Neurochemical Research, Vol. 11, No. 3, 1986, pp. 453–463

SUBCELLULAR DISTRIBUTIONS OF CALCIUM/CALMODULIN-STIMULATED AND GUANINE NUCLEOTIDE-REGULATED ADENYLATE CYCLASE ACTIVITIES IN THE CEREBRAL CORTEX

SHIRLEY T. BISSEN[1] and TETSUFUMI UEDA[1,2,3]
Departments of Pharmacology[1] and Psychiatry[2]
and
Mental Health Research Institute[3]
The University of Michigan,
Ann Arbor, Michigan 48109

Accepted September 18, 1985

The subcellular distribution of Ca^{2+}/calmodulin-stimulated adenylate cyclase activity was studied in comparison with that of guanine nucleotide-stimulated cyclase activity. The distributions of these activities were similar among the crude fractions but differed among the purified subsynaptosomal fractions. The specific activity of Ca^{2+}/calmodulin-stimulated cyclase was highest in a light synaptic membrane fraction, which has few, if any, postsynaptic densities, whereas that of guanine nucleotide-stimulated cyclase was highest in a heavier synaptic membrane fraction rich in postsynaptic densities. These results suggest that the Ca^{2+}/calmodulin-stimulated cyclase has, at least in part, a different cellular or subcellular location than the guanine nucleotide-stimulated cyclase.

INTRODUCTION

Numerous hormones and neurotransmitters initiate some of their biological effects by stimulating or inhibiting the activity of adenylate cyclase (EC 4.6.1.1), and both of these effects require guanosine 5′-triphosphate.

Shirley T. Bissen's present address: Department of Zoology, University of California, Berkeley, CA 94720.
Abbreviations used: CaM, calmodulin; GppNHp, guanosine 5′-(β,γ-imino) triphosphate.

0364-3190/86/0300-0453$05.00/0

The adenylate cyclase complex is considered to be composed minimally of three components: hormone receptor (R), guanine nucleotide-binding regulatory component (N), and catalytic subunit (C) (1, 2). Stimulatory and inhibitory N components have been functionally distinguished (1, 3–5), and two distinct proteins have been isolated and characterized: a stimulatory N protein (N_s) (6–9) and an inhibitory N protein (N_i) (10–12).

The mammalian brain is rich in another class of adenylate cyclase, namely, Ca^{2+}/calmodulin (CaM)-sensitive adenylate cyclase (13, 14). This cyclase is activated directly at the C subunit by the Ca^{2+}/CaM complex (15–17), without the involvement of guanine nucleotides or the N_s component (18, 19). The Ca^{2+}/CaM-sensitive cyclase has been resolved from Ca^{2+}/CaM-insensitive cyclase (20, 21), and guanine nucleotides stimulate both Ca^{2+}/CaM-sensitive and -insensitive cyclases (21–23).

In view of the possibility that these different forms of brain adenylate cyclase may be present in different types of cells or in different loci of the same cell, and therefore involved in different cellular functions, we have studied the subcellular distributions of the Ca^{2+}/CaM-stimulated and the guanine nucleotide-regulated cyclases in bovine cerebral cortex. Here we present evidence that Ca^{2+}/CaM-stimulated cyclase in the cerebral cortex is present, at least in part, in a membrane that is distinct from the membrane rich in guanine nucleotide-stimulated cyclase.

EXPERIMENTAL PROCEDURE

Fresh bovine brains were obtained from a local slaughterhouse, transported on ice, and processed immediately. Guanosine 5′-(β,γ-imino) triphosphate (GppNHp) was from International Chemical and Nuclear. [α-^{32}P] ATP (10-30 Ci/mmol) was from Amersham, and [2,8-^{3}H] cyclic AMP (30-50 Ci/mmol) was from New England Nuclear. Highly purified CaM was a generous gift of Drs. M. E. Gnegy and M. J. Welsh of the University of Michigan.

Various subcellular fractions of the entire bovine cerebral cortex were prepared as described (24), except that the crude synaptic membrane fraction (M_1) was centrifuged (50,000 *g*) on a discontinuous sucrose density gradient composed of layers of 0.8, 0.9, 1.0, 1.2, and 1.4 M sucrose.

Adenylate cyclase activity was measured in a mixture (100 μl final volume) containing 80 mM Tris-maleate (pH 7.4), 2 mM $MgSO_4$, 10 mM theophylline, 0.1 mM cyclic AMP, 0.5 mM [α-^{32}P] ATP (70-90 cpm/pmol), an aliquot of the appropriate fraction (15-35 μg protein) and test substances as indicated. Following preincubation at 30°C for 2 min, the reaction was initiated by the addition of ATP and allowed to proceed for 5 min at 30°C. The reaction was terminated and [^{32}P] cyclic AMP was isolated and quantitated by the method of Salomon et al. (25). All assays were performed in duplicate; the range of variation was generally less than 10%. Recovery of cyclic AMP ranged from 60% to 80%. Under the assay conditions used, the formation of cyclic AMP was linear with respect to protein concentration and time.

Protein concentrations were determined by the method of Lowry et al. (26) with bovine serum albumin as standard. Free or effective concentrations of Ca^{2+} were calculated using the program described by Fabiato and Fabiato (27).

RESULTS

Ca^{2+}/CaM-stimulated and GppNHp-stimulated adenylate cyclase activities in various subcellular fractions are shown in Table I. Among the primary and mitochondrial subfractions, these cyclase activities were primarily associated with the plasma membrane and synaptosome-containing fractions, P_1, P_2, and P_2 (1.2) (Table I, A and B). Among the synaptosome subfractions, these cyclase activities were highest and of comparable levels in the M_1 synaptic membrane fraction. In contrast, in the M_2 crude synaptic vesicle fraction, the Ca^{2+}/CaM-stimulated activity was more than 15 times greater than the GppNHp-stimulated activity (Table I, C).

Among the synaptic membrane subfractions, the majority of these cyclase activities were associated with the M_1(1.0) and M_1(1.2) fractions. However, the specific activity of the Ca^{2+}/CaM-stimulated cyclase was highest in the M_1(0.9) fraction, and that of the GppNHp-stimulated cyclase was highest in the M_1(1.2) fraction. In the light fractions of the gradient, M_1(0.8) and M_1(0.9), the Ca^{2+}/CaM-stimulated activity was five to ten times greater than the GppNHp-stimulated activity, but these cyclase activities were nearly equal in the heavy fractions of the gradient (Table I, D). In every synaptic vesicle subfraction, the Ca^{2+}/CaM-stimulated activity was much greater than the GppNHp-stimulated activity, and both activities were lowest in the lightest fraction, which is most enriched with synaptic vesicles (24, 28) (Table I, E). Essentially the same distribution pattern was observed in another experiment.

Since these data suggest that the lighter synaptic membrane fractions contain cyclase primarily stimulated by Ca^{2+}/CaM, and the heavier synaptic membrane fractions contain cyclase equally stimulated by Ca^{2+}/CaM or GppNHp, the effects of these ligands on cyclase activity in the M_1(0.9) and M_1(1.2) fractions were studied in more detail. The cyclase activity in either fraction was maximally stimulated by the same concentration of Ca^{2+}, which was calculated to be 0.26 μM Ca^{2+}, and was inhibited by higher concentrations of Ca^{2+} (Figure 1). The effects of various GppNHp concentrations on cyclase activity in the absence or presence of EGTA are shown in Figure 2. In the absence of EGTA, the cyclase activity in the M_1(0.9) fraction was inhibited by every concentration of GppNHp examined, whereas the cyclase activity in the M_1(1.2) fraction

TABLE I

DISTRIBUTIONS OF Ca^{2+}/CaM-STIMULATED AND GPPNHP-STIMULATED ADENYLATE CYCLASES IN SUBCELLULAR FRACTIONS OF BOVINE CEREBRAL CORTEX

Fraction	Protein (mg)	Adenylate cyclase activity: Total activity (nmol/min) Ca^{2+}/CaM-stimulated	Total activity (nmol/min) GppNHp-stimulated	Specific activity (pmol/min/mg) Ca^{2+}/CaM-stimulated	Specific activity (pmol/min/mg) GppNHp-stimulated	Specific activity (pmol/min/mg) Basal activity
A. *Primary subfractions*						
Starting material (II)	5,710	725	582	127	102	19.3
P_1 (nuclei, cell debris)	2,700	408	432	151	160	21.8
P_2 (mitrochondria, synaptosomes)	1,670	267	237	160	142	24.9
P_3 (microsomes)	956	90.8	27.1	95.0	28.3	13.3
S (soluble components)	1,040	5.97	5.06	5.74	4.87	2.26
B. *Mitochondrial subfractions*						
P_2 (0.85) (myelin)	384	35.9	18.0	93.4	46.9	12.6
P_2 (1.0) (synaptic membranes)	142	27.4	18.0	193	127	28.4
P_2 (1.2) (synaptosomes)	1,020	231	203	227	199	30.4
P_2 (ppt) (mitochondria)	450	60.8	60.3	135	134	29.1

TABLE I (*Continued*)

Fraction	Protein (mg)	Adenylate cyclase activity: Total activity (nmol/min) Ca^{2+}/CaM-stimulated	Total activity (nmol/min) GppNHp-stimulated	Specific activity (pmol/min/mg) Ca^{2+}/CaM-stimulated	Specific activity (pmol/min/mg) GppNHp-stimulated	Specific activity (pmol/min/mg) Basal activity
C. *Lysed synaptosomal subfractions*						
M_1 (synaptic memb. and mitochon.)	667	239	187	358	281	61.6
M_2 (synaptic vesicles and membranes)	35.1	8.60	0.54	245	15.5	33.0
M_3 (soluble components)	266	0.06	ND	0.22	ND	2.23
D. *Synaptic membrane subtractions*						
M_1 (0.8)	26.3	10.0	0.88	382	33.5	44.2
M_1 (0.9)	30.6	20.0	3.79	654	124	77.2
M_1 (1.0)	153	67.9	61.8	444	404	77.7
M_1 (1.2) (synaptic memb. and PSD)	200	82.6	86.8	413	434	74.6
M_1 (1.4)	126	38.9	32.4	309	257	59.3
M_1 (ppt) (mitochondria)	15.1	2.31	1.60	153	106	28.1
E. *Synaptic vesicle subtractions*						
M_2 (0.4) (synaptic vesicles)	11.9	1.56	ND	131	ND	16.1
M_2 (0.6)	10.7	3.92	ND	366	ND	55.0
M_2 (0.8)	4.54	1.85	0.85	408	18.8	67.1
M_2 (ppt)	4.23	1.87	0.21	443	49.3	81.0

Subcellular fractions were prepared, and aliquots (40 μl, 20–30 μg protein) of each fraction were assayed for adenylate cyclase activity as described under Materials and Methods. The Ca^{2+}/CaM-stimulated activity was measured in the presence of 150 μM EGTA, 125 μM $CaCl_2$, and 0.5 μg CaM. The GppNHp-stimulated activity was measured in the presence of 150 μM EGTA and 10 μM GppNHP. The basal activity was measured in the presence of 150 μM EGTA, and the activities above the basal activity in each fraction are presented. Each value is the mean of duplicate assays. ND = Not detectable.

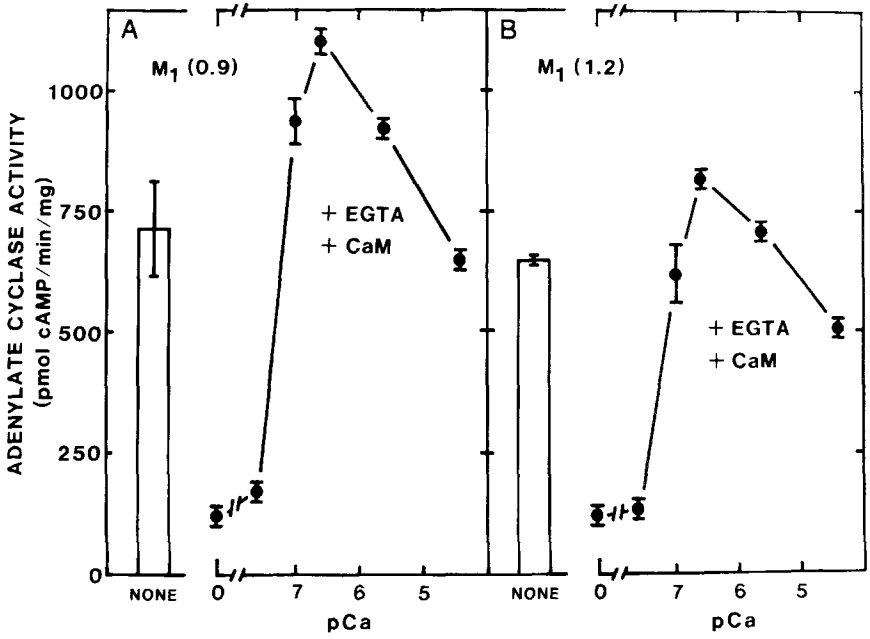

FIG. 1. Adenylate cyclase activity as a function of Ca^{2+} concentration. Adenylate cyclase activity was measured in the presence of 150 μM EGTA and 0.5 μg CaM alone or in the presence of various calculated free Ca^{2+} concentrations. The *open bars* represent the original cyclase activity, with no additions. The mean and range of duplicate determinations of adenylate cyclase activity in the $M_1(0.9)$ (*A*) and the $M_2(1.2)$ (*B*) fractions are presented. Data are representative of three separate experiments with two different membrane preparations. pCa = $-\log [Ca^{2+}]$.

was inhibited by low concentrations but slightly stimulated by high concentrations of GppNHp. In the presence of EGTA, the basal activity was substantially reduced and the GppNHp-induced inhibition was abolished, but the cyclase activity in both fractions was stimulated, although to different degrees, by high concentrations of GppNHp.

The differential effects of GppNHp on adenylate cyclase in the light and heavy synaptic membrane fractions prompted us to examine the GppNHp-inhibited as well as the Ca^{2+}/CaM-stimulated and GppNHp-stimulated cyclase activities in each of the synaptic membrane fractions. As shown in Figure 3, the specific activity of GppNHp-inhibited cyclase was highest in the $M_1(0.9)$ fraction, and its distribution among these fractions was similar to that of the Ca^{2+}/CaM-stimulated activity but different from that of the GppNHp-stimulated activity.

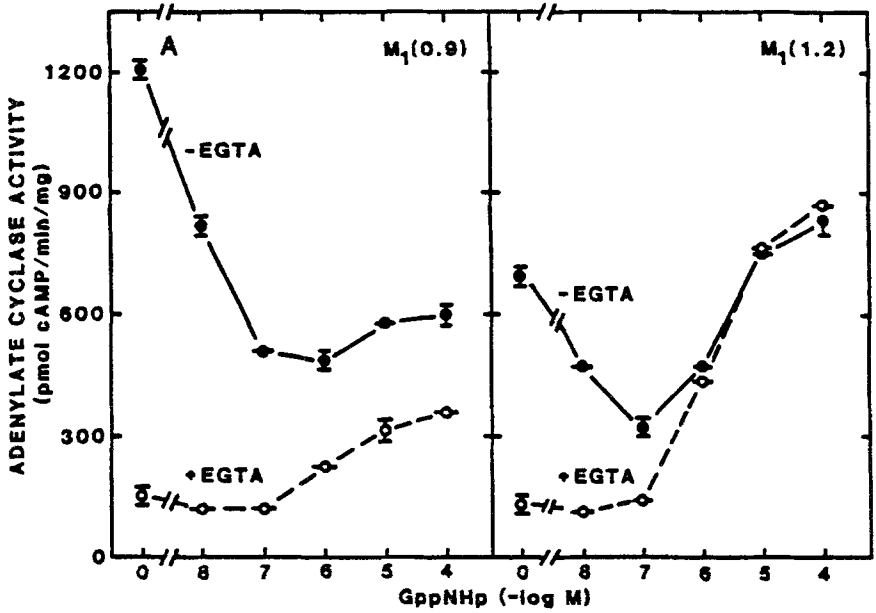

FIG. 2. Effect of GppNHp concentration on adenylate cyclase activity in the absence or presence of EGTA. Adenylate cyclase activity was measured in the absence (●---●) or presence (O---O) of 150 μM EGTA with various concentrations of GppNHp in the $M_1(0.9)$ (*A*) and the $M_1(1.2)$ (*B*) fractions. The mean and range of duplicate assays of adenylate cyclase activity are presented. Data are representative of two to four separate experiments with three different membrane preparations.

DISCUSSION

This study represents, to our knowledge, the first detailed studies on the subcellular distributions of Ca^{2+}/CaM-stimulated and guanine nucleotide-regulated adenylate cyclase activities in the brain. The results presented here indicate that some (or all) of the Ca^{2+}/CaM-stimulated cyclase is associated with a different type of synaptic membrane than the GppNHp-stimulated cyclase. These data also suggest that some of the GppNHp-inhibited cyclase may have a different subcellular location than the GppNHp-stimulated cyclase.

The GppNHp-stimulated cyclase is most abundant in the heavy synaptic membrane fractions, and it has previously been shown that fluoride-stimulated and dopamine-stimulated adenylate cyclase activities are highest in the heavy synaptic membrane fractions (29, 30). These observations suggest that guanine nucleotide-stimulated and neurotransmitter-stimu-

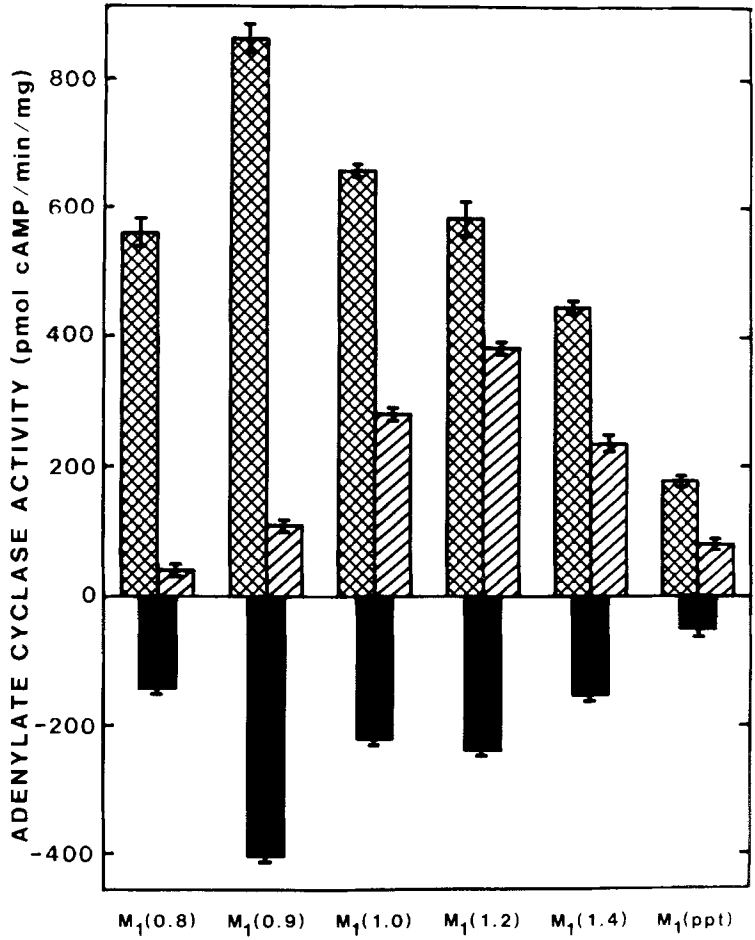

FIG. 3. Distributions of Ca^{2+}/CaM-stimulated, GppNHp-stimulated, and GppNHp-inhibited adenylate cyclases in synaptic membrane fractions. The Ca^{2+}/CaM-stimulated activity (*crosshatched bars*) was measured in the presence of 150 μM EGTA, 125 μM $CaCl_2$, and 0.5 μg CaM. The GppNHp-stimulated activity (*striped bars*) was measured in the presence of 150 μM EGTA and 10^{-5} M GppNHp. The corresponding basal activity was measured in the presence of 150 μM EGTA. The GppNHp-inhibited activity (*solid bars*) was measured in the presence of 10^{-7} M GppNHp, and the corresponding basal activity was measured with no additions. Presented are the mean and range of duplicate determinations of adenylate cyclase activity, above or below the corresponding level of basal activity. Data are representative of three separate fractionations. Basal activities, in the presence or absence of EGTA, are: M_1(0.8), 60.1, 414; M_1(0.9), 90.8, 741; M_1(1.0), 92.7, 507; M_1(1.2), 93.2, 542; M_1(1.4), 70.4, 397; M_1(ppt), 34.0, 146 pmol cyclic AMP/min/mg.

lated cyclase activities are associated with the same limited membrane. The heavy fractions of the gradient are known to be rich in postsynaptic density-attached synaptic membrane complexes (24, 31, 32). The abundance of the GppNHp-stimulated adenylate cyclase in these fractions suggests that this cyclase is present in post-synaptic membranes in high concentrations.

The heavy synaptic membrane fractions are also rich in the Ca^{2+}/CaM-stimulated cyclase. The specific activity of this enzyme, however, is higher in the lighter membrane fraction, $M_1(0.9)$, and is not parallel to that of the GppNHp-stimulated cyclase among the subcellular fractions tested. This suggests that the Ca^{2+}/CaM-stimulated cyclase activity in the heavy membrane fractions may not be entirely associated with the guanine nucleotide-stimulated cyclase or with the post-synaptic membrane. Evidence suggests that guanine nucleotides or the stimulatory guanine nucleotide-binding protein are not required for the Ca^{2+}/CaM-induced stimulation of adenylate cyclase (15, 18, 19). Moreover, the heavy synaptic membrane fractions are known to contain other types of membranes, including presynaptic membranes, which are a part of the synaptic junctional complexes (24, 31, 32).

The subcellular origin of the membrane fragments present in the light membrane fractions is not well understood. However, they contain few, if any, synaptic membranes that are associated with post-synaptic densities (31, 33, 34). Based on this and other evidence, it has been suggested that these fractions are rich in presynaptic membranes (35). These observations raise the possibility that the Ca^{2+}/CaM-stimulated cyclase may be located, at least in part, in the presynaptic membrane, and that it may be involved in the Ca^{2+}-induced increase in the intraterminal concentration of cyclic AMP. Further studies are required, however, to elucidate the nature of all the membranes in these fractions and to evaluate this notion.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation Grant 8207999 and a Michigan Memorial-Phoenix Project Grant. S.T.B. was a recipient of a Predoctoral Fellowship from NSF and a Rackham Dissertation Fellowship from the University of Michigan. We thank Ms. Mary Roth for excellent assistance in the preparation of the manuscript.

REFERENCES

1. RODBELL, M. 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284:17–22.

2. Ross, E. M., and Gilman, A. G. 1980. Biochemical properties of hormone-sensitive adenylate cyclase. Ann. Rev. Biochem. 49:533–564.
3. Jakobs, K. H., Aktories, K., and Schultz, G. 1981. Inhibition of adenylate cyclase by hormones and neurotransmitters. Adv. Cyclic Nucleotide Res. 14:173–187.
4. Londos, C., Cooper, D. M. F., and Rodbell, M. 1981. Receptor-mediated stimulation and inhibition of adenylate cyclases: the fat cell as a model system. Adv. Cyclic Nucleotide Res. 14:163–171.
5. Cooper, D. M. F. 1982. Bimodal regulation of adenylate cyclase. FEBS Lett. 138:157–163.
6. Pfeuffer, T. 1977. GTP-binding proteins in membranes and the control of adenylate cyclase activity. J. Biol. Chem. 252:7224–7234.
7. Northup, J. K., Sternweis, P. C., Smigel, M. D., Schleifer, L. S., Ross, E. M., and Gilman, A. G. 1980. Purification of the regulatory component of adenylate cyclase. Proc. Natl. Acad. Sci. USA 77:6516–6520.
8. Sternweis, P. C., Northup, J. K., Smigel, M. D., and Gilman, A. G. 1981. The regulatory component of adenylate cyclase. Purification and properties. J. Biol. Chem. 256:11517–11526.
9. Hanski, E., Sternweis, P. C., Northup, J. K., Dromerick, A. W., and Gilman, A. G., 1981. The regulatory component of adenylate cyclase. Purification and properties of the turkey erythrocyte protein. J. Biol. Chem. 256:12911–12919.
10. Katada, T., and Ui, M. 1982. ADP ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. J. Biol. Chem. 257:7210–7216.
11. Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L., and Gilman, A. G. 1983. Identification of the predominant substrate for ADP-ribosylation by islet activating protein. J. Biol. Chem. 258:2072–2075.
12. Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R. D., and Manclark, C. R. 1983. Pertussis toxin substrate, the putative N_i component of adenylyl cyclases, is an $\alpha\beta$ heterodimer regulated by guanine nucleotide and magnesium. Proc. Natl. Acad. Sci. USA 80:4276–4280.
13. Brostrom, C. O., Huang, Y.-C., Breckenridge, B. M., and Wolff, D. 1975. Identification of a calcium-binding protein as a calcium-dependent regulator of brain adenylate cyclase. Proc. Natl. Acad. Sci. USA 72:64–68.
14. Cheung, W. Y., Bradham, L. S., Lynch, J. J., Lin, Y. M., and Tallant, E. A. 1975. Protein activator of cyclic 3′:5′-nucleotide phosphodiesterase of bovine or rat brain also activates its adenylate cyclase. Biochem. Biophys. Res. Comm. 66:1055–1062.
15. Salter, R. S., Krinks, M. H., Klee, C. B., and Neer, E. J. 1981. Calmodulin activates the isolated catalytic unit of brain adenylate cyclase. J. Biol. Chem. 256:9830–9833.
16. Bitonti, A. J., Moss, J., Hjelmeland, L., and Vaughn, M. 1982. Resolution and activity of adenylate cyclase components in a Zwitter-ionic cholate derivative [3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate]. Biochemistry 21:3650–3653.
17. Andreasen, T. J., Heideman, W., Rosenberg, G. B., and Storm, D. R. 1983. Photoaffinity labeling of brain adenylate cyclase preparations with azido [^{125}I] iodo calmodulin. Biochemistry 22:2757–2762.
18. Heideman, W., Wierman, B. M., and Storm, D. R. 1982. GTP is not required for calmodulin stimulation of bovine brain adenylate cyclase. Proc. Natl. Acad. Sci. USA 79:1462–1465.
19. Seamon, K. B., and Daly, J. W. 1982. Calmodulin stimulation of adenylate cyclase in rat brain membranes does not require GTP. Life Sci. 30:1457–1464.
20. Westcott, K. R., LaPorte, D. C., and Storm, D. R. 1979. Resolution of adenylate cyclase sensitive and insensitive to Ca^{2+} and calcium-dependent regulatory protein

(CDR) by CDR-Sepharose affinity chromatography. Proc. Natl. Acad. Sci. USA 76:204–208.

21. SANO, M., and DRUMMOND, G. I. 1981. Properties of detergent-dispersed adenylate cyclase from cerebral cortex. Presence of an inhibitor protein. J. Neurochem. 37:558–566.
22. BROSTROM, M. A., BROSTROM, C. O., and WOLFF, D. J. 1978. Calcium-dependent adenylate cyclase from rat cerebral cortex: activation by guanine nucleotides. Arch. Biochem. Biophys. 191:341–350.
23. TREISMAN, G. J., BAGLEY, S., and GNEGY, M. E. 1983. Calmodulin-sensitive and calmodulin-insensitive components of adenylate cyclase activity in rat striatum have differential responsiveness to guanyl nucleotides. J. Neurochem. 41:1398–1406.
24. UEDA, T., GREENGARD, P., BERZINS, K., COHEN, R. S., BLOMBERG, F., GRAB, D. J., and SIEKEVITZ, P. 1979. Subcellular distribution in cerebral cortex of two proteins phosphorylated by a cAMP-dependent protein kinase. J. Cell Biol. 83:308–319.
25. SALOMON, Y., LONDOS, C., and RODBELL, M. 1974. A highly sensitive adenylate cyclase assay. Anal. Biochem. 58:541–548.
26. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193:265–275.
27. FABIATO, A., and FABIATO, F. 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J. Physiol. (Paris) 75:463–505.
28. WHITTAKER, V. P., MICHAELSON, I. A., and KIRKLAND, R. J. A. 1964. The separation of synaptic vesicles from nerve ending particles ("synaptosomes"). Biochem. J. 90:293–303.
29. DE ROBERTIS, E., RODRIGUEZ DE LORES ARNAIZ, G., ALBERICI, M., BUTCHER, R. W., and SUTHERLAND, E. W. 1967. Subcellular distribution of adenyl cyclase and cyclic phosphodiesterase in rat brain cortex. J. Biol. Chem. 242:3487–3493.
30. CLEMENT-CORMIER, Y. C., PARRISH, R. G., PETZOLD, G. L., KEBABIAN, J. W., and GREENGARD, P. 1975. Characterization of a dopamine-sensitive adenylate cyclase in the rat caudate nucleus. J. Neurochem. 25:143–149.
31. RODRIGUEZ DE LORES ARNAIZ, G., ALBERICI, M., and DE ROBERTIS, E. 1967. Ultrastructural and enzymic studies of cholinergic and noncholinergic synaptic membranes isolated from brain cortex. J. Neurochem. 14:215–225.
32. COTMAN, C. W., BANKER, G., CHURCHILL, L., and TAYLOR, D. 1974. Isolation of postsynaptic densities from rat brain. J. Cell Biol. 63:441–455.
33. MORGAN, I. G., WOLFE, L. S., MANDEL, P., and GOMBOS, G. 1971. Isolation of plasma membranes from rat brain. Biochim. Biophys. Acta 241:737–751.
34. GURD, J. W., JONES, L. R., MAHLER, H. R., and MOORE, W. J. 1974. Isolation and partial characterization of rat brain synaptic plasma membranes. J. Neurochem. 22:281–290.
35. SORENSON, R. G., and MAHLER, H. R. 1982. Localization of endogenous ATPases at the nerve terminal. J. Bioenerg. Biomembr. 14:257–277.