Interactions of Forskolin and Adenylate Cyclase

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

Joseph A. Awad and Roger A. Johnson†
From the Department of Physiology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232

Karl H. Jakobs and Gunter Schultz
From the Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, Germany

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 7.6 $\mu M$ 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 cyclc variants. The adenylate cyclase of bovine spine, 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 association 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).

* This work was supported by the Deutsche Forschungsgemeinschaft and Grant AM 18185 from the United States National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of an Alexander von Humboldt Foundation fellowship, on sabbatical leave to Heidelberg University. To whom correspondence and reprint requests should be addressed.

† Recipient of an Alexander von Humboldt Foundation fellowship.


dissected by nitrogen cavitation in medium containing $150 \text{ mm NaCl, } 20 \text{ mm } 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 2 \text{ mm } MgCl}_2, \text{ and } 1 \text{ mm EDTA. EDTA and } \beta\text{-mercaptoethanol were added to the broken cells to give final concentrations of } 3 \text{ and } 15 \text{ mm, respectively. The broken cells were centrifuged at } 200 \times g \text{ for } 10 \text{ min. The resulting supernatant fraction was recentrifuged for } 10 \text{ min at } 30,000 \times g \text{. The subsequent pellet fraction was resuspended in } 20 \text{ mm } 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 0.1 \text{ mm EDTA, and } 5 \text{ mm } \beta\text{-mercaptoethanol and was stored in aliquots at } -70 \text{ °C or was used for adenylate cyclase determinations.}

Erythrocytes—Human erythrocyte membranes were prepared as described by Steck and Kant (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 $\times g$. 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

† The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
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 epididymal ducts from testes obtained from a local slaughterhouse. Sperm from both sources behaved similarly in these studies. Sperm were washed in isotonic buffer (150 mM NaCl, 10 mM NaF, 3 mM 2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.0) several times and were frozen in small aliquots (approximately 106 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 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 [γ-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 cAMP also present, whereas the solubilized brain cyclase was assayed with 4.2 mM excess MnCl₂ and no EGTA or cAMP. Reactions were for 10 min at 37°C unless otherwise stated. Reactions were terminated by precipitation with ZnCO₃, and [³²P]cAMP was purified as previously described (15).

**Determination of Dissociation Constants**—Dissociation constants for forskolin were determined as described by Scrutton and Utter (16). The interaction of an enzyme subjected to denaturation conditions and a stabilizing molecule can be described as follows:

\[ E + A \rightleftharpoons EA; K_d = \frac{[E][A]}{[EA]} \]

\[ E + k_s \rightarrow D; \]

\[ EA + k_s \rightarrow D; \]

where \( E \) = free enzyme, \( D \) = denatured enzyme, and \( A \) = stabilizing molecule. \( K_d \) is the dissociation constant of EA. \( k_s \) = rate constant for denaturation of \( E \), and \( k_d \) = rate constant for denaturation of EA. If the equilibrium between \( E, A, \) and \( EA \) is rapid compared with the denaturation process, then the following relationship applies. \( V_e/V_i = k_s/k_d \times K_d(\times V_o/V_0)\times[A] \), \( V_e \) and \( V_i \) represent, respectively, the rates for inactivation of \( E \) in the presence and absence of \( A \). When the ratios of inactivation rates \( (V_e/V_i) \) are plotted against \( (1 - V_e/V_i)\times[A] \), the ordinate intercept represents \( k_s/k_d \), and the slope is \( K_d \). The ratio \( k_s/k_d \) reflects the degree of protection afforded by the stabilizing ligand.

**Materials**

[γ-³²P]ATP was prepared enzymatically as described by Walseth 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 in the highest purity available from commercial sources. Calmodulin was isolated from pig testes by an ammonium sulfate precipitation procedure developed by Keravis and Wells2 and was purified to near homogeneity by chromatography on 2-trifluoro-10-(3-amino-propyl)phenothiazine-Sepharose CL-4B, essentially as described by Jamieson and Vanaman (18). The phenothiazine analog was generously provided by Dr. J. N. Wells, Vanderbilt University. Forskolin was a generous gift of Dr. H. Metager, Hoechst AG, Frankfurt, Germany.

**RESULTS**

**Protection against Inactivation by N-Ethylmaleimide**—Calmodulin- and forskolin-sensitive adenylate cyclase of the 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 5 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. When adenylate 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. \( k_s/k_d \) values were 6.3 and 0.5 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-

---

2 Excess MnCl₂ or MgCl₂ implies the concentration of metal in excess of the ATP concentration. Earlier studies (25) indicated that for these metals and ATP the excess concentration closely approximates the free concentration of metal ion.

3 T. Keravis and J. N. Wells, personal communication.

---

**Fig. 1. Forskolin protection of brain adenylate cyclase against inactivation by N-ethylmaleimide.** Soluble brain adenylate cyclase, following chromatography on DEAE-Sephadex, was incubated at 30°C with a calculated excess of 0.93 mM N-ethylmaleimide over the 3 mM dithiothreitol present in the enzyme and with either 100 μM forskolin or an amount of ethanol equivalent to that donated by the forskolin solution. The enzyme was prewarmed at 30°C for 2 min, aliquots for zero time were taken, and water or N-ethylmaleimide was added. At the indicated times, aliquots were immediately diluted into 4.75 volumes of ice-cold 50 mM triethanolamine-HCl, pH 7.4, and 3 mM dithiothreitol to give a molar excess of 15.5 over the amount of N-ethylmaleimide. Aliquots of the diluted enzymes were then used for assay. Forskolin carried into the assay was 8.7 μM. Values shown were assayed with 200 μM MnATP without added forskolin. Values are averages from duplicate determinations from one of four similar experiments. Initial activities were, in nanomoles of cAMP formed/minute-milligram of protein 2.68 for control enzyme, and 11.9 for enzyme preincubated with 100 μM forskolin.
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 incubation 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 549 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 with 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. 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. 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.
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 \(v\) versus [forskolin] \(I\) were concave downward (plot not shown). A Hill plot of these data (Fig. 6) was linear and gave a value of \(n = 0.59\).

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...

---

### Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cont. 0 min</th>
<th>Cont. 4 min</th>
<th>Cont. 8 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet</td>
<td>45.5</td>
<td>46.3</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0.187</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>S49 wild type</td>
<td>4.30</td>
<td>4.84</td>
<td></td>
</tr>
<tr>
<td>S49 cyc*</td>
<td>27.2</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Bovine sperm*</td>
<td>1.24</td>
<td>1.13</td>
<td></td>
</tr>
</tbody>
</table>

* Membranes from the indicated sources were suspended in 10 mM triethanolamine-HCl, pH 7.4. Preincubations were at 35 °C in the presence of 167 μM forskolin (forsk.) or an amount of ethanol equivalent to that carried into the incubation by the forskolin solution (cont.). At the indicated times, aliquots were removed into prechilled tubes and were then assayed for adenylate cyclase.

* Activity determinations were made as described under "Experimental Procedures" in the presence of 200 μM forskolin, 100 μM ATP, and 10 mM MnCl\(_2\) as cation. Reactions were for 20 min at 30 °C and were initiated by the addition of enzyme. Values are averages from triplicate incubations.

* Sperm preincubations were conducted for 10 min at 45 °C.

---

**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.

**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 \(K_f\) for forskolin as per Scrutton and Utter (16).
Interactions of Forskolin and Adenylate Cyclase

Table II

Effect of forskolin on the kinetic parameters of the platelet adenylate cyclase

<table>
<thead>
<tr>
<th>Addition</th>
<th>$K_m$(MgATP$^{2-}$)</th>
<th>$K_a$(MgATP$^{2-}$)</th>
<th>$K_m$(Mg$^{2+}$)</th>
<th>$K_a$(Mg$^{2+}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol control*</td>
<td>21 ± 5</td>
<td>16 ± 4</td>
<td>2.5 ± 0.3</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>Forskolin (10 μM)</td>
<td>82 ± 6</td>
<td>41 ± 5</td>
<td>3.0 ± 0.7</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

* 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). $K_m$ is the Michaelis constant for the given substrate and $K_a$ 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.

**Forskolin (10 mM) stock solutions were made in 95% ethanol. Dilution of the forskolin into the assay resulted in a contribution to the assay of 0.095% ethanol. This concentration of ethanol was used in control experiments.

excess MnCl$_2$, 100 μM forskolin increased the apparent $K_m$ 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$^{-1}$ 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 $K_{m_{app}}$ for MnATP of 20 μM. The brain adenylate cyclase is essentially a Mn$^{2+}$-requiring enzyme, but since forskolin caused a shift in the $K_{m_{app}}$ 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.

**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 $K_{m_{app}}$ 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 $K_f$ 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.

Acknowledgments—We are very appreciative of the superb technical assistance of Christa Steinmeyer and Gabriele Gabel and of the helpful suggestions of Dr. David L. Garbers during the preparation of this manuscript. We would like to thank Dr. H. Metzger of Hoechst AG for providing the forskolin. We are indebted to Dr. Ulrich Gehring for his help in growing the S49 lymphoma cells.

REFERENCES

Interactions of forskolin and adenylate cyclase. Effects on substrate kinetics and protection against inactivation by heat and N-ethylmaleimide.
J A Awad, R A Johnson, K H Jakobs and G Schultz


Access the most updated version of this article at http://www.jbc.org/content/258/5/2960.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/5/2960.citation.full.html#ref-list-1