MOLECULAR PHARMACOLOGY, 15, 484-491

Effect of Gangliosides on Adenylate Cyclase Activity in Rat Cerebral Cortical Membranes

C. R. PARTINGTON[1] AND J. W. DALY

Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, 20014

(Received August 10, 1978)
(Accepted November 20, 1978)

SUMMARY

PARTINGTON, C. R. & DALY, J. W. (1979) Effect of gangliosides on adenylate cyclase activity in rat cerebral cortical membranes. *Mol. Pharmacol.* **15**, 484-491.

The addition of 50 μM mixed brain gangliosides to membrane preparations from rat cerebral cortex caused a 50-95% increase in the basal adenylate cyclase activity. The activation was rapid at all temperatures and was relatively irreversible, as evidenced by the fact that repeated washing of the membrane after exposure to gangliosides failed to restore adenylate cyclase activity to its basal level. The V_{max} of the enzyme was increased with no apparent change in the K_m for the substrate, ATP. The expression of activation of adenylate cyclase by gangliosides showed a marked temperature dependence, with maximal effects seen in the 30-40° range and no activation seen at either 10° or 50°. The activation of adenylate cyclase by brain gangliosides was additive with respect to activation by NaF and detergents, but was not additive with respect to activation by GppNHp or lysolecithin. The activation of the enzyme by gangliosides was unaffected by the presence of calcium ions, calcium-dependent activator protein, or EGTA. The addition of brain gangliosides to the membranes caused a consistent restoration of the responsiveness of the adenylate cyclase system to activation by β-adrenergic agents, an effect similar in magnitude to that observed by addition of GppNHp to the membrane preparations.

INTRODUCTION

The formation, degradation, and function of cyclic AMP in the nervous system have been investigated extensively (1, 2). Formation of this nucleotide in the nervous system is regulated by the activity of adenylate cyclase (E.C. 4.6.1.1.), and has been found to be markedly enhanced by putative neurotransmitters, thus implicating cyclic AMP[2] and adenylate cyclase as central characters in nerve transmission. Other endogenous substances, however, have been found to affect the basal activity of adenylate cyclase and the sensitivity of this enzyme to activation by neurotransmitters. Notable are calcium ions, which are stimulatory at low concentrations and inhibitory at high concentrations (3); magnesium and manganese ions, which are stimulatory (4); GTP, which is not only stimulatory (5) but perhaps even essential for hormone stimulation; and various soluble protein factors that are stimulatory to the enzyme (6, 7). In spite of the recent interest in the regulation of membrane bound enzymes by other membrane components, adenylate cyclase of the central nervous system has not yet been thoroughly studied in this

[1] Fellow of the Pharmacology Research Associate Training Program of the NIGMS, NIH.

[2] The abbreviations used are: cyclic AMP, cyclic adenosine 3',5'-monophosphate; GppNHp, 5'-guanylylimidodiphosphate.

0026-895X/79/030484-08$02.00/0

regard. The role of gangliosides in the control of adenylate cyclase activity has here been investigated in membranes prepared from rat cerebral cortex. Gangliosides are sialic acid containing glycolipids that are present in high concentrations in neuron plasma membranes in the central nervous system (8).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (150–175 g) were obtained from Taconic Farms (Germantown, N.Y.).

Drugs and reagents. [^{3}H]ATP (24 Ci/mmole) was from Amersham/Searle; cyclic AMP, creatine phosphate, creatine phosphokinase, ATP, bovine serum albumin, adenosine, and L-norepinephrine bitartrate from Calbiochem; histamine dihydrochloride, DL-propranolol·HCl, and Lubrol PX from Sigma Chemical Co.; phentolamine·HCl from Ciba; phenylephrine·HCl from Sterling-Winthrop Research Institute; Cutscum (isooctylphenoxypolyethanol) from Fisher Scientific Co.; guanylylimidodiphosphate (GppNHp) from P-L Biochemicals; mixed brain gangliosides (Type III) were obtained from Sigma and dialyzed against 1.0 mM EGTA and against 10 mM Tris·HCl, pH 7.4, prior to use. Other compounds were from standard commercial sources.

Preparation of cerebral cortical membranes. A male Sprague-Dawley rat was killed by decapitation, and the brain quickly removed, chilled briefly in ice-cold Krebs-Ringer buffer, and placed on a chilled glass plate. The cerebral cortical grey matter was dissected free with a razor blade and the tissue (*ca.* 300 mg/animal) homogenized in 15 volumes ice-cold 50 mM Tris·HCl buffer, pH 7.4, with a teflon-glass homogenizer. This suspension was centrifuged at 3000 × *g* for 15 min at 4°, the supernatant discarded, the pellet resuspended in 15 volumes cold 50 mM Tris, and the suspension again centrifuged at 3000 × *g* for 15 min at 4°. The resulting pellet was resuspended in 10 volumes 50 mM Tris·HCl, pH 7.4, to produce a membrane suspension with a protein concentration of between 80 and 100 μg/25 μl.

Membranes deficient in calcium-dependent activator protein were prepared by the method of Lynch *et al.* (9). The membrane suspension (see above) was centrifuged at 20,000 × *g* for 20 min at 4°, and the pellet washed by suspension (teflon-glass homogenizer) in 15 volumes 50 mM Tris·HCl, pH 7.4, containing 1 M NaCl and 2 mM EGTA, followed by centrifugation at 20,000 × *g* for 15 min. This washing was repeated three times, and the resulting pellet was washed twice (suspension followed by centrifugation) with 15 volumes of 50 mM Tris·HCl buffer to remove the NaCl and EGTA. The final pellet was suspended in 10 volumes of 50 mM Tris buffer to produce a membrane suspension with a protein concentration between 80 and 100 μg/25 μl.

In order to avoid inconsistencies associated with membrane storage, fresh membranes were always prepared immediately prior to use.

Adenylate cyclase assay. The enzyme assay used was a modification of the Perkins and Moore procedure (10) reported by Skolnick *et al.* (11). Incubations were carried out in a total volume of 250 μl, containing 50 mM Tris·HCl buffer (pH 7.4), 0.5 mM isobutylmethylxanthine, 5 mM $MgCl_2$, 20 mM creatine phosphate, 10 units creatine phosphokinase, and 0.5 mM [^{3}H]ATP (1 μCi; spec. act., 8 Ci/mole). The assay was initiated by the addition of 25 μl of the membrane suspension to the warmed (37°) assay mixture containing the various agents. After incubation, usually at 37° for 10 min, the reaction was terminated by the addition of 500 μl of 10% trichloroacetic acid. Carrier cyclic AMP (250 μl of a 2 mM solution) was added, the mixture centrifuged, and the supernatant applied to a Bio-Rad AG 50 × 8 (200–400 mesh, H^+ form) ion exchange column (bed volume 1.0 ml). The column was washed with 3 ml water, and the cyclic AMP eluted directly onto a 300 mg alumina column with an additional 3 ml water. The alumina was washed with 1 ml 0.1 M imidazole·HCl buffer, pH 7.4, and the cyclic AMP eluted directly into scintillation vials with 4 ml imidazole buffer. A 1 ml aliquot was removed, and the recovery determined by the absorbance at 258 nm. To the remainder of the eluate was added 10 ml Aquasol, and

the [^{3}H] cyclic AMP determined by liquid scintillation counting. Cyclic AMP recovery was 65–90%. Reactions were linear with time and with protein concentration in the ranges studied.

Calcium-dependent activator protein. The calcium-binding protein was prepared from bovine brain through the DEAE-cellulose chromatography step of the procedure reported by Lin *et al.* (12).

Protein determination. The trichloroacetic acid precipitates from the adenylate cyclase assays were dissolved by heating in a boiling water bath with 250 μl 1 N NaOH, and the protein determined by the method of Lowry *et al.* (13), using bovine serum albumin as a protein standard. The presence of mixed brain gangliosides was found to interfere with the Lowry (13), Biuret (14), and Coomassie (15) protein assays. Therefore, protein was not determined for samples containing gangliosides; instead an average protein value from the control samples, which did not contain added gangliosides, was used. For each membrane preparation, protein contents of 25 μl aliquots were found to be within 6% of the mean value for that membrane preparation.

RESULTS

The addition of bovine brain gangliosides to membrane preparations from rat cerebral cortex produced an immediate 50–95% increase in the basal adenylate cyclase activity. The extent of activation was dependent upon the dose of gangliosides (Fig. 1). Half maximal activation occurred at about 125 μg gangliosides per ml assay mixture (30 μg gangliosides/100 μg membrane protein). This would correspond to a concentration of about 50 μM. The increase in adenylate cyclase activity was maximal after 1.0 min incubation, and remained constant throughout the standard 10 min incubation (data not shown). The pattern of activation by gangliosides was similar with normal and EGTA-inhibited adenylate cyclase. In both cases the activity of adenylate cyclase in ganglioside-treated preparations was 125–150 pmol/min/mg protein. This level of activity represents about a threefold activation of the enzyme by gangliosides in the presence of EGTA (Fig. 1).

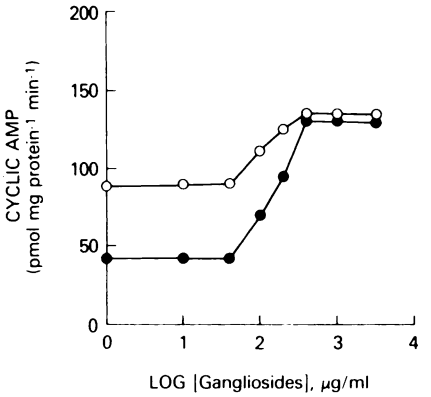

FIG. 1. *Dose-response relationship for activation of adenylate cyclase by mixed brain gangliosides in rat cerebral cortical membranes*

Membranes were prepared as described in METHODS, and adenylate cyclase activity measured under the standard assay conditions in the presence of various concentrations of mixed brain gangliosides, both in the presence (filled symbols) and absence (unfilled symbols) of 200 μM EGTA. Values are the means of triplicate determinations.

The interaction of gangliosides with the membrane preparation occurred readily, even at 0° (data not shown), and was irreversible as evidenced by the fact that repeated washing of the membrane after exposure to gangliosides failed to restore the adenylate cyclase activity to its basal level (Table 1). The presence of gangliosides caused an increase in the V_{max} of the enzyme, with no significant alteration in the apparent K_m for ATP (data not shown).

The expression of activation of the adenylate cyclase by gangliosides in cerebral cortex membranes showed a clear temperature dependence, with only a 14% increase above basal levels at 20° and a 75% increase above basal levels at 30° (Fig. 2). At high (50°) and low (10°) temperatures, no significant activation of the cyclase was expressed in the ganglioside-treated preparation.

The activation of adenylate cyclase by gangliosides was additive to the activation of the enzyme by NaF and detergents, but gangliosides had no effect on enzyme activated by GppNHp or lysolecithin (Table 2, Fig. 3). The activation of the enzyme by gangliosides was unaffected by the presence of calcium-dependent activator protein, the

enzyme being activated to approximately the same level of activity in normal membranes and in membranes deficient in activator protein (Table 3).

The presence of gangliosides was found to have a significant effect on the activation of adenylate cyclase by β-adrenergic agents in homogenates (Fig. 4). In the presence of gangliosides, a 50–60 pmol/mg protein/min activation of adenylate cyclase was *always* observed with norepinephrine or isoproterenol, while in the absence of gangliosides the effect was inconsistent, varying from preparation to preparation between no effect and a 30 pmol/mg protein/min activation by norepinephrine. This effect was shown to have the characteristics of a β-adrenergic response in that the activation was blocked by propranolol but not by phentolamine (Table 4). Isoproterenol caused a slightly greater activation than norepinephrine, while a variety of other agents (histamine, dopamine, phenylephrine, adenosine) had no effect. A response to norepinephrine was also consistently observed in the presence of GppNHp (Fig. 4).

TABLE 1

Activation of adenylate cyclase activity in rat cerebral cortical membranes: effect of washing

Membranes were either assayed with no pretreatment; preincubated with 500 μg mixed brain gangliosides per ml for 15 min at 0°, centrifuged at 4000 × *g*, taken up in 10 volumes 50 mM Tris buffer at pH 7.4, and assayed; or preincubated with gangliosides as above, washed three times by suspension in 10 volumes Tris buffer followed by centrifugation at 4000 × *g*, and the final membrane pellet taken up in 10 volumes buffer and assayed for adenylate cyclase activity under the standard assay conditions. Basal values were obtained in the absence of a stimulating agent, while ganglioside-stimulated values were obtained in assay mixtures containing 400 μg mixed brain gangliosides per ml. Values reported are the means of triplicate assays.

Treatment	Adenylate Cyclase Activity	
	Basal	Gangliosides-Present
	(pmol/mg protein/min)	
None	89.1	140.5
Preincubated with gangliosides	147.7	146.4
Preincubated with gangliosides and washed	152.7	148.7

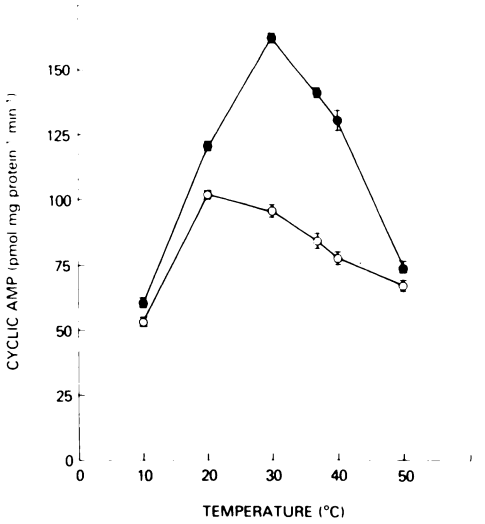

FIG. 2. *Effect of temperature on adenylate cyclase activity in rat cerebral cortical membranes*

Membranes were prepared as described in METHODS and adenylate cyclase activity measured in standard assay solutions at various temperatures. Assays were performed in the absence (unfilled circles) or in the presence (filled circles) of 400 μg mixed brain gangliosides/ml. Values are means ± SE of three separate experiments.

DISCUSSION

Several lines of evidence support the concept that adenylate cyclase is influenced by the composition of its membrane environment. Treatment of membrane preparations with membrane-active agents such as filipin (16), phospholipases (17, 18), organic solvents (19), fatty acids (20, 21), and phospholipids (22, 23) has been shown to produce marked changes in the basal adenylate cyclase activity, the hormonal responsiveness, or both. Manipulation of the membrane composition *in vivo* through dietary alteration (24) or growth media alteration (23) has demonstrated that these membrane alterations and the concomitant adenylate cyclase changes can occur and have significant effects in living organisms.

In the present study, mixed brain gangliosides were found to produce a rapid activation of the adenylate cyclase in membranes prepared from rat cerebral cortex.

TABLE 2

Effects of combinations of gangliosides and other agents on adenylate cyclase activity in rat cerebral cortical membranes

Membranes were assayed for adenylate cyclase activity under the standard conditions both in the absence and in the presence of 400 μg mixed brain gangliosides per ml. Doses of other agents were those which produced maximal effects. Values reported are the means of triplicate assays for a typical experiment.

Agent	Adenylate Cyclase Activity	
	No Gangliosides	Gangliosides present
	(% basal)	
None	100	193
10 μM GppNHp	339	333
5 mM NaF	569	671
200 μM Lysolecithin	182	180
0.1% Cutscum	307	363
0.1% Lubrol PX	24	77
10 μM L-Norepinephrine	104	232
200 μM Lysolecithin + 10 μM L-Norepinephrine	184	—
0.1% Cutscum + 10 μM L-Norepinephrine	310	—
200 μM Lysolecithin + 10 μM GppNHp	312	—
200 μM Lysolecithin + 5 mM NaF	571	—

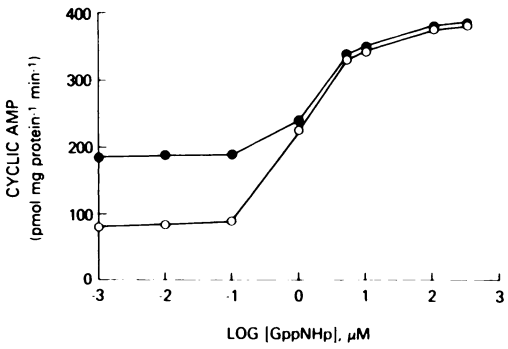

FIG. 3. *Dose-response relationship for activation of adenylate cyclase by GppNHp in rat cerebral cortical membranes*

Membranes were prepared as described in METHODS, and adenylate cyclase activity assayed under the standard conditions in the presence of various concentrations of GppNHp. Assays were performed in the absence (unfilled circles) or in the presence of 400 μg mixed brain gangliosides/ml (filled circles). Values are the means of triplicate assays which showed a variation of less than 7%.

TABLE 3

Effect of calcium-dependent activator protein and gangliosides on adenylate cyclase activity

Activator protein deficient membranes (see METHODS) were suspended in 10 volumes Tris buffer, and assayed for basal and ganglioside-stimulated adenylate cyclase activity under the standard assay conditions, both in the presence and in the absence of activator protein (4.5 μg/assay). Mixed brain gangliosides were used at 400 μg per ml. Values reported are the means ± SE of three separate experiments.

Agent	Adenylate cyclase activity	
	Basal	Gangliosides present
	(pmol/mg protein/min)	
None	78.9 ± 5.3	151.9 ± 6.2
Calcium-dependent activator protein	131.8 ± 1.6	156.7 ± 4.8

The dose of mixed brain gangliosides required to produce activation of the cyclase system was relatively large (Fig. 1). This might indicate that a minor component of the ganglioside mixture was responsible for the observed effect and would, by itself, be very potent. Preliminary data indicate that the major brain gangliosides G_{M1} and G_{D1a}[3] are not particularly more potent than the mixture of brain gangliosides. Alternatively, rapid nonspecific incorporation of exogenous gangliosides into the membrane (cf. ref. 25) might compete with specific incorporation sites, thereby decreasing the apparent potency of the gangliosides. The Lubrol PX solubilized adenylate cyclase from rat cerebral cortex was not activated by gangliosides (data not shown), indicating the importance of the membrane environment of the enzyme for the activation to be observed. The activating effect of gangliosides was specific for the adenylate cyclase of neuronal tissue. The particulate adenylate cyclase from rat liver was not activated by gangliosides (data not shown). Membrane bound phosphodiesterase present in rat cerebral cortex membranes was not activated by gangliosides (Davis, C. W. and J. W. Daly, unpublished observations).

The ganglioside-activated adenylate cy-

[3] These gangliosides were provided by Dr. L. Cohn, NIH.

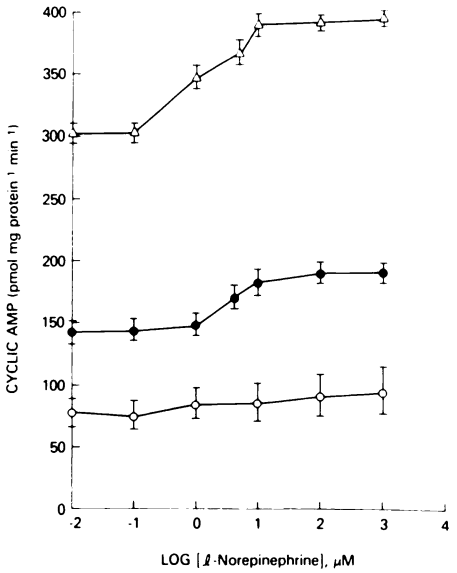

FIG. 4. *Dose-response relationship for activation of adenylate cyclase by L-norepinephrine in rat cerebral cortical membranes*

Membranes were prepared as described in METHODS and adenylate cyclase activity assayed under the standard conditions in the presence of various concentrations of L-norepinephrine. Assays were performed in the absence of exogenous gangliosides or guanyl nucleotides (unfilled circles), in the presence of 400 μg mixed brain gangliosides/ml (filled circles), or in the presence of 10 μM GppNHp (unfilled triangles). Values are means ± SE of six separate experiments. The response in the presence of both 400 μg mixed brain gangliosides/ml and 10 μM GppNHp did not differ significantly from that observed with 10 μM GppNHp alone.

clase showed classical Michaelis-Menton kinetics with respect to the substrate, ATP, in the presence of excess Mg^{++}, which indicated that the enzyme underwent a change in the maximum velocity but not in the affinity for the substrate. The expression of ganglioside activation of adenylate cyclase showed a marked dependence on the temperature at which the incubation was carried out (Fig. 2). Maximal effects were seen between 30 and 40°.

The activation of adenylate cyclase in brain membranes was not related to the ability of gangliosides to sequester calcium ions, since the level of activity to which the cyclase was raised was identical in the presence or absence of EGTA (Fig. 1). Exhaustive dialyses of the gangliosides against EGTA failed to remove all of the calcium ions which the commercial preparation contained, but the amount of non-dialyzable calcium which remained (3 μg calcium/1 mg gangliosides as determined by atomic adsorption spectrometry) had no effect on the adenylate cyclase activity in these membrane preparations (data not shown).

The observation that gangliosides activated the adenylate cyclase through a mechanism that was independent of calcium ions and the calcium-dependent activator protein (Table 3) introduces the possibility that the presence of gangliosides could have activated the enzyme *via* interaction with the same site at which the activator protein normally functions. This possibility is consonant with the fact that the maximal activation of enzyme in salt-washed membranes by either activator protein or gangliosides resulted in approximately equal levels of adenylate cyclase activity. The maximal activations by activator protein were consistently in the 150–160 pmol/mg protein/min range (data not shown).

Gangliosides activated adenylate cyclase,

TABLE 4

Effect of norepinephrine on adenylate cyclase activity in the presence and absence of gangliosides

Cerebral cortical membranes were assayed for adenylate cyclase activity under the standard conditions. Other agents including 100 μM histamine, 10 and 100 μM adenosine, 100 μM phenylephrine, and 10 μM dopamine had no effect on adenylate cyclase activity, either in the presence or absence of 400 μg of gangliosides per ml. Values reported are the means of triplicate assays.

Stimulating Agent	Adenylate cyclase activity	
	No gangliosides	Gangliosides present
	(pmol/mg protein/min)	
None	61.3	107.6
10 μM DL-isoproterenol	66.2	139.2
10 μM L-norepinephrine	64.6	130.1
10 μM L-norepinephrine + 10 μM propranolol	62.6	108.2
10 μM L-norepinephrine + 20 μM phentolamine	72.3	129.1

even in the presence of detergents (Table 2), suggesting that the activation was a reasonably specific phenomenon, not related to generalized surfactant properties. The ganglioside effect remained constant throughout the dose-response curves for activation and inhibition of the enzyme by various detergents (data not shown), so that even the maximally detergent-activated (0.1% Cutscum in Table 2) and maximally detergent-inhibited (0.1% Lubrol PX in Table 2) enzymes were susceptible to ganglioside activation.

The presence of gangliosides restored consistency to the response of adenylate cyclase in rat cerebral cortex membranes to stimulation by norepinephrine (Fig. 4). The response to norepinephrine varied greatly from experiment to experiment in the samples which contained no added gangliosides, but the response was always consistent in the samples which contained gangliosides. The response observed was mediated by a β-adrenergic receptor (Table 4 and RESULTS), and was a specific effect of gangliosides, not seen with other activators of adenylate cyclase such as lysolecithin or detergents. It is, however, similar to the effect of GppNHp (Fig. 4). GTP also causes an activation of adenylate cyclase in rat cerebral cortical membranes but unlike gangliosides, GTP is inhibitory to the GppNHp-response (data not shown).

Gangliosides are incorporated into biological membranes with the lipophilic ceramide portion embedded in the membrane and the hydrophilic carbohydrate portion protruding into the surrounding cytoplasm (8). This structural feature provides the potential for selective control of membrane characteristics both through fluidity changes and through specific interaction of the carbohydrate moiety with membrane surface associated components such as hormone receptors. Thus, gangliosides could conceivably be involved in the organization of the components of the mosaic of the membrane structure and thus in the regulation of adenylate cyclase in the central nervous system. Gangliosides are concentrated in synaptic plasma membranes (26). Furthermore, the developmental appearance of certain gangliosides (e.g., G_{D1a}) closely parallels the appearance of a functional β-adrenergic sensitive adenylate cyclase in the central nervous system (27–30). Clearly, gangliosides must be considered potentially important for the physiological regulation of central cyclic AMP-generating systems.

REFERENCES

1. Daly, J. W. *Cyclic Nucleotides in the Nervous System.* (1977) Plenum Publishing Co., New York.
2. Daly, J. W. (1977) The formation, degradation, and function of cyclic nucleotides in the nervous system. *Internat. Rev. Neurobiol.,* **20,** 105–168.
3. Bradham, L. S., Hold, D. A. & Sims, M. (1970) The effect of Ca^{2+} on the adenyl cyclase of calf brain. *Biochim. Biophys. Acta,* **201,** 250–260.
4. Johnson, R. A. & Sutherland, E. W. (1973) Detergent-dispersed adenylate cyclase from rat brain; effects of fluoride, cations, and chelators. *J. Biol. Chem.,* **248,** 5114–5121.
5. VanInwegen, R. G., Strada, S. J. & Robison, G. A. (1975) Effects of prostaglandins and morphine on brain adenyl cyclase. *Life Sci.,* **16,** 1875–1876.
6. Kauffman, F. C., Harkonen, M. H. A. & Johnson, E. C. (1972) Adenyl cyclase and phosphodiesterase activity in cerebral cortex of normal and undernourished neonatal rats. *Life Sci.,* **11,** 613–621.
7. Izumi, H., Oyama, H. & Ozawa, H. (1975) The stimulatory effect of the boiled supernatant on cyclic AMP formation in synaptosomes from rat cerebral cortex. *Jap. J. Pharmacol.,* **25,** 375–382.
8. Irwin, L. N. (1974) Glycolipids and glycoproteins in brain function. *Rev. Neuroscience,* **1,** 137–180.
9. Lynch, T. J., Tallant, E. A. & Cheung, W. Y. (1977) Rat brain adenylate cyclase; further studies on its stimulation by a Ca^{2+} binding protein. *Arch. Biochem. Biophys.,* **182,** 124–133.
10. Perkins, J. P. & Moore, M. M. (1971) Adenyl cyclase of rat cerebral cortex: activation by sodium fluoride and detergents. *J. Biol. Chem.,* **246,** 62–68.
11. Skolnick, P., Stalvey, L. P., Daly, J. W., Hoyler, E. & Davis, J. N. (1978) Binding of α- and β-adrenergic ligands to cerebral cortical membranes; effect of 6-hydroxydopamine treatment and relationship to the responsiveness of cyclic AMP generating systems in two rat strains. *Eur. J. Pharmacol.,* **47,** 201–210.
12. Lin, Y. M., Lin, Y. P. & Cheung, W. Y. (1974) Cyclic 3′,5′-nucleotide phosphodiesterase; purification, characterization, and active form of the protein activator from bovine brain. *J. Biol.*

Chem., **249**, 4943–4954.

13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
14. Layne, E. (1957) Spectrophotometric methods for measuring proteins. *Methods Enzymol.*, **III**, 447–454.
15. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–259.
16. Puchwein, G., Pfeuffer, T. & Helmreich, E. J. M. (1974) Uncoupling of catecholamine activation of pigeon erythrocyte membrane adenylate cyclase by filipin. *J. Biol. Chem.*, **249**, 3232–3240.
17. Rubalcara, B. & Rodbell, M. (1973) The role of acidic phospholipids in glucagon action on rat liver adenylate cyclase. *J. Biol. Chem.*, **248**, 3831–3837.
18. Pohn, S. L., Krans, H. M. J., Koeyreff, V., Birnbaumer, L. & Rodbell, M. (1971) The glucagon sensitive adenylate cyclase in plasma membranes from rat liver; evidence for a role of membrane lipids. *J. Biol. Chem.*, **246**, 4447–4454.
19. Rethy, A., Tomasi, V. & Tervisani, A. (1971) Role of lipids in activity of adenylate cyclase of rat liver plasma membranes. *Arch. Biochem. Biophys.*, **147**, 36–40.
20. Anderson, W. B. & Jaworski, C. J. (1977) Modulation of adenylate cyclase activity of fibroblasts by free fatty acids and phospholipids. *Arch. Biochem. Biophys.*, **180**, 374–382.
21. Fain, J. N. & Shepherd, R. E. (1975) Free fatty acids as feedback regulators of adenylate cyclase in rat fat cells. *J. Biol. Chem.*, **250**, 6586–6592.
22. Houslay, M. D., Hesketh, R. T., Smith, G. A., Warren, G. B. & Metcalf, J. C. (1976) The lipid environment of the glucagon receptor regulates adenylate cyclase activity. *Biochim. Biophys. Acta*, **436**, 495–504.
23. Englehard, V. H., Esko, J. D., Storm, D. R. & Glaser, M. (1976) Modification of adenylate cyclase activity in LM cells by manipulation of the membrane phospholipid composition *in vivo*. *Proc. Natl. Acad. Sci. USA*, **73**, 4482–4486.
24. Brivio-Haugland, R. P., Louis, S. L., Musch, K., Waldeck, N. & Williams, M. A. (1976) Liver plasma membranes from essential fatty acid deficient rats. *Biochim. Biophys. Acta*, **433**, 150–163.
25. Sharom, F. J. & Grant, C. W. M. (1978) A model for ganglioside behaviour in cell membranes. *Biochim. Biophys. Acta*, **507**, 280–293.
26. Morgan, I. G., Wolfe, L. S., Mandel, P. & Gombos, G. (1971) Isolation of plasma membranes from rat brain. *Biochim. Biophys. Acta*, **241**, 737–743.
27. Rösner, H. (1977) Gangliosides, sialoglycoproteins, and acetylcholinesterase of developing mouse brain. *Rouxs Arch. Develop. Biol.*, **183**, 325–335.
28. Suzuki, K. (1965) Developmental pattern of brain gangliosides in rat. *J. Neurochem.*, **12**, 969–974.
29. VonHungen, K., Roberts, S. & Hill, D. F. (1974) Developmental and regional variations in neurotransmitter sensitive adenylate cyclase systems in cell free preparations from rat brain. *J. Neurochem.*, **22**, 811–819.
30. Savaki, H. E. & Levis, G. M. (1977) Changes in rat brain gangliosides following active avoidance conditioning. *Pharmacol. Biochem. Behav.*, **7**, 7–15.