Interactions of Forskolin and Adenylate Cyclase

EFFECTS ON SUBSTRATE KINETICS AND PROTECTION AGAINST INACTIVATION BY HEAT AND N-ETHYLMALEIMIDE

The interaction of forskolin with adenylate cyclase was studied by evaluating its effect on metal and metal-ATP kinetics and by measuring its protective effect when the enzyme was subjected to denaturation conditions. The solubilized calmodulin- and forskolin-sensitive adenylate cyclase from brain and the particulate enzyme from platelets were inactivated upon preincubation with N-ethylmaleimide. Forskolin protected against this inactivation in a concentration-dependent manner and demonstrated $K_d$ values of 6.3 and 7.8 $\mu M$ for the brain and platelet adenylate cyclases, respectively. Protection against N-ethylmaleimide inactivation of the brain enzyme was also afforded by calmodulin, but not to the extent seen with forskolin.

Forskolin also protected against thermal inactivation of the adenylate cyclases from brain, platelets, erythrocytes, and S49 lymphoma wild type and cyc- variants. The adenylate cyclase of bovine sperm, which is insensitive to activation by forskolin, was not protected by forskolin against inactivation by either N-ethylmaleimide or heat. Half-maximal activation of the platelet adenylate cyclase was seen with 3 to 10 $\mu M$ forskolin, and the $K_d$ for forskolin, determined from heat inactivation kinetics, was 9 to 11 $\mu M$. Activation of the platelet adenylate cyclase by forskolin was negatively cooperative ($n = 0.59$) with respect to forskolin. This activation occurred without change in Michaelis or dissociation constants for free Mg$^{2+}$, but coincided with a 5-fold increase in the corresponding constants for MgATP and a 2-fold increase in the $K_{app}$ for MnATP. However, forskolin did not affect the $K_{app}$ for MnATP of the solubilized adenylate cyclase from brain.

These results imply binding of forskolin by adenylate cyclase. The data suggest that the same binding site for forskolin is involved in both protection and activation and that this binding site is distinct from those to which the substrates bind.

Forskolin is a diterpene derivative from the Indian plant Coleus forskohlii and has been found to exert a positive inotropic effect on the heart and to lower blood pressure (1). These effects of forskolin led to studies showing that forskolin activates nearly all mammalian adenylate cyclases by a mechanism independent of hormone receptors or a fully functional guanine nucleotide regulatory component (2-6). Consequently, Seamon and Daly (5) suggested that stimulation of adenylate cyclase by forskolin may be a direct effect of forskolin on the catalytic subunit of the enzyme. The catalytic activity of adenylate cyclase is sensitive to inactivation by heat and by treatment with sulfhydryl reactive agents such as N-ethylmaleimide (e.g., Refs. 7 and 8). In this report, the effects of forskolin on the catalytic unit have been further examined by subjecting the enzyme to such denaturation conditions and by evaluating its effects on the kinetic behavior of the enzyme with respect to metal and metal-ATP.

In the case of the brain enzyme, comparison has been made with protective effects of calmodulin. Calmodulin has also been suggested to activate directly the catalytic unit of a brain adenylate cyclase (9), by a mechanism independent of a fully functional guanine nucleotide regulatory component, and it is known that calmodulin protects this cyclase against thermal inactivation (9, 10).

**EXPERIMENTAL PROCEDURES**

**Methods**

**Platelet Adenylate Cyclase**—Membranes from human platelets were prepared as previously described (11).

**S49 Lymphoma Wild Type and cyc- Adenylate Cyclases**—S49 lymphoma cells and their cyc- variants were grown in Dulbecco's modified Eagle's medium with 10% (v/v) horse serum. Cells were disintegrated by nitrogen cavitation in medium containing 150 mM NaCl, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 2 mM MgCl$_2$, and 1 mM EDTA. EDTA and $\beta$-mercaptoethanol were added to the broken cells to give final concentrations of 3 and 15 mM, respectively. The broken cells were centrifuged at 200,000 X g for 10 min. The resulting supernatant fraction was reconstituted in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 0.1 mM EDTA, and 5 mM $\beta$-mercaptoethanol and was stored in aliquots at $-70^\circ$C or was used for adenylate cyclase determinations.

**Erythrocytes**—Human erythrocyte membranes were prepared as described by Steck and Kunt (12).

**Brain Adenylate Cyclase**—Detergent-dispersed adenylate cyclase from rat brain was prepared as previously described (13) with the modification that initial centrifugation speeds were increased from 3,000 to 17,000 X g. The Lubrol-PX-dispersed enzyme was chromatographed on DEAE-Sephadex as previously described (14), with the following modification to separate calmodulin. Bound hydrophilic proteins, including calmodulin, were first eluted from the DEAE-Sephadex with 600 mM NaCl containing 1 mM EGTA. The column

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1 The abbreviation used is: EGTA, ethylene glycol bis(β-amino-ethyl ether)-N,N,N',N'-tetraacetic acid.
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was sequentially washed with 600 mM NaCl to remove EGTA and with 0.25% (w/v) Lubrol-PX containing neither salt nor EGTA to elute the adenylate cyclase.

Sperm Adenylate Cyclase—Bovine sperm were obtained either from the School of Veterinary Medicine in Giessen, Germany, or from a local slaughterhouse. Sperm from both sources behaved similarly in these studies. Sperm were washed in isotonic buffer (150 mM NaCl, 10 mM 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0) several times and were frozen in small aliquots (approximately 10^6 cells/ml) in liquid nitrogen. Frozen sperm were thawed with the addition of 1 or 2 volumes of 10 mM triethanolamine- HCl, pH 7.4, and were homogenized with a motor-driven glass-Teflon homogenizer. The particulate material was then collected by centrifugation at 30,000 × g for 10 min. The particles were resuspended in 10 mM triethanolamine-HCl, pH 7.4, and were then used for assay of adenylate cyclase.

Adenylate Cyclase Assay—Adenylate cyclase activities were determined with a reaction mixture containing 50 mM triethanolamine-HCl, pH 7.4, 1 mM 3-isobutyl,1-methylxanthine, 1 mM dithiothreitol, 1 mg of bovine serum albumin/ml, 10 to 200 μM ATP, 4 or 10 mM excess MnCl₂ or, for the kinetics experiments, 0.8 to 12 mM MgCl₂, 20 mM creatine phosphate, 200 μg of creatine kinase/ml, and [α-³²P]ATP (2 to 8 × 10⁶ cpm), in a volume of 100 μl. Particulate cyclases were assayed with 10 mM excess MnCl₂ and with 0.1 mM EGTA and 0.1 mM Ca⁴⁺ also present, whereas the solubilized brain cyclase was assayed with 4.2 mM excess MnCl₂ and no EGTA or Ca⁴⁺. Reactions were stopped by the addition of 10 mM EDTA and were then used for assay of adenylate cyclase.

Adenylyl Cyclase Assay—Adenylyl cyclase activities were determined with a reaction mixture containing 50 mM triethanolamine-HCl, pH 7.4, 1 mM 3-isobutyl,1-methylxanthine, 1 mM dithiothreitol, 1 mg of bovine serum albumin/ml, 10 to 200 μM ATP, 4 or 10 mM excess MnCl₂ or, for the kinetics experiments, 0.8 to 12 mM MgCl₂, 20 mM creatine phosphate, 200 μg of creatine kinase/ml, and [α-³²P]ATP (2 to 8 × 10⁶ cpm), in a volume of 100 μl. Particulate cyclases were assayed with 10 mM excess MnCl₂ and with 0.1 mM EGTA and 0.1 mM Ca⁴⁺ also present, whereas the solubilized brain cyclase was assayed with 4.2 mM excess MnCl₂ and no EGTA or Ca⁴⁺. Reactions were stopped by the addition of 10 mM EDTA and were then used for assay of adenylate cyclase.

Materials

[α-³²P]ATP was prepared enzymatically as described by Walsh and Johnson (17). Carrier-free [³²P]phosphoric acid was purchased at the highest concentration available from New England Nuclear. N-Ethylmaleimide was from Sigma, and other reagents were obtained at the highest purity available from commercial sources. Calmodulin was isolated from pig testes by an ammonium sulfate precipitation procedure developed by Keravis and Wells and was purified to near homogeneity by chromatography on 2-trifluoro-10-(3-amino-propyl)phenotheiazine-Sepharose CL-4B, essentially as described by Jamieson and Vanaman (18). The phenotheiazine analog was generously provided by Dr. J. N. Wells, Vanderbilt University. Forskolin was a generous gift of Dr. H. Metzger, Hoechst AG, Frankfurt, Germany.

RESULTS

Protection against Inactivation by N-Ethylmaleimide—The calmodulin- and forskolin-sensitive adenylate cyclase of rat brain was clearly protected by forskolin against inactivation by millimolar N-ethylmaleimide (Fig. 1). Basal activity and sensitivity to stimulation by forskolin were completely stable at 30 °C for at least 10 min (data not shown). The rate of inactivation of the forskolin-protected enzyme was linear for at least 8 min (up to 6 min shown in Fig. 1). The unprotected enzyme usually showed a brief initially rapid phase of inactivation followed by a slower linear phase of inactivation.

The activities shown in Fig. 1 were measured without a further addition of forskolin in the assay. When activities were measured in the presence of 100 μM additional forskolin, the rates of inactivation of the cyclase by N-ethylmaleimide were superimposable with these shown here. Moreover, inactivation by N-ethylmaleimide did not affect the degree of stimulation by forskolin. The ratio of inactivation rates of enzyme incubated with 100 μM forskolin to control enzyme was 3.75 in this experiment.

Adenylyl cyclase activities were subsequently assayed with a saturating concentration of forskolin, a similar ratio of inactivation rates was observed (ratio = 3.53). When similar experiments were conducted with 1 mM N-ethylmaleimide and 100 μM forskolin at 0 °C, protection ratios (3.0 to 3.7) were also comparable to those obtained at 30 °C.

Results similar to those shown in Fig. 1 for the brain adenylate cyclase were also obtained with enzyme from platelets (data not shown). For both tissues, the protective effect of forskolin was concentration-dependent. Kₐ and kᵣ/kᵣ values were 6.3 and 0.51 μM, respectively, for the brain enzyme and were 7.6 and 0.58 μM, respectively, for the platelet adenylate cyclase.

Calmodulin has been reported to protect the brain adenylate cyclase against thermal inactivation (9, 10), but compara-

![FIG. 1. Forskolin protection of brain adenylate cyclase against inactivation by N-ethylmaleimide.](http://www.jbc.org/)

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The enzyme from human erythrocytes was somewhat more stable (53% inactivation after 8 min). The enzyme from sperm, however, to be comparably inactivated required a 10-min exposure at 45 °C. In each instance, with the clear exception of the sperm adenylate cyclase, the presence of 167 μM forskolin offered protection against heat inactivation. By comparison, exposure of the solubilized brain cyclase to 45 °C for 4 min caused the loss of 80 and 50% of its activity when preincubated without or with 100 μM forskolin, respectively (data not shown). It should be noted that the degree of protection seen with forskolin was essentially identical for adenylate cyclases from both S49 wild type and cyc− cells despite the lack of a fully functional guanine nucleotide regulatory component in the cyc− variants (19). This lack may be responsible for the reported considerable difference in the sensitivity of these two cyclases to stimulation by forskolin (5).

The time course of heat inactivation of the platelet adenylate cyclase in the absence and presence of forskolin is shown in Fig. 4. In both cases, the rates of decay were linear with time for at least 10 min. In the experiments represented here, after 10 min, about 54% of the initial activity remained in the absence of forskolin, whereas about 84% remained in 200 μM forskolin. The corresponding first order rate constants for inactivation were 0.0272 min−1 for control and 0.0046 min−1 with forskolin, giving a ratio of slopes of 5.9. The concentration dependency of this protective effect of forskolin is shown in Fig. 5. For comparison, the concentration dependency of activation of this platelet adenylate cyclase by forskolin is also shown. The protection data were replotted (inset) giving values for Kd of 9.8 μM forskolin and k2/k1 of 0.32. Values from a second similar experiment were Kd = 10.9 μM and k2/k1 = 0.27. By comparison, half-maximal stimulatory effects of forskolin ranged between 3 and 10 μM.

**Forskolin on Metal and Metal-ATP Kinetics—**Stimulation of adenylate cyclase by some, but not all, agents suspected of being mediated by the guanine nucleotide regulatory component coincides with a shift in the enzyme’s sensitivity to divalent cation (e.g. Refs. 20–23). Since available evidence

![Fig. 2](http://www.jbc.org/)  
**Fig. 2. Protection by calmodulin and forskolin against N-ethylmaleimide (NEM) inactivation of brain adenylate cyclase.** Preincubations and assays were as described in the legend to Fig. 1. Calmodulin (100 μg/ml) and/or forskolin (200 μM) were present in the preincubation as indicated. Forskolin and calmodulin carried into the assays were 14 μM and 7.1 μg/ml, respectively. Values are averages from triplicate determinations from one of three similar experiments.

![Fig. 3](http://www.jbc.org/)  
**Fig. 3. Lack of protection by forskolin against N-ethylmaleimide inactivation of sperm adenylate cyclase.** Sperm particles were preincubated at 0 °C with 20 mM N-ethylmaleimide (NEM) or water and 100 μM forskolin or an amount of ethanol equivalent to that contributed by the forskolin solution. For all preincubation conditions, aliquots were taken at the indicated times, and activities were immediately determined in reaction mixtures containing 100 μM forskolin, 50 μM ATP, 10 mM MnCl2, 10 mM dithiothreitol (a 5-fold molar excess over the contributed N-ethylmaleimide). Assays were for 20 min at 37 °C. Values are averages from duplicate determinations from one of two similar experiments.

**Protection against Thermal Inactivation—**The sensitivity of adenylate cyclases from various sources to thermal denaturation was explored (Table I). The adenylate cyclases from platelets and from S49 lymphoma wild type and cyc− cells showed, for example, comparable loss of activity following incubation at 35 °C for 8 min (e.g. 78 to 71% inactivation).

In other experiments, we found that this protective effect of calmodulin was unaffected by the inclusion of additional calcium during the preincubation, due to the sufficient amounts of calcium contaminating cyclase preparations, the calmodulin, and buffers. A similar lack of effect of additional calcium to enhance the protective effect of calmodulin against thermal inactivation was observed by Salter et al. (9).

The enzyme from human erythrocytes was somewhat more stable (53% inactivation after 8 min). The enzyme from sperm, however, to be comparably inactivated required a 10-min exposure at 45 °C. In each instance, with the clear exception of the sperm adenylate cyclase, the presence of 167 μM forskolin offered protection against heat inactivation. By comparison, exposure of the solubilized brain cyclase to 45 °C for 4 min caused the loss of 80 and 50% of its activity when preincubated without or with 100 μM forskolin, respectively (data not shown). It should be noted that the degree of protection seen with forskolin was essentially identical for adenylate cyclases from both S49 wild type and cyc− cells despite the lack of a fully functional guanine nucleotide regulatory component in the cyc− variants (19). This lack may be responsible for the reported considerable difference in the sensitivity of these two cyclases to stimulation by forskolin (5).

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**Forskolin on Metal and Metal-ATP Kinetics—**Stimulation of adenylate cyclase by some, but not all, agents suspected of being mediated by the guanine nucleotide regulatory component coincides with a shift in the enzyme’s sensitivity to divalent cation (e.g. Refs. 20–23). Since available evidence
suggested that forskolin activation of adenylate cyclase may be due to an effect directly on the enzyme's catalytic unit. It was of interest to determine whether activation by forskolin would affect the kinetics of the enzyme with respect to its substrates Mg$^{2+}$ and MgATP$^{2-}$. Earlier studies showed that the kinetic behavior of both platelet and brain adenylate cyclases was straightforward and conformed to that of a sequential addition of metal and metal-ATP (24, 25). When forskolin was tested, it was found to exert no significant effect on the kinetic behavior of the platelet adenylate cyclase with respect to Mg$^{2+}$ (Table II and Fig. 6). This lack of effect of forskolin on the affinity of the enzyme for Mg$^{2+}$ was under conditions with which forskolin caused a 10 to 20-fold increase in enzyme activity (Fig. 6). The effect of forskolin on reaction velocity at near saturating concentrations of both substrates was negatively cooperative with respect to forskolin. That is, double reciprocal plots of velocity versus [forskolin]$^{-1}$ were concave downward (plot not shown). A Hill plot of these data (Fig. 7) was linear and gave a value of $n = 0.6$. In contrast with the lack of effect of forskolin on the kinetic parameters with respect to Mg$^{2+}$, forskolin caused a concentration-dependent increase in the Michaelis and dissociation constants for MgATP (Table II and Fig. 7). The concentration dependency of the effect on the apparent $K_m$ for MgATP is shown in the inset to Fig. 7. In similar experiments with MnATP as substrate, with a fixed concentration of 2 mM

![Fig. 4. Forskolin protection against thermal inactivation of platelet adenylate cyclase. Platelet particles were preincubated at 35 °C without or with 200 μM forskolin. At the indicated times, aliquots were diluted 2-fold into ice-cold 10 mM triethanolamine-HCl, pH 7.4. Diluted particles were then assayed for adenylate cyclase activity. All reaction mixtures contained 200 μM forskolin, 100 μM ATP, and 10 mM MnCl$_2$. Assays were for 15 min at 30 °C. Values are averages ± S.E. from five determinations.](http://www.jbc.org/)

![Fig. 5. Concentration dependency of forskolin activation of platelet adenylate cyclase and its protection against thermal inactivation. Platelet particles were preincubated for 6 min at 35 °C with the indicated concentrations of forskolin. Aliquots were removed into cold Eppendorf tubes, and adenylate cyclase activities were determined with a reaction mixture containing 100 μM ATP, 10 mM MnCl$_2$, and 200 μM forskolin at 30 °C for 20 min. Initial velocity was 3.57 nanomoles of cAMP formed/minute/milligram of protein. Concentration dependency for forskolin activation was determined with 50 μM ATP and 10 mM MnCl$_2$ with a 15-min incubation at 30 °C. Values are averages from triplicate determinations from one of two similar experiments. Inset, replot of protection data for the determination of $K_a$ for forskolin as per Scrutton and Utter (16).](http://www.jbc.org/)
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Effect of forskolin on the kinetic parameters of the platelet adenylate cyclase

<table>
<thead>
<tr>
<th>Addition</th>
<th>Kinetic parameter</th>
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<tr>
<td></td>
<td>$K_a$(MgATP$^{-1}$)</td>
</tr>
<tr>
<td>Ethanol control</td>
<td>21 ± 5</td>
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<tr>
<td>Forskolin (10 μM)</td>
<td>82 ± 6</td>
</tr>
</tbody>
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*Kinetic constants were determined as previously described (25) from linear regression analysis of slope and intercept replots of primary double reciprocal plots, as suggested by Cleland (26). $K_a$ is the Michaelis constant for the given substrate and $K$ is the dissociation constant. Values are the averages ± S.E. from the number of experiments given in parentheses, each of which was assayed in triplicate.

Fig. 6. Double reciprocal plot of Mg$^{2+}$ kinetics at varying concentrations of forskolin. Platelet adenylate cyclase activities were determined with 500 μM MgATP as substrate, 5 mM creatine phosphate, 400 μg of creatine kinase/ml, 0.1 mM CAMP, and 1 mM dithiothreitol, and otherwise as described under "Experimental Procedures." Forskolin dilutions were made such that the contribution in the assay under all conditions was 0.95% ethanol. Values are averages of triplicate determinations from one of two similar experiments. Dimensions for velocity are (nanomoles of CAMP formed)/10 min./tube). Inset, logarithmic Hill plot of forskolin activation. Values used were those obtained with 16 mM excess MgCl$_2$ (1/Mg$^{2+}$ = 0.0625) shown in the right-hand section.

excess MnCl$_2$, 100 μM forskolin increased the apparent $K_a$ for MnATP from 14 to 32 μM. While these shifts were clearly evident with the platelet adenylate cyclase (Fig. 7), a similar effect was not seen with a detergent-dispersed calmodulin-sensitive adenylate cyclase from brain which was also stimulated about 12-fold by 100 μM forskolin. With the brain enzyme, double reciprocal plots of velocity versus 1/[MnATP], with 2 mM excess MnCl$_2$ at several concentrations of forskolin, resulted in a series of lines intersecting on the abscissa and corresponding to a $K_{app}$ for MnATP of 20 μM. The brain adenylate cyclase is essentially a Mn$^{2+}$-requiring enzyme, but since forskolin caused a shift in the $K_{app}$ for both MgATP and MnATP with the platelet cyclase, it is not likely that the use of MnATP with the brain enzyme accounts for its different response to forskolin. An additional difference is that the brain enzyme does not require the use of an ATP-regenerating system for its assay. The difference in the effect of forskolin on the kinetic behavior of the brain enzyme and the platelet enzyme could have been due to an effect on the regenerating system. However, there was no significant effect of 100 μM forskolin on creatine kinase activity.

Fig. 7. Double reciprocal plot of MgATP$^{-2}$ kinetics at varying concentrations of forskolin. Platelet adenylate cyclase activities were determined with 20 mM excess MgCl$_2$ and otherwise as described in the legend to Fig. 6. Values are averages of triplicate determinations from one of two similar experiments. Dimensions for velocity are (nanomoles of CAMP formed)/10 min./tube). Inset, apparent $K_a$ for MgATP as a function of forskolin concentration. Values were calculated from abscissa intercepts of the lines shown in the right-hand section.

DISCUSSION

The studies reported here demonstrate a protective effect of forskolin against inactivation of adenylate cyclase by exposure to heat or to N-ethylmaleimide. In general, such protection implies first, that forskolin-adenylate cyclase interactions occur, and second, in the case of N-ethylmaleimide, that an important reactive sulfhydryl group is protected by forskolin. Activation of adenylate cyclase by forskolin occurred without affecting the interactions of the enzyme with metal, but significantly decreased the affinity of the platelet enzyme for MgATP and MnATP. Since forskolin did not increase the $K_{app}$ for MnATP of the brain enzyme, the increases seen with the platelet adenylate cyclase probably are not a characteristic of forskolin stimulation per se, but are likely due to the influence of other factors in these preparations. Thus, the data indicate that activation of adenylate cyclase by forskolin is due to a change in the enzyme’s $V_{max}$ without attendant obligatory changes in the enzyme’s kinetic parameters for either substrate and are consistent with a binding site for forskolin distinct from those of either metal-ATP or free metal. Since the $K_a$ for forskolin (6 to 11 μM), determined from inactivation kinetics, was essentially identical with the concentration of forskolin eliciting half-maximal activation (3 to 10 μM), it is likely that the same binding site for forskolin is involved in both protection and activation. However, the
negative cooperativity exhibited by forskolin activation, determined under cyclase assay conditions with essentially saturating substrate concentrations, would be consistent with either multiple interacting binding sites or with a mixture of binding sites with differing affinities. Thus, the protection data are consistent with a single binding site for forskolin, whereas the activation data would be consistent with more than one binding site for forskolin. One possible argument that could explain such an apparent discrepancy is that one of the forskolin binding sites involved in activation of the cyclase does not protect the enzyme or does so very weakly. Alternatively, it is conceivable that the forskolin may be contaminated with a factor which does not protect the enzyme but alters enzyme activity.

The adenylate cyclase of brain was activated by forskolin and by calmodulin, but these effects were less than additive (Fig. 2). The protective effect of forskolin against inactivation of this enzyme by N-ethylmaleimide, however, was considerably greater than that seen with calmodulin. Protection by forskolin was also considerably greater than the protective effect of calmodulin against thermal inactivation reported by others (9, 10). Thus, calmodulin and forskolin may stimulate or protect either the same cyclase population by distinct mechanisms or act on different cyclase populations. It is conceivable, for example, that the isolated catalytic unit of the calmodulin-sensitive adenylate cyclase may not be affected by forskolin.

Data presented here and available evidence from others (4, 5) suggest that forskolin protection and activation may be effects directly on the cyclase catalytic unit. Both effects of forskolin are seen with enzyme from a variety of tissues, significantly with cyclases from both S49 lymphoma wild type and cyc- cells, but notably not with the cyclase from bovine sperm. The adenylate cyclase from sperm may represent a distinct class of enzyme, but since forskolin activation of the purified catalytic unit of no mammalian cyclase has been demonstrated, it remains possible that an additional component may be required. This would be consistent with multiple binding sites for forskolin in the activation of the cyclase. However, the first order characteristics of the protective effects of forskolin against both thermal and N-ethylmaleimide denaturation reported here would be consistent with a site on the catalytic unit. Due to the complex structure of adenylate cyclase, it is premature to ascribe locus or mechanism to either the protective or activating actions of forskolin.

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