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DIFFERENTIATION OF FLUORIDE-STIMULATED AND NON-FLUORIDE-STIMULATED COMPONENTS OF BEEF BRAIN CORTEX ADENYLATE CYCLASE BY CALCIUM IONS, ETHYLENEGLYCOL-BIS-(β-AMINOETHYL ETHER) *N,N'*-TETRAACETIC ACID AND TRITON X-100

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Summary

Beef brain cortex adenylate cyclase (ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1) activity is 84—88% inhibited by $5 \cdot 10^{-5}$ M ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid in the absence of F^- but only 50—60% inhibited by $5 \cdot 10^{-5}$ M ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid in the presence of F^-. In either case, further increase in EGTA concentration did not alter the degree of inhibition. The inhibition can be completely reversed in both cases by addition of $3 \cdot 10^{-5}$ M Ca^{2+}, (yielding a [free Ca^{2+}] of approximately $2 \cdot 10^{-6}$ M) and $5 \cdot 10^{-5}$ M Mn^{2+} or Co^{2+} and partially by $5 \cdot 10^{-5}$ M Sr^{2+} but not by addition of $5 \cdot 10^{-5}$ M Ba^{2+}, Zn^{2+}, Ni^{2+} or Fe^{2+}. A [free Ca^{2+}] of $7.2 \cdot 10^{-5}$ M markedly inhibited cyclase activity in the presence of F^-. Solubilization by 1.8% Triton X-100 resulted in an enzyme preparation no longer stimulated by NaF and 100% inhibited by the addition of $5 \cdot 10^{-5}$ M ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid either in the absence or presence of NaF. However, in contrast to ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid, EDTA had no measurable effect on adenylate cyclase either in the presence or absence of NaF and ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid did not affect ATPase or phosphodiesterase activities.

The data is rationalized by the postulation of two independent enzyme components in brain cortex: one component is about six-fold activated by NaF and the NaF effect is enhanced by low concentrations of Ca^{2+} and Mg^{2+}. A second component is totally Ca^{2+} dependent and inhibited by high concentrations of F^-. Mn^{2+}, Co^{2+} and Sr^{2+} appear to be in vitro Ca^{2+} substitutes for both enzyme systems. On this basis, Triton X-100 treatment results in about a

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid.

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three-fold increase in specific activity of the Ca^{2+} dependent cyclase component but a complete abolition of the NaF stimulated component.

Introduction

Partial inhibition of beef brain adenylate cyclase [1,2] and rat brain cyclase [3] is exerted by EGTA, a powerful Ca^{2+} chelator [4]. In both cases, inhibition is reversible by either Ca^{2+} or Mn^{2+} and in beef brain was shown to be non-competitive in nature [2]. These studies have been suggestive of a role of Ca^{2+} in the modulation of brain cyclase activity. However, the beef brain cyclase studies were performed in the presence of NaF [1,2]. The partial nature of the inhibition is not understood and studies on the effect of EGTA on unstimulated beef brain adenylate cyclase are not available. It is the purpose of this communication to further elucidate this problem and to propose a two component brain adenylate cyclase model to rationalize the behavior of this enzyme in the presence of F^-, Ca^{2+}, EGTA and Triton X-100.

Experimental procedure

Materials

Disodium ATP, monosodium 3′,5′cyclic AMP and Triton X-100 were from Sigma Chemical Co. Magnesium sulphate, sodium fluoride, nicklous, zinc, cupric and ferrous chlorides, isopropanol and sodium silicotungstate were Baker Chemicals. Sodium metaperiodate and perchloric acid were from Analar and sodium borohydride was from British Drug Houses. Orcinol monohydrate, lead acetate, sodium molybdate and sodium, calcium, manganous, cobalt, strontium and barium chlorides were obtained from Fisher Chemical Co.

Methods

Preparation of adenylate cyclase from beef brain cortex. Whole cow brains were obtained within 30 min of killing from a local slaughter-house. The tissue was immediately immersed in ice-cold standard buffer consisting of glycylglycine $2 \cdot 10^{-3}$ M (pH 7.6), $MgSO_4$ 10^{-3} M, and NaCl $2 \cdot 10^{-2}$ M. The cortex was cut out, minced, and the tissue disrupted in a Teflon-glass Potter-Elvehjem homogenizer according to a modified procedure of Bitensky and co-workers [5]. 5—10 g of tissues were homogenized at the time by 9 passes through the apparatus, at the rate of 210 rev./min. The resulting homogenate was processed by the procedure of Sutherland et al. [6]. Eight volumes of homogenate in standard buffer were obtained from one volume of tissue. The suspension was strained through a double layer of No. 35 Curity gauze and centrifuged again at $600 \times g$ for 15 min. This procedure was repeated once more and the sediment was re-suspended and stored frozen at —20° or —70°C in 15-ml portions in sealed polyethylene tubes.

Solubilization of adenylate cyclase with Triton X-100. "Solubilization" of the sediment with Triton X-100 was accomplished using a method similar to that of Sutherland and co-workers [6], 10 ml of Triton solution was added slowly to 50 ml of washed 50% diluted membrane particles while stirring

constantly at 0°C to bring the final Triton concentration to 1.8%. After stirring for 10 min the suspension was centrifuged at 40 000 × *g* for 20 min in a Spinco model "L" centrifuge; the supernatant was recentrifuged at 100 000 × *g* for 2 h. The pellet formed was resuspended in 5 ml of ice-cold standard "wash buffer" which contained additional glycylglycine (0.2 M at pH 7.5). "Solubilized" preparation was stored at −20°C or −70°C.

In vitro synthesis of 3',5'cyclic AMP. The standard incubation medium (4 ml) consisted of ATP, 10^{-3} M $MgSO_4$, $3.6 \cdot 10^{-3}$ M or $6.0 \cdot 10^{-4}$ M; caffeine, $5 \cdot 10^{-2}$ M; glycylglycine $6.0 \cdot 10^{-2}$ M pH 7.5; NaF $6.0 \cdot 10^{-3}$ M (when designated) and approximately 4.0 mg particulate cortex preparation. All components of the reaction mixture except ATP were preincubated for 20 min and the reaction was initiated by the introduction of ATP. The incubation mixtures were shaken for 20 min at 25°C in a Dubnoff incubator and the reaction stopped by rapid immersion for 3.5 min in boiling water in 15 ml cellulose tubes.

Direct non-chromatographic assay for 3',5'cyclic AMP. After cooling to 0°C, the tubes were centrifuged at 10 000 × *g* for 40 min. The clear supernatant fluids were quantitatively transferred to 25 ml flasks and assayed directly for 3',5'cyclic AMP as previously described [7].

3',5'Cyclic AMP phosphodiesterase activity. 3',5'Cyclic AMP phosphodiesterase activity was assayed by the same method employed for adenylate cyclase activity except $5 \cdot 10^{-5}$ M 3',5'cyclic AMP was used as substrate instead of ATP. Caffeine at $5.0 \cdot 10^{-2}$ M was included and residual activity monitored.

Assay for ATPase activity. ATPase activity was measured according to Post and Sen [8] as modified by Roufogalis and Belleau [9] except the reaction mixture (4 ml) was identical to that for adenylate cyclase assays. Reactions were stopped by the addition of 1.4 ml perchloric acid solution [9].

Estimation of total $[Ca^{2+}]$, $[Mn^{2+}]$ and $[Co^{2+}]$ in adenylate cyclase preparation and reaction mixture components. Particulate preparation was solubilized by gentle heating in 1 ml conc nitric acid and adjusted to 10 ml with ethanol/water (1/1, v/v). Other solutions were treated similarly. Total Ca^{2+}, Mn^{2+} and Co^{2+} concentrations were estimated on a type AA5 Techtron atomic absorption spectrophotometer at 422.8 nm, 279.5 nm and 240.7 nm, respectively. An acetylene/nitrous oxide flame under 10 and 27 lb/inch^2 was employed. Appropriate blanks and standards were measured in parallel.

Estimation of protein. Protein concentrations were measured according to Lowry et al. [10].

Calculation of the [free Ca^{2+}] in reaction mixtures. All [free Ca^{2+}] values were calculated on the basis of the total Ca^{2+} (from all sources), total [EGTA] and the equilibrium value, log K = 11.0 [4].

Results

General properties of adenylate cyclase

Particulate beef brain cortex adenylate cyclase preparations were shown to remain active for 1 month (about 20% loss in activity) when stored at −20°C and for about 3 months when stored at −70°C. In contrast, Triton X-100 solubilized cyclase remained active for only 5–6 days at −20°C and

about 2 weeks at −70° C. Both preparations at protein concentrations less than 1.2 mg/ml were shown to synthesize 3′,5′cyclic AMP linearily for 20 min at 25° C either in the presence or absence of NaF. Although the synthesis of 3′,5′cyclic AMP was higher (about 1.6 nmol/min/mg protein) than observed in some earlier brain enzyme studies [1,6] it is comparable to others [2,7]. The primary difference between these sets of studies appears to be the concentration of methyl xanthine employed [7].

Inhibition of adenylate cyclase by EGTA and reversal by calcium

At a $[Mg^{2+}]$ of $3.6 \cdot 10^{-3}$ M, incubation with $5 \cdot 10^{-5}$ M EGTA abolished 84% ± 3% of unstimulated cyclase activity while in the presence of NaF an equal molar concentration of EGTA abolished only 63% ± 3% of the stimulated activity (Fig. 1). Similarly at a $[Mg^{2+}]$ of $6 \cdot 10^{-4}$ M, the same concentration of EGTA abolished 88% ± 3% and 50% ± 2% of the activity in the absence and presence of NaF respectively. In all cases, the partial inhibition was optimal at $5 \cdot 10^{-5}$ EGTA and no further inhibition was obtainable by increasing the [EGTA] to 10^{-4} M. NaF stimulation was reproducibly greater (approximately three-fold as opposed to two-fold) at the lower $[Mg^{2+}]$ (Fig. 1) which was somewhat less than saturating (K_m for Mg^{2+} was estimated at $3.0 \cdot 10^{-4}$ M). Unlike EGTA, EDTA was not inhibitory. Partial inhibition by $5 \cdot 10^{-5}$ M

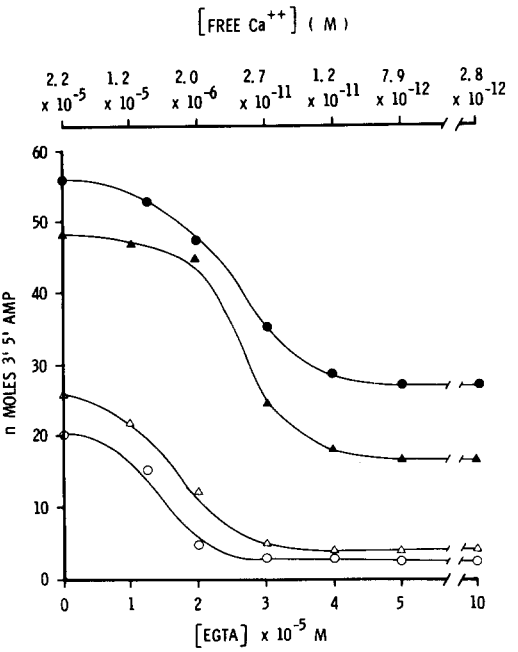

Fig. 1. Effects of EGTA and fluoride on adenylate cyclase activity. A 20 min preincubation at 25° C with EGTA was used prior to ATP addition; 3′,5′cyclic AMP synthesis at high $[Mg^{2+}]$ ($3.6 \cdot 10^{-3}$ M) in the presence of fluoride ($6 \cdot 10^{-3}$ M) (▲———▲) and the absence of fluoride (△———△) and at low $[Mg^{2+}]$ ($6 \cdot 10^{-4}$ M) in the presence of fluoride ($6 \cdot 10^{-3}$ M) (●———●) and the absence of fluoride (○———○) is plotted against EGTA concentration. Reagents: ATP $5 \cdot 10^{-4}$ M, Gly-Gly buffer 0.06 M, pH 7.5, caffeine $5 \cdot 10^{-2}$ M and protein 1.0 mg/ml, and $MgSO_4$ (concentrations as indicated above). Incubations for 20 min at 25° C. Calculated [free Ca^{2+}] is represented on the upper abcissa scale.

EGTA (either in the presence or absence of NaF) was completely reversible by the addition of $3 \cdot 10^{-5}$ M Ca^{2+} (giving a total $[Ca^{2+}]$, from all sources $\simeq$ $5.2 \cdot 10^{-5}$ M and a calculated total [free Ca^{2+}] $\simeq$ $2 \cdot 10^{-6}$ M in the incubation mixture). As shown in Fig. 2, a [free Ca^{2+}] of $7.2 \cdot 10^{-5}$ M was strongly inhibitory in the NaF stimulated system but only marginally inhibitory in the unstimulated system.

Reversal of EGTA inhibition by other divalent cation

Addition of either $5 \cdot 10^{-5}$ M Mn^{2+} or Co^{2+} could replace Ca^{2+} in fully reversing the inhibitory effect of $5 \cdot 10^{-5}$ M EGTA (Fig. 3). In the NaF activated systems the activity in the presence of Mn^{2+} and Co^{2+} exceeded that in the presence of Ca^{2+}. In accord with Bradham et al. [1], Sr^{2+} was only partially effective in restoring activity both in the control and in the NaF stimulated systems (Figs 2 and 3) while Ba^{2+}, Zn^{2+}, Ni^{2+} and Fe^{2+} were all ineffective (Fig. 3).

Effect of Triton X-100 treatment and EGTA on the NaF stimulation of adenylate cyclase

Treatment of particulate preparation with 1.8% Triton X-100 resulted in a 1.6-fold increase in specific activity of adenylate cyclase (Table I). In agree-

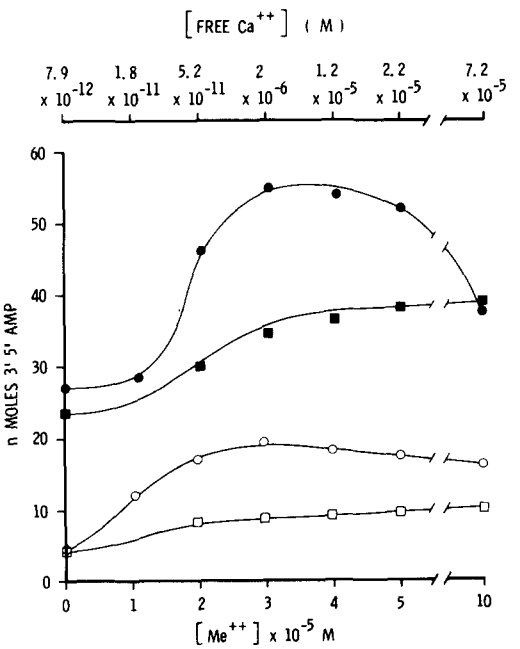

Fig. 2. Protection by Ca^{2+} and Sr^{2+} against EGTA inhibition. Adenylate cyclase activity in the presence of EGTA is plotted against the added bivalent metal ion concentration $[Me^{2+}]$ for both $CaCl_2$ in the presence of $6 \cdot 10^{-3}$ M fluoride (●——●) and the absence of fluoride (○——○) and for $SrCl_2$ in the presence of $6 \cdot 10^{-3}$ M fluoride (■——■) and its absence (□——□). Reagents: ATP $5 \cdot 10^{-4}$ M, Gly-Gly 0.06 M, pH 7.5, caffeine $5 \cdot 10^{-2}$ M, $MgSO_4$ $6 \cdot 10^{-4}$ M, EGTA $4 \cdot 10^{-5}$ M, protein 1.0 mg/ml, $SrCl_2$ and $CaCl_2$. Preincubation for 20 min at 25° C with EGTA and appropriate $[Me^{2+}]$ prior to ATP addition, followed by a 20 min assay. Calculated [free Ca^{2+}] for the Ca^{2+}-containing system is represented on the upper abcissa scale.

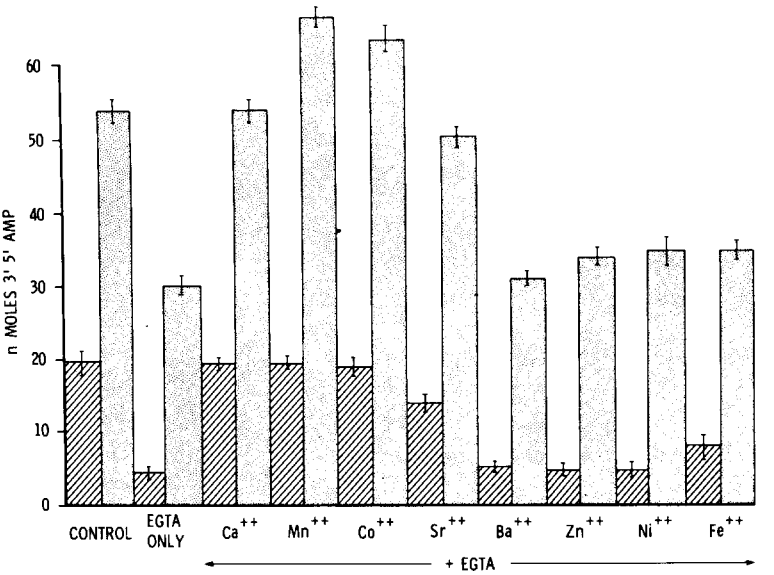

Fig. 3. Relative ability of various divalent metal ions to substitute for calcium in the presence of fluoride (dotted bars) and in the absence of fluoride (lined bars). Reagents ATP, $5 \cdot 10^{-4}$ M, $MgSO_4$ $3.6 \cdot 10^{-3}$ M, caffeine $5 \cdot 10^{-2}$ M, glycylglycine 0.06 M, pH 7.5, proteins 1.0 mg/ml, EGTA $5 \cdot 10^{-5}$ M (absent in the control, only), $5 \cdot 10^{-5}$ M total added bivalent metal ion chloride (as designated) and $6.3 \cdot 10^{-3}$ M sodium fluoride (dotted bars only). A preincubation of the reaction mixtures in the presence of EGTA (as designated) 20 min at 25° C in the absence of substrate preceded the 20 min 3',5'cyclic AMP synthesis period at 25° C.

ment with the observed effect of Triton X-100 on rat brain cyclase [3], a loss of the NaF stimulation effect is also observed in beef brain cyclase (Fig. 4). Moreover, the cyclase activity in the treated preparation is completely abolished by the introduction of EGTA into the reaction mixture. As in the particulate preparation, the loss of activity can be reversed by a free Ca^{2+} ion concentration of $2 \cdot 10^{-6}$ M. On the other hand, the effect of NaF on particulate cyclase is not abolished by the introduction of $5 \cdot 10^{-5}$ M EGTA but modified (Fig. 4). Concentrations of NaF in excess of 10^{-2} M inhibit in the

TABLE I

EFFECT OF TRITON X-100 TREATMENT AND EGTA ON ADENYLATE CYCLASE, 3',5'CYCLIC AMP PHOSPHODIESTERASE AND ATPase ACTIVITIES IN CEREBRAL CORTEX

Reaction system*	Specific activity (nmol/mg prot./min)		
	Adenylate cyclase	Residual phosphodiesterase	ATPase
Particulate (untreated and without EGTA)	1.6	1.0	19.0
"Solubilized" (Triton X-100 treated)	2.6	0.0	11.0
Particulate (+ $5 \cdot 10^{-5}$ M EGTA)	0.26	1.0	18.8

* In the presence of [ATP] = 10^{-3} M, [Mg^{2+}] = $3.6 \cdot 10^{-3}$ M, caffeine $5.0 \cdot 10^{-2}$ M glycylglycine = $6.0 \cdot 10^{-2}$ M pH 7.5 and absence of NaF (see Methods section)

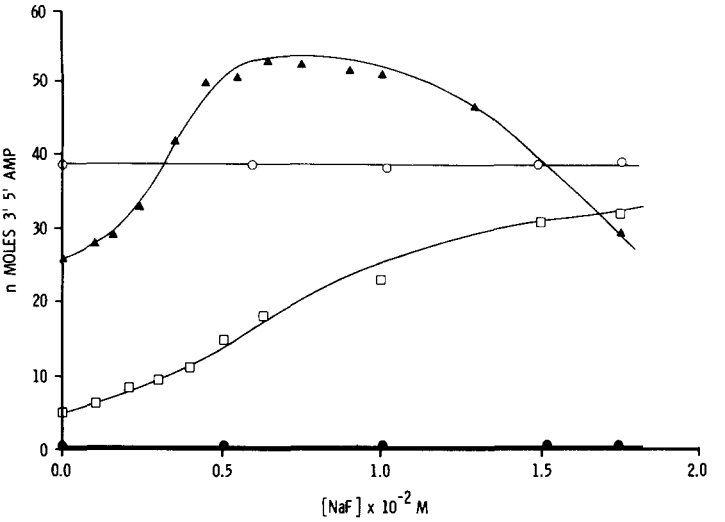

Fig. 4. Effects of EGTA and fluoride on particulate and Triton X-100 treated preparations. Adenylate cyclase activity, varying the fluoride concentration was studied on particulate preparation (proteins 1.0 mg/ml) in the presence of $5 \cdot 10^{-5}$ M EGTA (□———□) and in the absence of EGTA (▲———▲) and on "solubilized" preparations (proteins 1.1 mg/ml) in the presence of $5 \cdot 10^{-5}$ M EGTA (●———●) and the absence of EGTA (○———○). Reagents: $MgSO_4$ $3.6 \cdot 10^{-3}$ M, ATP $5 \cdot 10^{-4}$ M, caffeine $5 \cdot 10^{-2}$ M, Gly-Gly 0.06 M, pH 7.5, treated and untreated preparations and sodium fluoride. All reaction mixtures were preincubated 20 min at 25°C for the addition of ATP.

absence of EGTA while corresponding concentrations of NaF continue to activate in the presence of EGTA.

Effect of Triton X-100 treatment and EGTA on phosphodiesterase and ATPase

Triton X-100 treatment depressed the specific activity of ATPase and abolished the activity of 3',5'cyclic AMP phosphodiesterase (Table I). However, in agreement with others [1,3], EGTA had no measurable effect on either ATPase or 3',5'cyclic AMP phosphodiesterase.

Sources of Ca^{2+} in the cyclase reaction mixture

The Ca^{2+} derived from 4.0 mg particulate preparation (1.0 mg/ml) was estimated as $1.0 \cdot 10^{-5}$ M while the $[Ca^{2+}]$ derived from a blank reaction mixture (enzyme preparation excluded) was estimated at $1.2 \cdot 10^{-5}$ M. Over 85% of the Ca^{2+} was associated with the $MgSO_4$ while approximately 9% was associated with the ATP. Less than 5% was from the Gly-Gly/NaOH buffer, NaF and caffeine. Thus the total Ca^{2+} in a complete reaction mixture is approximately $2.2 \cdot 10^{-5}$ M. The total $[Mn^{2+}]$ and $[Co^{2+}]$ on a complete reaction mixture was immeasurably small ($<10^{-7}$ M).

Discussion

The results confirm and extend the previous observations on the behavior of beef brain cyclase in the presence of EGTA, divalent metal ions and NaF [1,2] using a chemical assay for the estimation of 3',5'cyclic AMP described

earlier [7]. In this system, the estimation of 3',5'cyclic AMP is completely quantitative and no interference problems were encountered with any components of the reaction system.

The experimental approach varied slightly from those in earlier brain cyclase studies [1,2]: 50 mM caffeine was employed to maximally inhibit phosphodiesterase [7] instead of 6.7 mM caffeine [1,6] or 50 mM theophyline [2]. Particulate brain preparation was preincubated with all reaction components (except ATP) for 20 min at 25°C before initiating the reaction with ATP, thus allowing time for the tissue to adequately sequester free divalent metal or for EGTA to adequately remove divalent metal from tissue. Thus inhibition and reactivation curves were highly reproducible under the prescribed conditions. The partial inhibition by excess EGTA at a $[Mg^{2+}]$ of $3.6 \cdot 10^{-3}$ M and in the presence of NaF (Fig. 1) was 63% and closely resembled the figure obtained by Bradham et al. [1] (approximately 65%). The partial nature of this inhibition in the presence of NaF is more obvious at a $[Mg^{2+}]$ of $6.0 \cdot 10^{-4}$ M (50%) (Fig. 1). The inhibition by EGTA in the absence of NaF is closer to completion but still partial (84—88%). However, regardless of the extent of inhibition by EGTA, the introduction of $3 \cdot 10^{-5}$ M Ca^{2+} (to give $2 \cdot 10^{-6}$ M free Ca^{2+}) restored the activity to its former level. The replacability of Ca^{2+} by Mn^{2+} and in part by Sr^{2+} has already been noted in beef brain [1,2] and by Mn^{2+} and Co^{2+} in rat brain [3] and rat heart [11]. The total concentration of Ca^{2+} required to fully reactivate the cyclase was lower than that observed by Bradham [2] who had included $1.0 \cdot 10^{-4}$ M EGTA into the reaction medium, but the [free Ca^{2+}] for optimal activity is essentially identical in his study and the present. As previously utilized in muscle studies [12] the ratio of $[Ca^{2+}]$ / [EGTA] in the "buffer" system determines the [free Ca^{2+}]. The biological significance of the increased degree of inhibition by excess Ca^{2+} in the NaF stimulated system remains speculative. This observation may be analogous to a similar behavior of fat cell adenylate cyclase (in the absence of EGTA) where the NaF containing reaction was also found more sensitive to Ca^{2+} inhibition than the control reaction [13].

The replaceability of Ca^{2+} by Mn^{2+}, Co^{2+} or Sr^{2+} could be due to either a direct or indirect effect. These ions may compete with varying degrees of success for the EGTA from the EGTA-Ca^{2+} complex. However, if these effects were totally indirect one would not expect an activation in the presence of NaF beyond that obtainable for Ca^{2+}. Secondly both Zn^{2+} and Ni^{2+}, which have higher equilibrium constants than Ca^{2+}, Mn^{2+} or Co^{2+}, should be successfully displacing Ca^{2+} from the complex but neither Zn^{2+} nor Ni^{2+} activate cyclase. Some selective sequestering action by the enzyme preparation of the EGTA-Ca^{2+} complex would explain an apparent direct effect by other divalent ions.

Data presented in Fig. 4 was particularly useful in rationalizing the total data represented in Figs 1—3. A two-component adenylate cyclase model is postulated in Table II on the basis of these findings when viewed collectively. One component is apparently activated six-fold by NaF and quantitatively deactivated by Triton X-100. Low concentrations of Ca^{2+} (Mn^{2+}, Co^{2+} or Sr^{2+}) and low concentrations of Mg^{2+} appear to enhance the NaF effect by varying degrees. The second component is Ca^{2+} dependent, totally deactivated by EGTA, and inhibited by excessively high concentrations by NaF ($>10^{-2}$ M).

TABLE II

A GENERAL MODEL INTERPRETING OBSERVED PROPERTIES OF BRAIN ADENYLATE CYCLASE

Component of activity (or "receptor")	Effectors	Properties
Fluoride Stimulated	F^-	Six fold activated by NaF (optimal at $1.5 \cdot 10^{-2}$ M)
	Ca^{2+}, Mn^{2+}	Enhanced NaF activation at low [Mg^{2+}] ($6 \cdot 10^{-4}$ M)
	Co^{2+}, Sr^{2+}	Enhanced NaF activation by Ca^{2+} (optimal at [free Ca^{2+}] $\simeq$ $2.0 \cdot 10^{-6}$ M)
		Substitutes for Ca^{2+} are $Mn^{2+} > Co^{2+} > Ca^{2+} > Sr^{2+}$
		Inhibition at a [free Ca^{2+}] $\simeq 7.2 \cdot 10^{-5}$ M
		Complete loss in activity by Triton X-100 treatment
Calcium Dependent	Ca^{2+}, Mn^{2+}	Inhibition by $> 10^{-2}$ M NaF
	Co^{2+}, Sr^{2+}	Activity totally dependent on Ca^{2+}
		Ca^{2+} totally replacable by Mn^{2+} or Co^{2+} and partially replacable by Sr^{2+}
		Not as sensitive to inhibition by excess Ca^{2+} (less inhibition at a [free Ca^{2+}] = $7.2 \cdot 10^{-5}$ M)
		Purification or activation by Triton X-100 treatment

Triton X-100 treatment results in a three-fold increase in specific activity of the latter component but abolishes the former component. This model is compatible with the present experimental data and with earlier observations for beef brain [1,2] and for rat brain cyclase [3]. Moreover, the activation of the rat cerebral cortex enzyme by Triton X-100 and that by NaF was unequivocally shown by Perkins and Moore [14] to be non-additive. They further suggest the existence of a multi-component adenylate cyclase system in this tissue. Three chemically distinguishable components have already been identified in fat cell adenylate cyclase [15] and two in liver enzyme [5]. Present data do not yet distinguish whether the increase in specific activity of the Ca^{2+}-dependent component by Triton X-100 represents a purification or activation process or both. Earlier experimental data have indicated the NaF effect to be a reversible activation [2,3] and data presented in Fig. 4 indicate the NaF effect to be an activation rather than a dependence of one activity component on F^-. Whether the NaF stimulated component in vitro would be in fact a hormonally regulated component in vivo is speculatory. This concept is none the less appealing in the light of observations of relationships between hormonal control, 3',5'cyclic AMP and Ca^{2+} [16].

It is, however, evident that future studies on brain adenylate cyclase must examine the properties of each component individually since the two-component model does not preclude the possible existence of subcomponents.

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