 278 ± 21 pmol cAMP × mg^{-1} × min^{-1}). One representative experiment of four independent experiments run in duplicate is shown. Similar results as shown in panels A,B and C were obtained with cyc$^-$ membranes, but the total activities were decreased by 36 ± 6%.

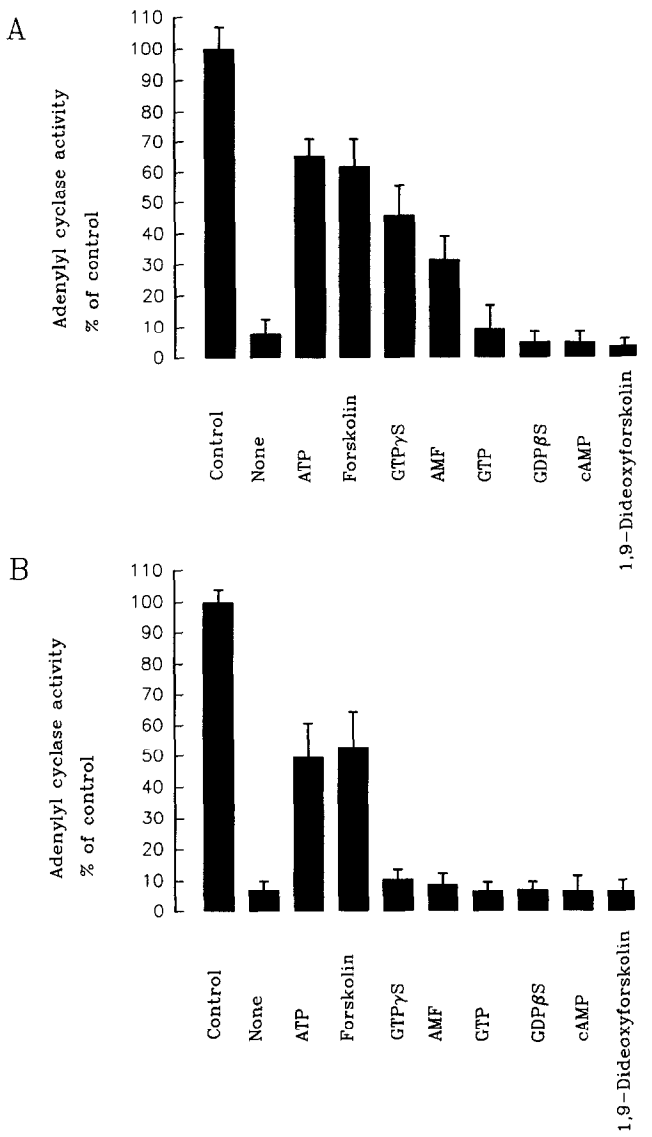

Fig. 2. Effect of different preservatives on the thermal inactivation of adenylyl cyclase in wt (panel A) and cyc$^-$ (panel B) plasma membranes. Plasma membranes (35 μg) prepared from wt and cyc$^-$ cells, respectively, were preincubated for 10 min at 0°C (control samples) or at 30°C in the presence of detergents and indicated preservatives and then the adenylyl cyclase activity was measured. Final concentrations of preservatives were: 100 μM GTPγS, AlF_4^- (10 mM NaF, 10 mM $MgCl_2$ and 15 μM $AlCl_3$), 100 μM GDPβS, 100 μM GTP, 100 mM cAMP, and 50 μM 1,9-dideoxyforskolin. Activities are expressed as percentage of the activities determined in the corresponding control samples. Control activities (pmol cAMP $\times$ mg^{-1} $\times$ min^{-1}) in wt membranes (A) were: 362 ± 14 (without inactivation) and 32 ± 8 (after the inactivating pretreatment without any preservative). The presence of preservatives during the inactivation procedure changed control activities to 288 ± 14 (ATP), 276 ± 12 (forskolin), 578 ± 32 (GTPγS), 436 ± 24 (AlF_4^-), 389 ± 33 (GTP), 264 ± 16 (GDPβS), 258 ± 18 (cAMP) and 83 ± 16 (1,9 dideoxyforskolin), respectively. Control activities in cyc$^-$ membranes (B) were: 242 ± 14 (without inactivation) and 15 ± 6 (after the inactivating pretreatment without any preservative). The presence of preservatives during the inactivation procedure changed control activities to 188 ± 17 (ATP), 196 ± 8 (forskolin), 148 ± 16 (GTPγS), 156 ± 14 (AlF_4^-), 159 ± 9 (GTP), 164 ± 12 (GDPβS), 158 ± 10 (cAMP) and 53 ± 7 (1,9 dideoxyforskolin), respectively. Data represent the mean ± S.D. of three independent experiments performed in duplicate.

4. Discussion

This study describes effects of ATP, forskolin, G proteins and some other factors on the time course of the thermal inactivation of adenylyl cyclase in plasma membranes isolated from S49 lymphoma cells. In the absence of preservatives adenylyl cyclase was inactivated in a time-dependent manner by increased temperature. The presence of detergents accelerated the inactivation process. The protective effect of increased protein concentration on adenylyl cyclase against thermal inactivation was considerably higher at lower temperatures (up to 20°C) than at higher temperatures (20°C–37°C). Adenylyl cyclase was inactivated only slightly during a prolonged storage (several hours) of plasma membranes on ice (approx. 5% per hour, data not shown). No significant difference was found between the enzyme stability characteristics in wt and cyc$^-$ plasma membranes at our experimental conditions.

ATP (substrate of adenylyl cyclase) as well as forskolin (direct activator) reduced the rate of the thermal inactivation of adenylyl cyclase in a dose–response manner. Adenylyl cyclase in plasma membranes (35 μg) was almost completely preserved by 0.5 mM ATP and/or 50 μM forskolin in the absence of detergents, and by 1.25 mM ATP and/or 250 μM forskolin in the presence of 0.033% lubrol PX and 0.33% sodium cholate during the inactivation treatment. Whereas cAMP (product of adenylyl cyclase activity) as well as 1,9 dideoxyforskolin

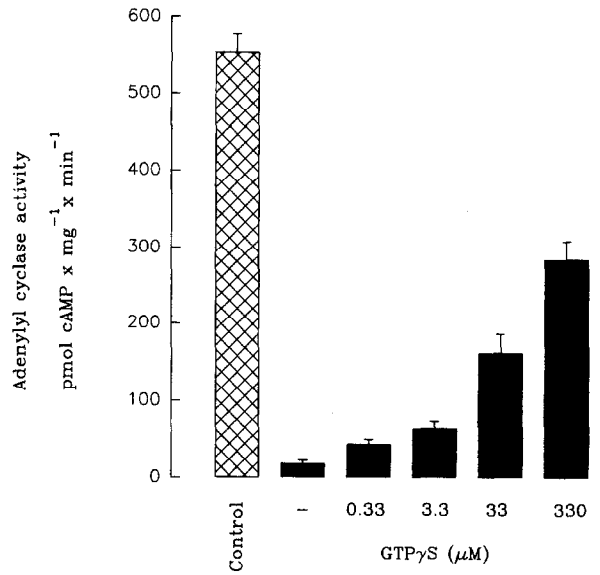

Fig. 3. Effect of concentration of GTPγS on the thermal inactivation of adenylyl cyclase in wt membranes. wt plasma membranes (35 μg) were incubated in the presence of detergents at 0°C (control) or at 30°C for 10 min in the presence of indicated concentrations of GTPγS. Afterwards, buffer A and solution of GTPγS (to equilibrate its final concentration to 10^{-4} M) were added and the adenylyl cyclase assay was performed. Data represent the mean ± S.D. of three independent experiments performed in duplicate.

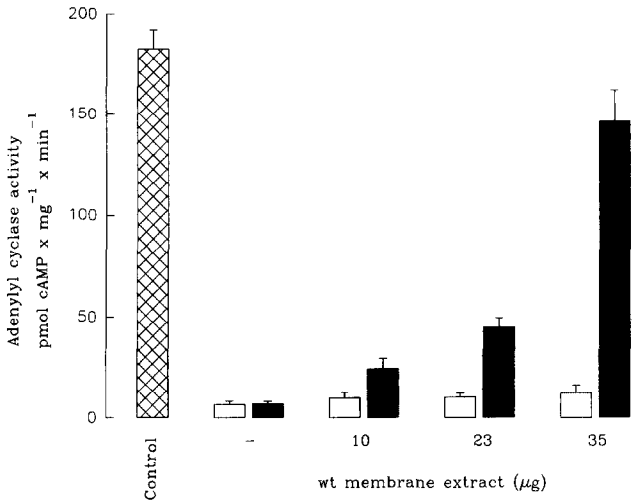

Fig. 4. Effect of wt extract and GTPγS on the thermal inactivation of adenylyl cyclase in cyc$^-$ plasma membranes. cyc$^-$ plasma membranes (35 μg) were complemented with indicated amounts of sodium cholate extract from wt plasma membranes and incubated for 10 min at 30°C in the presence of detergents without (▩) or with (■) GTPγS (3.33×10^{-4} mol/l). Afterwards, buffer A and solution containing appropriate amount of wt sodium cholate extract (to equilibrate the level of wt extract) were added and the adenylyl cyclase assay was performed. The activity of control sample pretreated for 10 min at 0°C (□) in the presence of wt extract (35 μg) and GTPγS (3.33×10^{-4} mol/l) was 186 ± 13 pmol cAMP × mg^{-1} × min^{-1}. Data represent the mean ± S.D. of three independent experiments performed in duplicate.

(analogue of forskolin, which does not activate adenylyl cyclase but activates protein kinases [21]) decreased the maximal adenylyl cyclase activity, the inactivation process was not influenced. These findings suggest that phosphorylation does not play a crucial role in the inactivation process.

Direct stimulatory effects of $G_{s\alpha}$ proteins or modulatory effects of $\beta\gamma$ and/or some other G_α subunits on the activity of some subtypes of adenylyl cyclases were previously demonstrated [12–14,22]. Recently recombinant $G_{i\alpha}$ was shown to inhibit the $G_{s\alpha}$ stimulatory effect in cyc$^-$ cells as well as the stimulatory effects of forskolin or $G_{s\alpha}$ on some of the recombinant adenylyl cyclases, but the mechanism of this inhibition remains unclear [6,22]. Here we used different activators of G proteins as preservatives during the inactivative treatment of adenylyl cyclase in wt and cyc$^-$ (lacking $G_{s\alpha}$ protein) plasma membranes in order to investigate possible differences in the characteristics of interaction of $G_{s\alpha}$ and $G_{i\alpha}$ with adenylyl cyclase. Adenylyl cyclase in wt but not in cyc$^-$ plasma membranes was partially protected against thermal inactivation when GTPγS or AlF_4^- were present during the inactivation procedure. Interestingly, complementation of cyc$^-$ plasma membranes with extracts from wt plasma membranes (no adenylyl cyclase activity detected) followed by activation by GTPγS protected adenylyl cyclase against thermal inactivation. Although pertussis toxin catalyzed ADP-ribosylation of $G_{i\alpha}$ did not influence the inactivating process of adenylyl cyclase, cholera toxin catalyzed ADP-ribosylation of $G_{s\alpha}$ increased significantly the protective effect of GTP and decreased the protective effect of AlF_4^-. Taken together, these results suggest that the interaction of adenylyl cyclase with $G_{s\alpha}$ has a quite different character than its interaction with $G_{i\alpha}$.

Acknowledgements: This work was supported by Grant MFR-08640 from the Swedish Medical Research Council.

References

[1] Tang, W.-J., Iniguez-Lluhi, J.A., Mumby, S. and Gilman, A.G. (1992) Cold Spring Harbour Symp. Quant. Biol. 57, 135–144.
[2] Tang, W.-J., Krupinski, J. and Gilman, A.G. (1991) J. Biol. Chem. 266, 8595–8603.
[3] Iyengar, R. (1993) FASEB J. 7, 768–775
[4] Pfeuffer, T. (1977) J. Biol. Chem. 252, 7224–7234.
[5] Bokoch, G.M., Katada, T., Northup, J.K., Ui, M. and Gilman, A.G. (1984) J. Biol. Chem. 259, 11517–11526.
[6] Tang, W.-J. and Gilman, A.G. (1992) Cell 70, 869–872.
[7] Simon, M.I., Strathmann, M.P. and Gautam, N. (1991) Science 252, 802–808.
[8] Milligan, G. (1992) Hoppe-Seyler Biochem. Soc. Transc. 20, 135–140.
[9] Hepler, J.R. and Gilman, A.G. (1992) Trends Biochem. Sci. 17, 383–387; Brown, A.M. (1990) Annu. Rev. Physiol. 52, 197–213.
[10] Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–648.
[11] Ransnäs, L.A. and Insel, P.A. (1988) J. Biol. Chem. 263, 17239–17242.
[12] Lee, E., Taussig, R. and Gilman, A.G. (1992) J. Biol. Chem. 267, 1212–1218.
[13] Wong, Y.H., Federman, A., Pace, A.M., Zachary, I., Evans, T., Poyssegur, J. and Bourne, H.R. (1991) Nature 351, 63–65.
[14] Tang, W.-J. and Gilman, A.G. (1991) Science 254, 1500–1503.
[15] Ross, E.M. and Gilman, A.G. (1977) J. Biol. Chem. 252, 6966–6969.
[16] Ross, E.M., Maguire, M.E., Sturgill, T.W., Biltonen, R.L. and Gilman, A.G. (1977) J. Biol. Chem. 252, 5761–5775.
[17] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 258, 7059–7063.
[18] Sternweis, P.C., Northup, J.K., Smigel, M.D. and Gilman, A.G. (1981) J. Biol. Chem. 256, 11517–11526; Johnson, R.A. and Salomon, Y. (1991) in: Methods in Enzymology (Johnson, R.A. and Corbin, J.D., Eds.) Vol. 195, pp. 3–21, Academic Press, London.
[19] Murayama, T., Tsai, S.-Ch., Adamik, R., Moss, J. and Vaughan, M. (1993) Biochemistry 32, 561–566.
[20] Robishaw, J.D., Russel, D.W., Harris, B.A., Smigel, M.D. and Gilman, A.G. (1986) Proc. Natl. Acad. Sci. USA 83, 1251–1255.
[21] Anderson, R.J., Breckon, R. and Colston, D. (1991) Biochem. J. 279, 23–27.
[22] Taussig, R., Iniguez-Lluhi, J.A. and Gilman, A.G. (1993) Science 261, 218–221.