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Biochemistry

Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells

(cyclic AMP/guanyl nucleotides/brain/fluoride/neurotransmitters)

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ABSTRACT **The diterpene, forskolin [half-maximal effective concentration (EC_{50}), 5–10 μM] activates adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in rat cerebral cortical membranes in a rapid and reversible manner. Activation is not dependent on exogenous guanyl nucleotides and is not inhibited by guanosine 5′-*O*-(2-thiodiphosphate) when assayed with adenosine 5′-[β,γ-imido]triphosphate as substrate. GTP and GDP potentiate responses to forskolin. The activations of adenylate cyclase by forskolin and guanosine 5′-[β,γ-imido]triphosphate p[NH]ppG are not additive, whereas activations by forskolin and fluoride are additive or partially additive. The responses of adenylate cyclase to forskolin or fluoride are not inhibited by manganese ions, whereas the response to p[NH]ppG is completely blocked. Activation of adenylate cyclase by forskolin is considerably greater than the activation by fluoride in membranes from rat cerebellum, striatum, heart, and liver, while being about equal or less than the activation by fluoride in other tissues. Forskolin (EC_{50}, 25 μM) causes a rapid and readily reversible 35-fold elevation of cyclic AMP in rat cerebral cortical slices that is not blocked by a variety of neurotransmitter antagonists. Low concentrations of forskolin (1 μM) augment the response of cyclic AMP-generating systems in brain slices to norepinephrine, isoproterenol, histamine, adenosine, prostaglandin E_2, and vasoactive intestinal peptide. Forskolin would appear to activate adenylate cyclase through a unique mechanism involving both direct activation of the enzyme and facilitation or potentiation of the modulation of enzyme activity by receptors or the guanyl nucleotide-binding subunit, or both.**

Cyclic nucleotides regulate many cellular events (1), but it has proven difficult to firmly establish the relationship of cyclic AMP (cAMP) levels to physiological functions in intact cells, tissues, and organisms. In part, this is due to the lack of a satisfactory *general* activator for adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in intact cells. Receptor-mediated activation of the enzyme is highly specific and is further dependent on interactions of an intracellular guanyl nucleotide-binding subunit with the catalytic unit of adenylate cyclase (2). Sodium fluoride (3), guanyl nucleotides (4), and divalent cations such as manganese (5, 6) and calcium (7) activate the enzyme independent of specific cell-surface receptors, but fluoride is ineffective with intact cells, and guanyl nucleotides and divalent cations require access to intracellular sites. Cholera toxin and other enterotoxins activate adenylate cyclase both in crude membranes and in intact cells, but the process is irreversible and requires interaction with a ganglioside cell-surface site (8).

The hypotensive (9) diterpene forskolin (Fig. 1) from the roots of *Coleus forskohlii* (10) has been reported to activate cardiac and brain adenylate cyclase (11, 12). In the present paper, forskolin is shown to be a potent and unique activator of adenylate cyclase in membranes from brain and other tissues and to elevate cAMP levels in intact cells of brain slices. Thus, forskolin represents an invaluable agent for the general activation of adenylate cyclase in intact cells and, hence, for the investigation of the relationship of cAMP levels to physiological functions in a variety of systems.

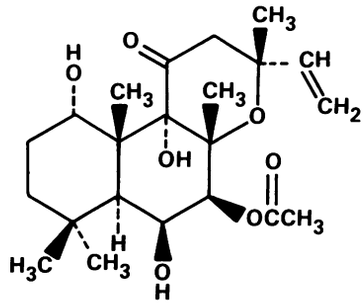

FIG. 1. Structure of forskolin.

MATERIALS AND METHODS

[2,8-^{3}H]Adenine (16 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and [2,8-^{3}H]ATP (29.7 Ci/mmol) were from New England Nuclear; adenosine 5′-[β,γ,-imido]triphosphate (p[NH]ppA), guanosine 5′-[β,γ-imido]triphosphate (p[NH]ppG), and [2,8-^{3}H]p[NH]ppA (18 Ci/mmol) were from ICN Chemical and Radioisotope Division, Irvine, CA; guanosine 5′-*O*-(2-thiodiphosphate) (GDP[βS]) was from Boehringer Mannheim. Prostaglandin E_2 was from Upjohn; 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone, coded ZK 62771, was provided by W. Kehr of Schering, Berlin. Forskolin (7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one, $C_{22}H_{34}O_7$; M_r, 410) was generously provided by Hoechst Pharmaceuticals, Bombay, India. Forskolin is now available from CalBiochem-Behring, La Jolla, CA, and has activity equivalent to that reported in this paper for the Hoechst sample. Forskolin (15 mM) was dissolved in 95% ethanol and was stable for at least 4 mo in solution. All other reagents were of the highest quality available from standard commercial sources.

Preparation of Membranes. A male Sprague–Dawley rat (150–175 g) was killed by decapitation, and the brain was removed quickly, chilled briefly in ice-cold Krebs–Ringer bicarbonate/glucose buffer, and placed on a glass plate. Cerebral cortical grey matter was dissected with a razor and homogenized in 50 mM Tris·HCl buffer, pH 7.5/0.1 mM $CaCl_2$ (5 ml per rat cortex) in a Dounce homogenizer (10 strokes). The homogenate

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Abbreviations: p[NH]ppA, adenosine 5′-[β,γ-imido]triphosphate; p[NH]ppG, guanosine 5′-[β,γ-imido]triphosphate; GDP[βS], guanosine 5′-*O*-(2-thiodiphosphate); cAMP, cyclic AMP.

was centrifuged at 10,000 × *g* for 20 min, and the supernatant was discarded. The pellet was washed once in 10 ml of ice-cold Tris·HCl buffer and centrifuged at 10,000 × *g* for 20 min. The final pellet was resuspended in 1.5 ml of Tris·HCl buffer per rat cortex and kept at 0°C on ice. Membranes from other tissues were prepared in an identical manner, except in some cases the tissue was disrupted with a Brinkman Polytron homogenizer. Fresh membranes were used in all experiments.

cAMP Levels in Brain Slices. Rat cerebral cortical slices were prepared, labeled with 30 μM [2,8-^{3}H]adenine (20–60 μCi) for 40 min at 37°C in Krebs–Ringer bicarbonate/glucose buffer, washed, incubated a further 15 min, divided into 15 portions, and incubated with agents for 10 min as described (13). Data are reported as percentage of conversions (i.e., the percentage of total radioactive adenine taken up that was converted to radioactive cAMP). The prelabeling technique has afforded results completely consonant with those based on measurement of endogenous levels of cAMP (14). Radioactive cAMP was isolated as described by Salomon *et al.* (15).

Assay of Membrane Adenylate Cyclase. Membrane adenylate cyclase assays were carried out essentially as described by Perkins and Moore (16). Incubations were carried out in a total volume of 250 μl containing 50 mM Tris·HCl buffer (pH 7.5), 1.0 mM 3-isobutyl-1-methylxanthine, 5 mM $MgCl_2$, 0.2 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 20 mM creatine phosphate, 10 units of creatine kinase, and 0.5 mM ATP containing 1 μCi of [2,8-^{3}H]ATP. When 0.15 mM p[NH]ppA containing 2 μCi [2,8-^{3}H]p[NH]ppA was used as a substrate, the assay conditions were as described with the exception that no creatine phosphate/creatine kinase regenerating system was used. Assays were initiated by the addition of 25 μl of membrane suspension to assay tubes that had been prewarmed at 37°C for 1 min. Assays were carried out at 37°C for 10 min and were terminated with 0.5 ml of 10% (wt/vol) trichloroacetic acid. Carrier cAMP (250 μl of 2 mM cAMP) was added, the mixture was centrifuged, and cAMP in the supernatant was isolated and analyzed by the method of Salomon *et al.* (15). Protein was determined in the trichloroacetic acid pellets by the Lowry method (17) as modified by Miller (18) with a Technicon AutoAnalyzer. Assays were carried out in triplicate, and activity is expressed as pmol of cAMP formed per min per mg of protein.

RESULTS

Effects of Forskolin on Membrane Adenylate Cyclase. Forskolin caused a 10-fold increase in the activity of adenylate cyclase in rat cerebral cortical membranes with ATP as substrate and in the presence of a nucleotide-regenerating system (Fig. 2*A*). Maximal activation occurred at about 100 μM forskolin with half-maximal activation occurring at about 10 μM forskolin. Membranes that were pretreated at 37°C for up to 5 min with 100 μM forskolin and subsequently washed showed no persistent activation of adenylate cyclase but rather exhibited activity almost identical to activity in control membranes preincubated without forskolin (data not shown). After a prior forskolin treatment and washing, forskolin restimulated the adenylate cyclase activity to an extent identical to stimulation of enzyme in control membranes (data not shown). Preincubation of membranes with 10 μM forskolin for up to 5 min prior to the addition of the radioactive ATP to initiate the assay of the enzyme did not lead to greater activation of adenylate cyclase (data not shown). Thus, the activation of brain adenylate cyclase by forskolin was rapid and completely reversible and did not lead to a desensitization of the enzyme with respect to reactivation by forskolin. Other agents, such as cholera toxin, p[NH]ppG, and NaF, that activate adenylate cyclase in broken cell preparations result in a virtual irreversible activation of the enzyme (8, 16, 19).

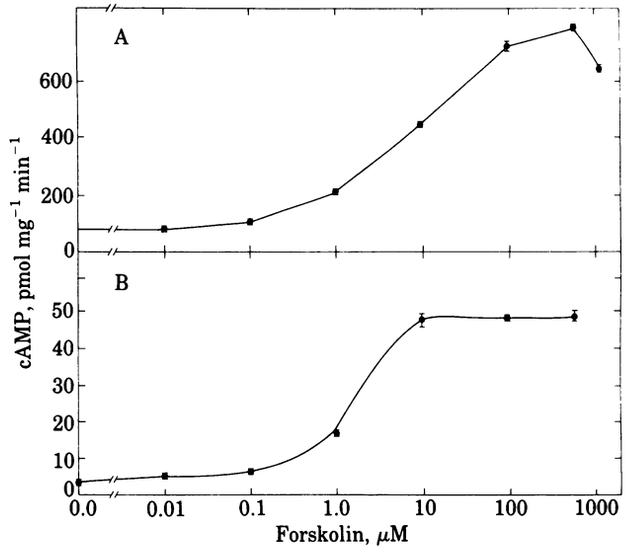

FIG. 2. Stimulation of rat cerebral cortical adenylate cyclase by forskolin. Assays were performed at 37°C for 10 min with 0.5 mM ATP and a nucleotide-regenerating system (*A*) or 0.15 mM p[NH]ppA with no regenerating system (*B*) as substrate. Values are the means ± SEM for three determinations.

When adenylate cyclase assays are carried out with ATP and nucleotide-regenerating systems, GTP is formed from endogenous guanyl nucleotides, making it difficult to assess the requirement of GTP for hormonal activation of the enzyme (20). Adenylate cyclase can be assayed in the absence of regenerating systems by using a nonhydrolyzable ATP analog, p[NH]ppA, as substrate, thereby circumventing any formation of GTP from endogenous GMP or GDP. Forskolin caused a 10-fold activation of adenylate cyclase with p[NH]ppA as substrate (Fig. 2*B*). The activation was rapid and reversible, with half-maximal activation occurring at about 5 μM and maximal activation occurring at about 10 μM. Thus, it would appear that the forskolin activation of adenylate cyclase does not depend on the formation or presence of GTP. This is quite unlike the activation of membrane adenylate cyclase by hormones or neurotransmitters, which in most instances requires the presence of GTP (2). Activation of adenylate cyclase in brain membranes by catecholamines could not be detected when assayed with p[NH]ppA under the conditions used (data not shown).

Sodium fluoride and the nonhydrolyzable guanine nucleotide p[NH]ppG activate adenylate cyclase through interactions that involve the guanyl nucleotide-binding subunit of the enzyme (5, 21, 22). Activation of the brain enzyme by p[NH]ppG was completely inhibited by GDP, GDP[βS], or GTP (Fig. 3), consonant with these nucleotides competitively inhibiting the interaction of p[NH]ppG at the guanyl nucleotide-binding site. Activation by fluoride was partially inhibited by GDP[βS], with no inhibition seen with GDP or GTP. Fluoride-activation of adenylate cyclase has been reported to be inhibited in other systems by GDP[βS] (23, 24). In contrast to the results with fluoride and p[NH]ppG, the activation of brain adenylate cyclase by 10 μM forskolin was not inhibited by GDP[βS] and was potentiated by both GDP and GTP (Fig. 3).

The response to maximal stimulatory concentrations of forskolin (100 μM) and fluoride (10 mM) was almost completely additive with p[NH]ppA as substrate, whereas the response to forskolin and a maximal stimulatory concentration of p[NH]ppG (100 μM) was not additive (Table 1). The lack of potentiation

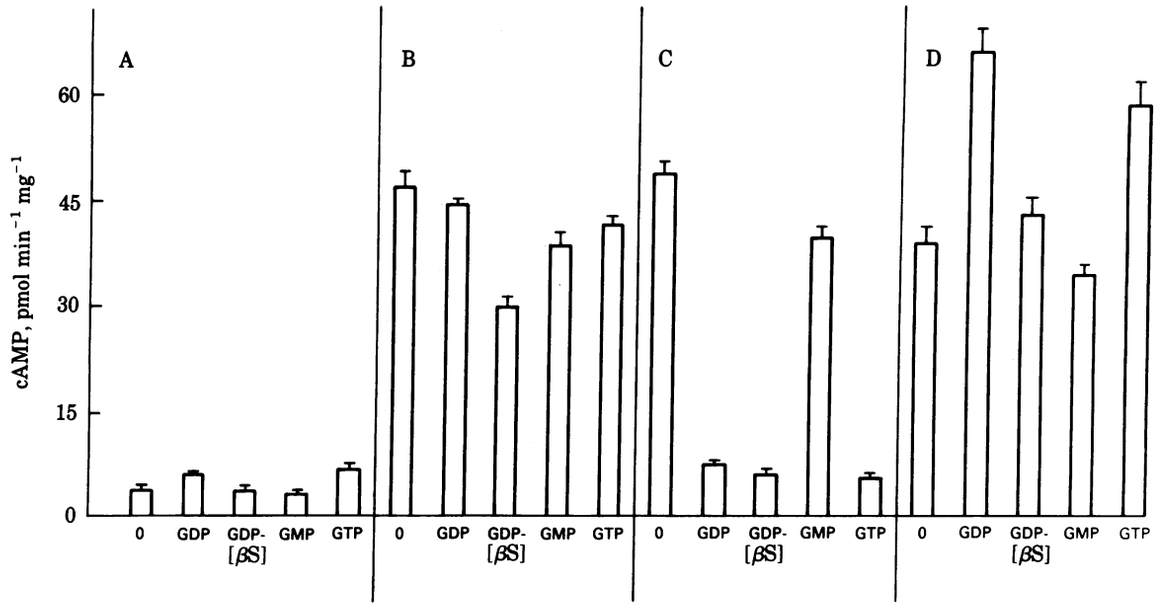

FIG. 3. Effect of guanine nucleotides on forskolin, fluoride, and p[NH]ppG stimulation of rat cerebral cortical adenylate cyclase. Membranes were prepared, and adenylate cyclase was assayed with 0.15 mM p[NH]ppA in the absence of a nucleotide-regenerating system. Membranes were incubated with no additions (*A*), 10 mM NaF (*B*), 10 μM p[NH]ppG (*C*), or 10 μM forskolin (*D*). The indicated guanine nucleotides (100 μM) were added at 0°C to the assays and warmed to 37°C for 1 min before the addition of the membrane suspension. Values are means ± SEM for three determinations.

of responses to forskolin by p[NH]ppG (Table 1) contrasted with the potentiation of the response to 10 μM and 100 μM forskolin by GTP and GDP (Fig. 3; unpublished data). The results for combinations of forskolin and fluoride with ATP as substrate were not as clear as the results were with p[NH]ppA as substrate. Forskolin (10 μM) still caused a further activation of the enzyme at both less than maximal and at maximal concentrations of fluoride with ATP as substrate (data not shown). However, the response to the combination of forskolin and fluoride was less than additive with ATP as substrate. The p[NH]ppG activation of adenylate cyclase was maximal at 100 μM with ATP as substrate, and forskolin caused no further activation of the enzyme (data not shown) as was the case with p[NH]ppA as substrate (Table 1). Adenylate cyclase partially activated by p[NH]ppG was, however, further activated by forskolin (data not shown).

Manganese ions are known to block the activation of adenylate cyclases by p[NH]ppG although having little effect on activation by fluoride (6, 22). When brain adenylate cyclase was assayed with increasing concentrations of manganese ions, there was some increase in basal activity of the enzyme, reaching a maximum at 5 mM manganese (Fig. 4). At this concentration of manganese ions there was virtually no response to 10 μM p[NH]ppG. In contrast, both the forskolin and fluoride-responses were not inhibited by manganese ions, but instead a slight potentiation occurred which was maximal at about 5 mM manganese.

Table 1. Stimulation of rat cerebral cortical adenylate cyclase by forskolin, fluoride, and p[NH]ppG

Agent	cAMP, pmol min^{-1} mg^{-1}
None	7.8 ± 1.4
Forskolin (100 μM)	55.1 ± 3.4
NaF (10 mM)	57.7 ± 1.4
p[NH]ppG (100 μM)	56.4 ± 2.8
NaF with forskolin	87.6 ± 2.9
p[NH]ppG with forskolin	51.8 ± 1.4

Rat cerebral cortical membranes were prepared and adenylate cyclase activity was assayed with 0.15 mM p[NH]ppA as substrate and no regenerating system. Values are means ± SEM for three determinations.

The effect of forskolin and fluoride on the activity of adenylate cyclase in membranes from a variety of rat tissues was investigated (Table 2). Adenylate cyclases of membranes from rat cerebellum, striatum, heart, and liver exhibited a 2- to 4-fold

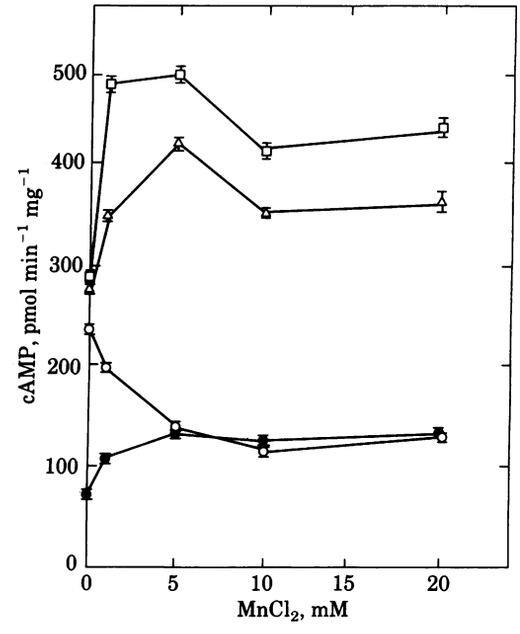

FIG. 4. Effect of manganese ions on fluoride, p[NH]ppG, and forskolin stimulation of rat cerebral cortical adenylate cyclase. Rat cerebral cortical membranes were prepared and adenylate cyclase activity was assayed with 0.5 mM ATP and a regenerating system. Membranes were incubated with the indicated concentrations of $MnCl_2$ in addition to the standard assay mixture with no additions (●), with 10 μM p[NH]ppG (○), 10 mM NaF (△), or 10 μM forskolin (□). Values are means ± SEM for three determinations.

Table 2. Effect of forskolin and fluoride on membrane adenylate cyclase activity from different tissues

Tissue	cAMP, pmol min^{-1} mg^{-1}		
	Basal	NaF	Forskolin
Cerebral cortex	65 ± 6	450 ± 8	630 ± 20
Cerebellum	110 ± 2	260 ± 16	810 ± 4
Striatum	130 ± 8	230 ± 12	1970 ± 90
Heart	10 ± 0.6	41 ± 3	100 ± 5
Liver	11 ± 0.4	54 ± 12	150 ± 4
Skeletal muscle	9 ± 2	38 ± 3	19 ± 0.2
Adrenal	24 ± 0.8	200 ± 9	81 ± 2
Pancreas	14 ± 0.5	120 ± 2	33 ± 0.6
Small intestine	20 ± 1	68 ± 1	44 ± 11
Spleen	37 ± 3	190 ± 20	160 ± 6
Kidney	22 ± 2	104 ± 10	98 ± 3
Stomach	13 ± 1	85 ± 5	23 ± 1
Testes	15 ± 1	60 ± 2	37 ± 2
Lung	40 ± 3	310 ± 30	64 ± 3

Tissue membranes were prepared and adenylate cyclase was assayed with 0.5 mM ATP and a nucleotide regenerating system. Basal activities were obtained with the standard assay mixture. The concentrations of NaF and forskolin were 10 mM and 100 μM, respectively. Membranes were added to the reaction mixture at 0°C for 10 min, and the assay was initiated at 37°C by the addition of the substrate. Incubations were carried out for 10 min. Values are means ± SEM for three determinations.

Table 3. Effect of forskolin on accumulations of cAMP in adenine-labeled rat cerebral cortical slices: Augmentation of responses to amines, vasoactive intestinal peptide, adenosine, prostaglandin E_2, and a phosphodiesterase inhibitor, ZK 62771

Agent, μM	Radioactive cAMP, % conversion	
	No forskolin	1 μM forskolin
None	0.21 ± 0.09	0.68 ± 0.28
Norepinephrine (100)	4.58 ± 0.53	9.40 ± 0.91
Isoproterenol (30)	1.37 ± 0.34	2.78 ± 0.30
Histamine (100)	0.57 ± 0.07	1.26 ± 0.20
Vasoactive intestinal peptide (0.05)	0.68 ± 0.04	4.58 ± 0.81
Adenosine (100)	2.41 ± 0.33	3.71 ± 0.48
Prostaglandin E_2 (50)	0.74 ± 0.13	3.29 ± 0.48
ZK 62771 (100)	1.64 ± 0.27	4.27 ± 0.79

Slices were prepared, labeled with radioactive adenine and incubated for 10 min with various agents in the presence or absence of forskolin; radioactive cAMP was determined and reported as percentage of total radioactivity present as cAMP. Values are means ± SEM of three experiments. In a separate set of three experiments, forskolin at concentrations from 1–300 μM elicited the following percentage conversions: 1 μM, 0.53 ± 0.1%; 5 μM, 1.7 ± 0.1%; 25 μM, 3.4 ± 0.4%; 150 μM, 6.6 ± 0.2%; 300 μM, 7.1 ± 0.3%.

activation by fluoride and a considerably greater activation by forskolin. In some tissues, the activations with fluoride were significantly greater than the activations elicited by forskolin. These tissues were skeletal muscle, adrenal, pancreas, stomach, and lung. Adenylate cyclases of all tissues examined were significantly activated by forskolin.

Effects of Forskolin on cAMP-Levels in Intact Cells. Forskolin elicited a maximal 35-fold increase in the accumulation of radioactive cAMP in adenine-labeled rat cerebral cortical slices (Table 3, legend). The forskolin-elicited increase in cAMP levels was dose dependent, with half-maximal effects at about 25 μM forskolin and maximal effects at 150 μM. The response to forskolin in brain slices was rapid, reaching a maximum within about 5 min (data not shown), a time course consonant with the time for penetration of a small molecule into brain slices (25). The increased accumulation of cAMP due to forskolin was maintained in brain slices for at least 40 min and was rapidly reversed after washing, with cAMP-levels returning to control values with a half-time of less than 5 min (data not shown). The response to 150 μM forskolin was not blocked by a variety of antagonists of neuromodulators or neurotransmitters (data not shown). These included the adenosine-antagonist 8-phenyltheophylline (10 μM), the α-adrenergic blocker phentolamine (10 μM), the β-adrenergic blocker propranolol (10 μM), and the histamine antagonists cimetidine (10 μM) and diphenhydramine (10 μM). The local anesthetic tetracaine (100 μM) had no effect on the response to forskolin. Forskolin at a concentration of 1 μM caused only a 3-fold accumulation of cAMP, but significantly augmented the accumulations of cAMP elicited by a variety of agents (Table 3). These agents included amines, such as norepinephrine, isoproterenol and histamine, vasoactive intestinal peptide, adenosine, and prostaglandin E_2. In the presence of a potent phosphodiesterase inhibitor, coded ZK 62771 (26), forskolin still elicited a marked accumulation of cAMP (Table 3).

DISCUSSION

Forskolin is a unique activator of adenylate cyclase, which can be distinguished from other stimulating agents by a number of criteria. The unique ability of forskolin to reversibly activate cAMP-generating systems in intact cells clearly distinguishes it from activators such as cholera toxin, which activates cAMP-generating systems in intact cells in an irreversible fashion by covalently modifying the guanyl nucleotide subunit (27–30). Activation of adenylate cyclase by forskolin does not appear to be related to an interaction with any of the major classes of cell-surface receptors, as a variety of receptor blockers do not inhibit forskolin-elicited accumulations of cAMP in brain slices. The activation of adenylate cyclase by forskolin in rat cerebral cortical membranes is independent of guanyl nucleotides—a characteristic that is not shared by neurotransmitters which require GTP or p[NH]ppG. Such neurotransmitters cause rather small activations of adenylate cyclase in membrane preparations from rat cerebral cortex (ref. 31 and unpublished data) in marked contrast to the 10-fold activation by forskolin. Activation of brain membrane adenylate cyclase by forskolin does not depend on calcium/calmodulin interactions because assays were carried out in the presence of 0.2 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'* -tetraacetic acid, which inhibits calcium/calmodulin activation of adenylate cyclase (32, 33). Forskolin does not increase apparent rates of formation of cyclic AMP by inhibiting phosphodiesterases because the membrane adenylate cyclase assays were performed in the presence of 1 mM 3-isobutyl-1-methylxanthine, a potent phosphodiesterase inhibitor (34). Furthermore, forskolin elicited marked accumulations of cyclic AMP in rat cerebral cortical slices, even in the presence of ZK 62771, another potent phosphodiesterase inhibitor (Table 3). Forskolin has been stated to have no effect on the activity of phosphodiesterases (9), but no details were reported. Forskolin thus appears to act directly at the adenylate cyclase enzyme complex, consisting of the guanyl nucleotide binding subunit and the catalytic subunit (2).

Activation of adenylate cyclase in membranes by forskolin is rapid and completely reversible—characteristics that distinguish it from fluoride activation of the enzyme, which is relatively irreversible (16). Furthermore, activation by forskolin of adenylate cyclase with p[NH]ppA as substrate is not inhibited by GDP[βS], whereas the response to fluoride is significantly inhibited (Fig. 3). GTP and GDP have little effect on the re-

sponse to fluoride, although potentiating the response to forskolin. With p[NH]ppA as substrate, activation of adenylate cyclase by forskolin is additive with fluoride activation (Table 1) and partially additive with ATP and a nucleotide regenerating system. Adenylate cyclases from liver and certain regions of brain, such as cerebellum and striatum, are greatly activated by forskolin, although showing a much smaller response to fluoride (Table 2). The converse is true in certain other tissues (for example, adrenal, pancreas, stomach, and lung). Thus, considerable evidence suggests that the mechanisms involved in the activation of adenylate cyclase by forskolin and fluoride are different. However, there are some similarities. For example, manganese ions do not inhibit the response to either forskolin or fluoride. Furthermore, the lack of additivity of the responses to forskolin and fluoride under certain conditions remains to be adequately explained, if the mechanisms involved are completely different. It should be noted that the most important difference between the responses to forskolin and fluoride is that forskolin causes a marked activation of adenylate cyclase in intact cells (Table 3), whereas fluoride does not (3).

The reversibility of the forskolin activation of adenylate cyclase distinguishes it from the p[NH]ppG activation of the enzyme, which is relatively irreversible (19). Furthermore, p[NH]ppG activation of brain adenylate cyclase is inhibited by other guanine nucleotides (GDP, GDP[βS], and GTP), none of which inhibit activation by forskolin (Fig. 3). Finally, p[NH]ppG activation of rat cerebral cortical adenylate cyclase is completely inhibited by 5 mM manganese, whereas that of forskolin is slightly potentiated (Fig. 4). Therefore, although p[NH]ppG and forskolin are not additive at their maximal doses, these two activators of adenylate cyclase clearly act through distinct mechanisms. This does not necessarily imply that activation of adenylate cyclase by forskolin does not affect interactions mediated through the guanyl nucleotide-binding subunit. Indeed, the responses to forskolin are affected by guanyl nucleotides, being potentiated by both GTP and GDP (Fig. 3). Hormonal activation of adenylate cyclase appears to be related to a facilitated exchange of guanyl nucleotides at the guanyl nucleotide-binding subunit (2). Forskolin appears to augment this hormonal activation not only in brain slices (Table 3) but also in crude membrane homogenates (35). *In toto*, the results suggest that forskolin not only activates adenylate cyclase directly in a guanyl nucleotide-independent manner, but also forskolin in some manner facilitates or potentiates the modulation of the enzyme activity by hormones or guanyl nucleotides, or both.

Forskolin activates adenylate cyclase in all tissues as yet tested. The unique action of forskolin on adenylate cyclase provides a powerful tool for investigation of this complex enzyme. In addition, the ability of forskolin to rapidly and reversibly increase cAMP levels in intact cells and tissues and to augment hormonal responses provides a hitherto unavailable tool for assessing the role of adenylate cyclase and cAMP in cell physiology.

Note Added in Proof. Forskolin will activate a water-soluble adenylate cyclase from rat testes and adenylate cyclase in membranes from the cyc$^-$ mutant of S49 murine lymphoma cells. These enzymes do not contain a functional guanine nucleotide-binding protein. Thus, forskolin appears to activate adenylate cyclase by direct interactions with the catalytic subunit, and this interaction is not due to a general perturbation of membrane structure.

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