THE INHIBITORY EFFECT OF METHANOL ON FORSKOLIN-ACTIVATED ADENYLATE CYCLASE IN RAT ERYTHROCYTE MEMBRANES DEPENDENT ON THE STATE OF THE GUANINE NUCLEOTIDE-BINDING STIMULATORY AND REGULATORY PROTEIN

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Forskolin-activated adenylate cyclase activity in rat erythrocyte membranes was markedly inhibited by methanol in a concentration dependent manner. On the contrary, no change was observed in the basal cyclase activity in the presence of methanol. The methanol inhibition forskolin-activated cyclase activity was protected by the stimulation of Gs in the presence of NaF in a concentration dependent manner. These data indicate that low- and high-affinity binding sites of forskolin in rat erythrocyte membranes have different sensitivities to methanol.

KEYWORDS- adenylate cyclase; forskolin; methanol; forskolin binding site; activated Gs; rat erythrocyte membrane

INTRODUCTION

Forskolin, a diterpene from the root of Coleus forskolii, directly activates the catalytic unit of the adenylate cyclase system.¹⁾ This activation may be mediated through a low affinity binding site for forskolin.^{2,3)} In addition, the maximal activation of this enzyme system by forskolin requires the guanine nucleotides stimulatory and regulatory protein (Gs).^{4,5)} Also, forskolin and some hormones can synergistically activate hormone-sensitive adenylate cyclase activity.^{5,6)} A high affinity binding site for forskolin is increased in these cases. Thus, the effect of forskolin seems to depend on the formation of a complex between the activated Gs and the catalytic unit. Furthermore, we have reported that forskolin has a stabilizing effect on the interaction between Gs and the catalytic unit of the adenylate cyclase system in rat erythrocyte membranes.⁷⁾

On the other hand, short-chain alcohols which are used as a forskolin

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solvent inhibit forskolin-activated cyclase activity in competition for forskolin,⁸⁾ though they have a little stimulatory effect on the basal cyclase activity. This suggests that the inhibition is effected by low affinity binding sites for forskolin. We attempted to determine, the effect of methanol on forskolin-activated cyclase activity in the presence or absence of NaF to evaluate the effect of the activated Gs using rat erythrocyte membranes. In the present study, we find that methanol markedly inhibits forskolin-activated cyclase activity without changing the basal activity. We also report that the activation of Gs by NaF effectively blocks the methanol inhibition of forskolin-activated cyclase activity, depending on a concentration of NaF.

MATERIALS AND METHODS

Materials - ATP, phosphocreatine, creatine phosphokinase and bovine serum albumin were purchased from Sigma, forskolin was from Calbiochem-Behring, and [³H] ATP was from Amersham International Ltd. All other agents were of reagent grade from standard commercial sources.

Preparation of Rat Erythrocyte Membranes - Rat erythrocyte membranes were prepared by the previously reported procedure.⁸⁾ Briefly, rat erythrocytes were treated with a hypotonic solution containing 5 mM Tris-HCl/1 mM EDTA (pH 7.4). The lysate was centrifuged at 20,000 × g for 10 min. The precipitate was suspended with 50 mM Tris-HCl buffer (pH 7.4) and the suspension was used as the membrane preparation in the following experiments.

Measurements of Adenylate Cyclase Activity and Protein - Adenylate cyclase activity was measured according to the method of Salomon et al.⁹⁾ with some modifications.¹⁰⁾ In the indicated cases, 10 μM forskolin, 5 mM NaF/20 μM AlCl₃ and methanol were added to the regular assay system. Protein was determined by the method of Lowry et al.¹¹⁾ using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Figure 1 shows the concentration-response curve of the basal and 10 μM forskolin-activated adenylate cyclase activities to methanol. The forskolin-activated cyclase activity was markedly inhibited in a concentration-dependent manner, but no change was observed in the basal activity. These results were consistent with those of the previous studies using a short-chain alcohol.¹²⁾ As shown in Table I, 5% methanol inhibited 5 mM NaF/20 μM AlCl₃-activated cyclase activity by approximately 20%. This suggests that methanol also slightly influenced the function of the Gs in rat erythrocyte membranes. The results coincide with the previous data, indicating that ethanol has an inhibitory effect on NaF-stimulated adenylate cyclase activity in bovine corpus luteum.⁸⁾ Interestingly, 10 μM forskolin plus 5 mM NaF/20 μM AlCl₃-activated cyclase activity was not affected by 5% methanol. This sug-

gests that a complex formed between the activated Gs and the catalytic unit becomes resistant to methanol.

To further determine the reduction of the inhibition by methanol of forskolin-activated cyclase activity coupled with the activated Gs, a 10 μ M concentration of the activity was measured in the presence of various concentrations of NaF with or without methanol. Figure 2A shows the concentration-response curve of NaF in the presence of 20 μ M of $AlCl_3$. The inhibition by methanol of forskolin-activated cyclase activity decreased depending on the concentration of NaF (Fig. 2B). Recent reports demonstrate that forskolin binds to two different sites in the

Table 1. Effect of NaF in Preventing the Inhibition of Forskolin-Activated Adenylate Cyclase Activity by Methanol

Addition to membranes	Adenylate cyclase activity (μ mol/min/mg protein)		% of control
	Control	5% methanol	
none	2.6	3.0	115.4
10 μ M forskolin	44.1	22.6	51.2
5 mM NaF/20 μ M $AlCl_3$	36.1	29.1	80.6
10 μ M forskolin + 5 mM NaF/20 μ M $AlCl_3$	61.8	59.9	96.9

Each value represents the mean of two determinations.

Fig. 1. Effect of Methanol on Forskolin-Activated and Basal Adenylate Cyclase Activities in Rat Erythrocyte Membranes

Rat erythrocyte adenylate cyclase activity was measured in the presence of indicated concentrations of methanol with (O) or without (●) 10 μ M forskolin.

Fig. 2. Effect of NaF on Forskolin-Activated Adenylate Cyclase Activity in Rat Erythrocyte Membranes

A: Rat erythrocyte adenylate cyclase activity was measured in the presence of 10 μ M forskolin and increasing concentrations of NaF with (●) or without (O) 5% methanol.

B: Change in the activity with methanol versus the activity without methanol as calculated from values in A.

adenylate cyclase system: a low affinity binding site, which is responsible for a direct activation of the catalytic unit, and a high affinity binding site, which is increased by the coupling with the activated Gs and the catalytic unit in rat brain¹³⁾ and human platelet.¹⁴⁾ On the other hand, our previous study⁷⁾ indicated that forskolin had a stabilizing effect, on the Gs-activated state of the adenylate cyclase system in rat erythrocyte membranes through a high affinity binding site. Our present results indicate that a low affinity binding site of forskolin can respond to methanol and that a high affinity binding site is less susceptible to methanol. Thus, the use of methanol may be useful in studies further of the mechanism of the activation by forskolin and properties of the two binding sites of forskolin in the adenylate cyclase system.

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