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Forskolin decreases sensitivity of brain adenylate cyclase to inhibition by 2',5'-dideoxyadenosine

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The effects of forskolin on the sensitivity of adenylate cyclase to 'P'-site-mediated inhibition were studied. Stimulation of crude and purified preparations of adenylate cyclase by forskolin led to decreased sensitivity to inhibition by 2',5'dideoxyadenosine with enzyme from rat and bovine brain. This is in contrast with the enhancement of P-site sensitivity induced by calmodulin, divalent cations, and stable GTP analogs and is in contrast with behavior seen with enzyme from liver and S49 cyc$^-$ membranes. The effect of forskolin on P-site sensitivity of the brain adenylate cyclase was not dependent on the presence of G-proteins or calmodulin. It was not the consequence of proteolysis nor was it due to an obvious artifact in the assay procedures. This distinct behavior of the brain enzyme is most likely due to a structural difference in the catalytic subunit.

Adenylate cyclase; P-site; Dideoxyadenosine, 2',5'-; Deoxyadenosine 3'-monophosphate, 2'-; Forskolin; Calmodulin; (Brain)

1. INTRODUCTION

Adenosine is known to modulate adenylate cyclase activity through three distinct sites. Two cell-surface receptors, the stimulatory R_a or A_2 and the inhibitory R_i and A_1 receptors, alter the activity of adenylate cyclase via the respective guanine nucleotide regulatory (G-) proteins, and a site on the intracellular surface of the membrane mediates inhibition of the catalytic subunit [1-6]. The latter adenosine site has been referred to as the 'P'-site [1] from its evident requirement for an intact purine moiety. Inhibition of adenylate cyclase via the P-site is independent of guanine nucleotides, is non-competitive with respect to metal-ATP, is metal dependent, and is accompanied by a 20-fold increase in the enzyme's apparent affinity for free metal [5-8]. In general, an important characteristic of P-site-mediated inhibition is that stimulated forms of adenylate cyclase are substantially more sensitive to inhibition than are unstimulated forms [6-9]. This sensitization of adenylate cyclase has been observed by reversibly stimulatory agents, e.g. forskolin, Mn^{2+}, or hormones and GTP, as well as by agents that lead to irreversible activation, e.g. fluoride, stable GTP analogs, or proteolysis in the presence of GTPγS [6-11]. A possible exception to this may be the sperm adenylate cyclase, which exhibits a P-site characterized by non-competitive, metal-dependent inhibition, but which exhibits substantially reduced sensitivity to established P-site agonists that is not enhanced by stimulatory agents [5,6,12]. The sensitization that occurs in most tissues is likely due to conformational changes in the enzyme's catalytic subunit since forskolin, known to act at the catalytic unit, has been reported to potentiate inhibition by the P-

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Abbreviations: 2'd3'AMP, 2'-deoxyadenosine 3'-monophosphate; 2'5'ddAdo, 2',5'-dideoxyadenosine; DTT, dithiothreitol; GTPγS, guanosine 5'-*O*-(3-thiotriphosphate); GPP(NH)P, guanyl-5'yl (β,γ-imino)diphosphate; IBMX, 3-isobutyl-1-methylxanthine; TEA-HCl, triethanolamine-HCl

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site agonist 2'5'ddAdo in rabbit liver and in wild-type and cyc$^-$ S49 lymphoma cells by Florio and Ross [11], and in pigeon breast muscle by Drummond [10]. We report here a contrasting decrease in the degree of inhibition by 2'5'ddAdo upon stimulation with forskolin in various adenylate cyclase preparations from brain. Possible causes for this counteractive action of forskolin and P-site agonists are discussed.

2. MATERIALS AND METHODS

2.1. *Enzyme preparations*

Particulate adenylate cyclase from rat brain was prepared as described [13]. Particulate and detergent-dispersed adenylate cyclases from rat brain and from bovine brain cortex were prepared similarly, except that for the bovine enzyme the homogenizing medium was changed to 20 mM TEA-HCl (pH 7.2), 250 mM sucrose, 1 mM EDTA, 3 mM DTT and 2.5 mM benzamidine during the first two homogenizing steps, then to 50 mM TEA-HCl (pH 7.4) in the third rehomogenizing step (washed particles), and to 50 mM TEA-HCl (pH 7.4) and 1% Lubrol-PX, without sucrose, in the detergent extraction step. Centrifugation in the first three steps was at 17 700 × g (r_{max}) for 30 min and in the detergent extraction step at 30 000 × g (r_{max}) for 60 min.

The catalytic subunit of adenylate cyclase was purified from bovine brain cortex to near homogeneity by forskolin affinity chromatography followed by chromatography on wheat-germ lectin Sepharose, essentially as described [14]. Commercially available 7-*O*-hemisuccinyl-7-deacetylforskolin was coupled to Affigel-10 as described [15]. The purified preparation was free of α_s, α_i, α_o and β subunits, as judged by immuno-blots.

Membranes from cyc$^-$ cells were obtained as in [16] and liver plasma membranes were prepared as in [17].

2.2. *Adenylate cyclase assay*

Adenylate cyclase activity was determined [8] in a reaction mixture containing 50 mM TEA-HCl, pH 7.5, 1 mM IBMX, 1 mM DTT, 0.1 mM or 1 mM EDTA, 2 mM purified creatine phosphate, creatine kinase (100 μg/ml), myokinase (100 μg/ml), adenosine deaminase (5 U/ml), 100 μM ATP [α-^{32}P]ATP (3–25 × 10^5 cpm), and the indicated concentrations of $MgCl_2$ or $MnCl_2$ in a volume of 100 μl. Prior to use myokinase and adenosine deaminase were centrifuged and the supernatant ammonium sulfate was removed. The pelleted enzymes were resuspended in water for use. After 15 min at 30°C reactions were stopped by the $ZnCO_3$ precipitation method [18] and the labelled cAMP was purified as in [19].

Results comparable to those shown were obtained in at least two separate experiments, each done in duplicate. IC_{50} values were determined graphically and are averages ± range from two experiments, each done in duplicate.

2.3. *Materials*

[α-^{32}P]ATP was purchased from ICN Pharmaceuticals. 2'5'ddAdo was synthesized by Dr S.-M.H. Yeung in our laboratory. 7-*O*-Hemisuccinyl-7-deacetylforskolin was obtained from Calbiochem. Affigel-10 was from BioRad and wheat germ lectin Sepharose 6MB was from Pharmacia. Cyc$^-$ cells were kindly provided by Dr G. Schultz, Freie Universität Berlin, Germany and antibodies to α_s, α_i, α_o and β subunits of the respective G-proteins were gifts from Dr A. Spiegel, National Institutes of Health, Bethesda, MD.

Other reagents were from commercial sources and were of the highest quality available.

3. RESULTS

3.1. *P-site sensitivity of crude and purified adenylate cyclase*

In agreement with observations of others [7–11], we found that sensitivity of the particulate rat brain adenylate cyclase to inhibition by 2'5'ddAdo was increased upon activation by metal ions and with prior activation by GTPγS or cholera toxin (table 1). For example, in the presence of 2.1 mM Mg^{2+} the IC_{50} for 2'5'ddAdo was reduced from 80 ± 9 μM for the unstimulated enzyme to approximately 40 μM for GTPγS- or cholera toxin-stimulated enzyme (table 1). Likewise, forskolin stimulated the brain enzyme, 6.0-fold with 2.1 mM Mg^{2+} and 5.3-fold with 10 mM Mg^{2+}. However, these effects were actually accompanied by an increase in the IC_{50} for inhibition by 2'5'ddAdo (table 1). This is in contrast with observations on enzyme from pigeon breast muscle, rabbit liver, and wild type or cyc$^-$ S49 lymphoma cells [10,11], in which forskolin potentiated inhibition by 2'5'ddAdo.

The reduced sensitivity to P-site-mediated inhibition of adenylate cyclase that was seen with forskolin was neither species specific nor was it confined to crude particulate preparations of brain enzyme. It was conceiveable, for example, that crude detergent extracts from brain tissue contained factors that altered the effects of forskolin on adenylate cyclase or mediated its effect to reduce P-site sensitivity. Alternatively, since it is well known that a predominant form of adenylate cyclase in brain tissue is calmodulin sensitive, it may have been that the effect of forskolin was confined to this form of the enzyme. The effects of forskolin on 2'5'ddAdo inhibition of crude and purified enzyme from bovine brain are illustrated in fig.1. Forskolin caused a 2-fold increase in the IC_{50} for 2'5'ddAdo in the presence of 3 mM Mg^{2+} (from 97 ± 3 to 200 μM) or 11 mM Mg^{2+} (from 32 ± 4 to 56 μM) (fig.1, upper panel). With 3.5 mM Mn^{2+}

Table 1

The effects of various stimuli on the relative sensitivity of particulate brain adenylate cyclase to inhibition by 2'5'-dideoxyadenosine

	IC_{50} values for 2'5'dideoxyadenosine (μM)					
	Control	Forskolin (100 μM)	GTPγS (10 μM)	CTx	Mn^{2+} (1 mM)	Forskolin + Mn^{2+}
Mg^{2+} (mM)						
2.1	80 ± 9	90 ± 10	43 ± 2	42 ± 4	2.8 ± 0.6	2.4 ± 0.2
10	19 ± 1	44 ± 12	20 ± 9	10 ± 0	4.9 ± 1.4	4.3 ± 0.3

Particulate adenylate cyclase from rat brain (8 to 14 μg protein/tube) was pretreated with GTPγS (10 μM; 15 min at 30°C) or cholera toxin (CTx 50 μg/ml + 500 μM GTP for 30 min at 20°C) and incubated as described in section 2.2 with a reaction mixture containing 100 μM GTP, 0.1 mM EDTA, 2.1 or 10 mM Mg^{2+}, and varying concentrations of 2'5'ddAdo (0.1 to 1000 μM). Initial activities in pmol cAMP/min per mg protein with 2.1 mM Mg^{2+} were: control, 119; forskolin, 714 ± 27; GTPγS, 284 ± 17; CTx, 203 ± 4; Mn^{2+}, 869 ± 5; forskolin + Mn^{2+}, 2916 ± 39; and with 10 mM Mg^{2+} were: control, 231 ± 23; forskolin, 1216 ± 181; GTPγS, 601 ± 47; CTx, 317 ± 34; Mn^{2+}, 1245 ± 48; forskolin + Mn^{2+}, 4734 ± 35. Values are expressed as averages ± range from two separate experiments, each done in duplicate

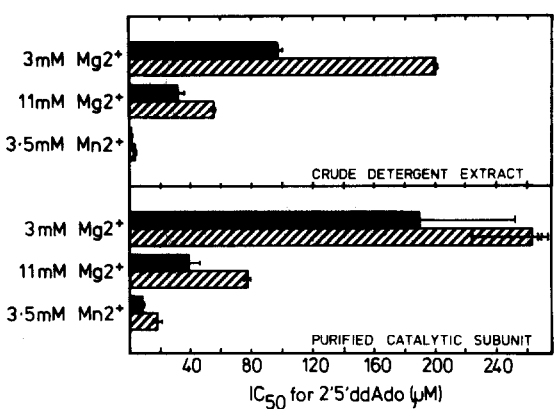

Fig.1. Effects of forskolin and divalent cations to alter sensitivity of crude and purified preparations of brain adenylate cyclase to inhibition by 2'5'ddAdo. Adenylate cyclase was assayed as in section 2.2 with a reaction mixture containing bovine serum albumin (1 mg/ml), 0.1% Lubrol PX, 1 mM EGTA, 100 μM ATP, and the indicated concentrations of metal ions, but without myokinase or adenosine deaminase. IC_{50} values are averages ± range from two experiments, each conducted in duplicate. Concentrations of 2'5'ddAdo were from 0.01 to 1000 μM. Solid bars: control; cross-hatched bars: 100 μM forskolin. Upper panel: detergent-dispersed adenylate cyclase from bovine brain (148 μg protein/ml) had the following initial activities, in pmol cAMP/min per mg protein, without forskolin: 3 mM Mg^{2+}, 43; 11 mM Mg^{2+}, 56; 3.5 mM Mn^{2+}, 1733; and with 100 μM forskolin: 3 mM Mg^{2+}, 308; 11 mM Mg^{2+}, 429; 3.5 mM Mn^{2+}, 6377. Lower panel: purified catalytic subunit from bovine brain was assayed without IBMX or ATP-regenerating system and had the following initial activities, in pmol cAMP/min per 10 μl enzyme, without forskolin: 3 mM Mg^{2+}, 0.46; 11 mM Mg^{2+}, 0.67; 3.5 mM Mn^{2+}, 1.73; and with 100 μM forskolin: 3 mM Mg^{2+}, 1.72; 11 mM Mg^{2+}, 2.26; 3.5 mM Mn^{2+}, 10.73.

forskolin increased the IC_{50} for 2'5'ddAdo from 1.4 ± 0.1 to 4.3 ± 0.3 μM. Although the purified enzyme from bovine brain exhibited a progressive loss in P-site sensitivity during its purification (fig.1, compare upper and lower panels [cf. 4,6]), it retained sensitivity to stimulation by forskolin (cf. [14]) and forskolin still caused a rightward shift in the concentration dependence for inhibition by 2'5'ddAdo (fig.1). This was observed at all Mg^{2+} and Mn^{2+} concentrations tested (fig.1, lower panel). These data imply that the effect of forskolin to counter inhibition by P-site agonists is not caused by factors present in the crude detergent extracts, but is a property of the catalytic subunit of the brain adenylate cyclase.

The effect of forskolin to decrease P-site sensitivity was concentration dependent (fig.2), with effects being evident with as little as 1 μM forskolin. The effect of forskolin on the IC_{50} for 2'5'ddAdo appears to be saturable, suggesting that the opposing actions of forskolin and 2'5'ddAdo are not competitive. Indeed, analysis of the kinetics of these effects on adenylate cyclase showed that 2'5'ddAdo and forskolin acted in a nonlinear, noncompetitive manner with the purified enzyme (not shown). Similar results were obtained with the crude detergent-dispersed enzyme. These data would be consistent with there being distinct binding domains on the catalytic subunit for forskolin and for P-site agonists.

Moreover, this effect of forskolin was not

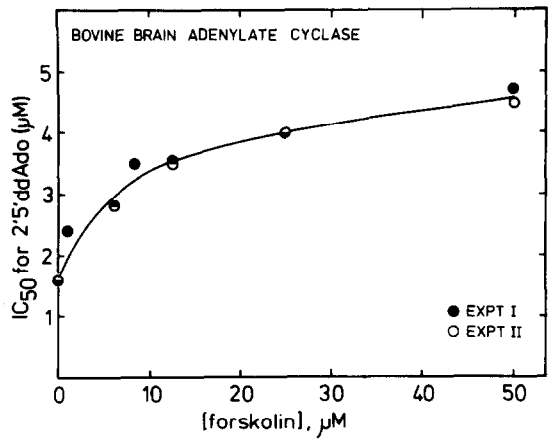

Fig.2. Concentration dependence of the effect of forskolin on the IC_{50} for 2'5'ddAdo. Detergent-dispersed adenylate cyclase was incubated as in section 2.2, with 3.5 mM Mn^{2+}. Concentrations of 2'5'ddAdo were from 0.01 to 1000 μM. IC_{50} values are from two separate experiments, each conducted in duplicate.

Table 3

Calmodulin-enhanced sensitivity to inhibition of purified adenylate cyclase by 2'5'-dideoxyadenosine

Metal	IC_{50} values for 2'5'-dideoxyadenosine (μM)	
	Control	Calmodulin (25 μg/ml)
$MgCl_2$ (10 mM)	31 ± 1	13 ± 3
$MnCl_2$ (2.5 mM)	5.5 ± 2.0	1.8 ± 0.5

Purified adenylate cyclase from bovine brain cortex was incubated as described in section 2.2 with a reaction mixture containing bovine serum albumin (1 mg/ml), 3 mM dithiothreitol, 0.1% Lubrol-PX, 100 μM ATP, and the indicated concentrations of metal. For reactions with calmodulin 50 μM $CaCl_2$ was added. Concentrations of 2'5'ddAdo were from 0.1 to 1000 μM. Values averages ± range from two experiments, each assayed in duplicate

restricted to inhibition by 2'5'ddAdo, but was also seen with 2'd3'AMP and adenosine (table 2). Thus, the forskolin-induced increase in the IC_{50} for 2'5'ddAdo is a characteristic of the P-site and not of a particular agonist. The consistent 2- to 3-fold increase in the IC_{50} for the P-site agonists we observed with the brain enzyme, contrasts with the decreased IC_{50} expected from forskolin's known effects on adenylate cyclase from other tissues (cf. [11] and below).

A possible explanation for this distinct behavior of the brain adenylate cyclase might be that this enzyme had undergone proteolysis during its isolation. That this was not the case could be concluded from experiments with enzyme from bovine brain that was prepared in the presence or absence of an array of protease inhibitors (antipain, aprotinin, soybean trypsin inhibitor, leupeptin, chymostatin, pepstatin, phenylmethylsulfonyl fluoride, *N*-tosyl-L-phenylalanine chloromethyl ketone, *Nα-p*-tosyl-L-lysine chloromethyl ketone, benzamidine, and EGTA). Specific activities of both preparations were comparable. Moreover, the effects of for-

Table 2

Opposing actions of forskolin and different P-site analogs on detergent-dispersed adenylate cyclase from brain

		Control		Forskolin (100 μM)	
		Enzyme activity (pmol/min per mg)	Inhibition (%)	Enzyme activity (pmol/min per mg)	Inhibition (%)
No addition		1403 ± 176		5081 ± 237	
2'5'ddAdo	(10 μM)	239 ± 17	82.9 ± 1.4	1938 ± 91	61.8 ± 2.7
	(100 μM)	88 ± 3	93.7 ± 0.7	668 ± 21	86.8 ± 0.7
2'd3'AMP	(10 μM)	241 ± 11	82.6 ± 1.5	1607 ± 36	68.3 ± 0.9
	(100 μM)	97 ± 6	93.0 ± 1.2	555 ± 33	89.1 ± 0.9
Adenosine	(10 μM)	975 ± 100	30.3 ± 1.8	4138 ± 189	18.4 ± 5.9
	(100 μM)	414 ± 29	70.4 ± 2.0	2640 ± 65	48.0 ± 1.2

Detergent-dispersed adenylate cyclase from bovine brain cortex (10 or 14.8 μg protein/tube) was incubated as described in section 2.2 with a reaction mixture containing bovine serum albumin (1 mg/ml), 0.1% Lubrol PX, 1 mM EGTA, and 3.5 mM Mn^{2+}, but without myokinase or adenosine deaminase. Results are expressed as means ± SD from three separate experiments, each done in duplicate

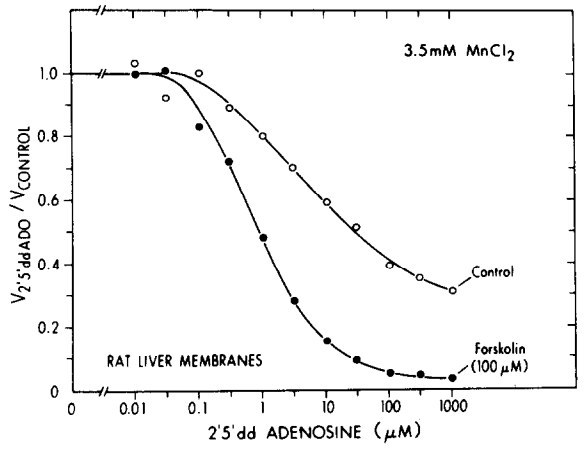

Fig.3. Forskolin-enhanced sensitivity of hepatic adenylate cyclase to inhibition by 2'5'ddAdo. Rat liver membranes (8.25 μg protein/tube) were incubated for 2 min at 37°C as described in section 2.2, in the presence of 3.5 mM Mn^{2+} and 1 mM EGTA. Initial activities in pmol cAMP/min per mg protein were: control, 13.6 (○); 100 μM forskolin, 223 (●). Values are averages of duplicate determinations in one of two similar experiments. See text for IC_{50} values.

skolin to stimulate the enzyme and to diminish sensitivity to P-site inhibition were also unaffected by use of the protease inhibitors (not shown).

3.2. *Calmodulin-enhanced sensitivity to P-site inhibition*

Since brain adenylate cyclase is stimulated by Ca^{2+}/calmodulin [4,14], the possible influence of calmodulin on the effects of forskolin and P-site sensitivity of the enzyme were examined. However, the results shown in fig.1 suggested that calmodulin did not alter the enzyme's response to forskolin, since these experiments had been conducted in the presence of 1 mM EGTA. The presence of EGTA reduced activity of the crude enzyme by 80% (not shown), due to the removal of calmodulin, but this did not alter the counteractive effects of forskolin and 2'5'ddAdo (fig.1, upper panel). Uncertain, though, was the effect of calmodulin on P-site sensitivity. When a saturating concentration of calmodulin (25 μg/ml) was added to the purified catalytic subunit, adenylate cyclase activity increased 3.2- and 2.1-fold with 10 mM Mg^{2+} and 2.5 mM Mn^{2+}, respectively, in agreement with observations by Smigel [14]. This stimulation by calmodulin was accompanied by a 2- to 3-fold increased sensitivity to inhibition by 2'5'ddAdo (table 3), which contradicts the findings of Yeager et al. [4], also with adenylate cyclase from bovine brain. These workers reported no significant effect of calmodulin on the IC_{50} (60 to 80 μM) for 3'dAdo as P-site agonist. Although these workers reported increased sensitivity to P-site-mediated inhibition by preactivation with GPP(NH)P, the IC_{50} values for 2'5'ddAdo (24 μM) and adenosine (260 μM) [4] were significantly higher than we observed (cf. table 2), suggesting qualitative differences in the two enzyme preparations. Our data suggest that, as with

Table 4

Potentiative interaction of forskolin and 2'5'-dideoxyadenosine in particulate adenylate cyclase from S49 cyc^- cells

	Adenylate cyclase activity (pmol cAMP/min per mg protein)		
	Control	2'5'ddAdo (100 μM)	Inhibition (%)
Mn^{2+} (3.5 mM)	7.3 ± 0.9	6.0 ± 0.4	17 ± 6
Mn^{2+} (20 mM)	7.0 ± 0.4	4.9 ± 0.6	30 ± 7
Mn^{2+} (3.5 mM) + forskolin (100 μM)	343 ± 71	65 ± 7	81 ± 2
Mn^{2+} (20 mM) + forskolin (100 μM)	279 ± 48	104 ± 13	62 ± 2

Membranes (20 to 40 μg protein/tube) from cyc^- variants of S49 lymphoma cells were incubated as described in section 2.2 with a reaction mixture containing 1 mM EDTA instead of 0.1 mM. Results are expressed as means ± SD from three separate experiments, each done in duplicate

other activators of adenylate cyclase, calmodulin enhances sensitivity to P-site-mediated inhibition and this is opposite to the effect of forskolin, although both effects are evidently on the catalytic subunit of the brain enzyme.

3.3. *Forskolin-enhanced sensitivity to P-site inhibition in other tissues*

To rule out the possibility that the unique effect of forskolin on the brain enzyme might be due to an artifact introduced in our laboratory, we tested the effects of forskolin on P-site sensitivity in rat liver membranes and S49 cyc$^-$ membranes (cf. [11]). With the adenylate cyclase of rat liver membranes (fig.3), forskolin induced a pronounced leftward shift in the inhibition curve for 2′5′ddAdo (IC_{50} 1.0 ± 0.1 μM) relative to the control (IC_{50} 19 ± 6 μM). With S49 cyc$^-$ membranes (table 4), forskolin also potentiated inhibition of adenylate cyclase by 2′5′ddAdo, consistent with observations by Florio and Ross [11]. The data indicate that adenylate cyclases from these tissues and from brain differ significantly in the character of the effects that forskolin induces in the enzyme's catalytic subunits.

4. DISCUSSION

Adenylate cyclase from brain exhibits the typical metal-dependent, non-competitive inhibition by P-site specific agents, as well as enhancement of sensitivity to inhibition by 2′5′ddApo upon activation via the stimulatory G_s protein by cholera toxin and upon proteolytic activation with the sperm protease ninhibin in the presence of GTPγS [6]. However, the effect of forskolin to potentiate P-site-mediated inhibition that is typical of adenylate cyclase preparations from a variety of tissues [10,11] was absent in the brain enzyme. This unusual behavior of the brain enzyme was not due to an obvious artifact in the assay procedure, nor was it likely a consequence of proteolysis of the brain enzyme. The effect of forskolin to diminish the inhibitory potency of 2′5′ddAdo was seen with both crude and purified preparations of the brain adenylate cyclase and in the presence or absence of calmodulin and/or functionally active G-proteins. Since other effects of forskolin on adenylate cyclase, such as enzyme activation or protection against inactivation by heat or *N*-ethylmaleimide, seem to be similar in brain and other tissues [11,16], it was surprising that the forskolin-stimulated form of the brain enzyme was actually less sensitive to P-site-mediated inhibition than was the unstimulated form.

From available evidence the loci for inhibition of adenylate cyclase by P-site agonists and activation of the enzyme by forskolin or calmodulin are on the enzyme's catalytic subunit itself [3–6,8,11,14]. It is thus likely that for the adenylate cyclases of liver or S49 cyc$^-$ cells, for example, one consequence of activation of the enzyme by forskolin is a conformational change in the catalytic unit that specifically affects the P-site domain, resulting in increased affinity for the P-site specific ligands. Data reported here would suggest further that an analogous conformational change in the catalytic subunit of the brain enzyme may be induced by Ca^{2+}/calmodulin (table 3). However, it is this structural change affecting the P-site domain that apparently does not occur when the brain adenylate cyclase is stimulated by forskolin. These observations lend further support to the idea that the calmodulin-sensitive form of brain adenylate cyclase is structurally different from hormone-sensitive forms of the enzyme in this and other tissues.

Other exceptions exist to the general rule that stimulated forms of adenylate cyclase are more sensitive to inhibition by P-site agonists than are unstimulated forms. We have observed with adenylate cyclase from rat brain, for example, that NH_4^+ ions increased activity but decreased its sensitivity to P-site-mediated inhibition [20]. This effect, while not directly on the catalytic unit is likely due to altered interactions of the catalytic unit and $G_s\alpha$, thereby affecting the conformation of the catalytic unit and consequently the sensitivity to inhibition by P-site agonists. In other studies, although millimolar Ca^{2+} inhibits adenylate cyclases, Ca^{2+} was reported to enhance sensitivity of the enzyme from guinea pig lung to inhibition by P-site agonists [21]. This may be due to calcium's mimicking or substituting for Mn^{2+} or Mg^{2+} at the metal-binding domain of the catalytic unit and inducing sufficient conformational changes in the catalytic unit for the enzyme to exhibit P-site sensitivity. Divalent cation, though, may not induce these changes in the catalytic units of all adenylate cyclases. The enzyme from ram [5] or bovine [6,12]

sperm, for example, exhibits poor P-site sensitivity (IC_{50} values of 0.5 to 1 mM for adenosine), even in the presence of optimally stimulatory concentrations of Mn^{2+}. The sperm enzyme, though, does not exhibit the same structure-activity relationship for inhibitory agonists as does the enzyme from somatic cells, in that adenosine was typically more potent than 2′dAdo or 2′5′ddAdo [5,6,12]. Thus, while virtually all mammalian adenylate cyclases exhibit a P-site, the enzymes may be distinguished by the structures and characteristics of these inhibitory binding domains.

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REFERENCES

[1] Londos, C. and Wolff, J. (1977) Proc. Natl. Acad. Sci. USA 74, 5482–5486.
[2] Wolff, J., Londos, C. and Cooper, D.M.F. (1981) Adv. Cyclic Nucleotide Res. 14, 199–214.
[3] Neer, E. and Salter, R.S. (1981) J. Biol. Chem. 256, 12102–12107.
[4] Yeager, R.E., Nelson, R. and Storm, D.R. (1986) J. Neurochem. 47, 139–144.
[5] Henry, D., Ferino, F., Tomova, S., Ferry, N., Stengel, D. and Hanoune, J. (1986) Biochem. Biophys. Res. Commun. 137, 970–977.
[6] Johnson, R.A., Yeung, S.-M.H., Stübner, D., Bushfield, M. and Shoshani, I. (1989) Mol. Pharmacol., in press.
[7] Johnson, R.A., Saur, W. and Jakobs, K.H. (1979) J. Biol. Chem. 254, 1094–1101.
[8] Londos, C. and Preston, M.S. (1977) J. Biol. Chem. 252, 5951–5956.
[9] McKenzie, S.G. and Bär, H.P. (1973) Can. J. Physiol. Pharmacol. 41, 190–196.
[10] Drummond, G.I. (1984) Arch. Biochem. Biophys. 235, 427–437.
[11] Florio, V.A. and Ross, E.M. (1983) Mol. Pharmacol. 24, 195–202.
[12] Brown, M.A. and Casillas, E.R. (1984) J. Androl. 5, 361–368.
[13] Johnson, R.A. and Sutherland, E.W. (1973) J. Biol. Chem. 248, 5114–5121.
[14] Smigel, M.D. (1986) J. Biol. Chem. 261, 1976–1982.
[15] Pfeuffer, E., Mollner, S. and Pfeuffer, T. (1985) EMBO J. 4, 3675–3679.
[16] Awad, J.A., Johnson, R.A., Jakobs, K.H. and Schultz, G. (1983) J. Biol. Chem. 258, 2960–2965.
[17] Pilkis, S.J. and Johnson, R.A. (1974) Biochim. Biophys. Acta 341, 388–395.
[18] Jakobs, K.H., Saur, W. and Schultz, G. (1976) J. Cyclic Nucleotide Res. 2, 381–392.
[19] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[20] Yeung, S.-M.H., Shoshani, I., Stübner, D. and Johnson, R.A. (1989) Arch. Biochem. Biophys., in press.
[21] Welton, A.F. and Simko, B.A. (1980) Biochim. Biophys. Acta 615, 252–261.