Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 605–609, February 1979
Biochemistry

Opiates and enkephalins inhibit synthesis of gangliosides and membrane glycoproteins in mouse neuroblastoma cell line N4TG1

(sialoglycosphingolipid biosynthesis/cell surface charge modulation/stereospecific inhibition/naloxone-reversible inhibition/adenylate cyclase)

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Communicated by Albert Dorfman, October 25, 1978

ABSTRACT When mouse neuroblastoma clonal cell line N4TG1 cells were cultured in the presence of opiates or enkephalins, in the range 10^{-6}–10^{-10} M for 24 hr, a dose-dependent inhibition of the incorporation of [^{3}H]glucosamine and [^{14}C]galactose into sialoglycosphingolipids and glycoproteins was observed. The gangliosides most affected comigrated in thin-layer chromatographic systems with G_{M2} (GalNAc[AcNeu]-Gal-Glc-ceramide), G_{M1} (Gal-GalNAc[AcNeu]Gal-Glc-ceramide), and G_{D1a} (AcNeu-Gal-GalNAc[AcNeu]Gal-Glc-ceramide). The effects were stereospecific and naloxone-reversible. Polyacrylamide gel electrophoresis revealed that the synthesis of a large number of membrane glycoproteins was also stereospecifically inhibited. Synthesis of other proteins and glycoproteins, proteoglycans, DNA, and membrane phospholipids and the rate of cell division were not altered in any specific or stereospecific manner. Moreover, clonal cell lines (neuroblastomas and oligodendroglioma) and human skin fibroblasts, which do not possess opiate receptors, did not respond to opiates or enkephalins in a stereospecific manner.

Studies on the molecular basis of opiate action have been facilitated by the use of cell and organ culture systems. However, only two cell lines have been described that contain substantial numbers of opiate (enkephalin) receptors. These are a neuroblastoma–glioma hybrid line (NG108-15) (1) and a neuroblastoma line (N4TG1) (2). Clonal line N4TG1 was derived from a spontaneous C1300 neuroblastoma in an AJ strain mouse on the basis of its resistance to 6-thioguanine. Its characterized neuronal properties include expression of acetylcholine esterase activity, neurites that become impregnated with silver, electrically excitable membranes (3), and the presence of approximately 20,000 opiate receptors per cell as assessed by the stereospecific binding of the enkephalin analogue ^{125}I-labeled Tyr-DAla-Gly-Phe-DLeu (2). Recently, we have demonstrated that sulfatide (sulfogalactosylceramide) does not appear to be a constituent of the enkephalin receptor in N4TG1 cells (4).

Studies in other laboratories with NG108-15 cells have demonstrated that opiates and enkephalins inhibit PGE_1-stimulated adenylate cyclase activity in a stereospecific, naloxone-reversible manner (5–8). Similar effects are also seen in N4TG1 cells (unpublished observations). However, other cell lines that do not contain opiate receptors, such as the parental cell lines C680-1 (glioma) and N18TG2 (neuroblastoma), do not respond to opiates in this way. Continued culture of opiate receptor-positive NG108-15 cells in the presence of opiates and enkephalins produces a gradual restoration of adenylate cyclase activity until normal levels are achieved (5–8). Addition of naloxone at this point then produces a rapid accumulation of cyclic AMP in the cells (5). Such observations have been used to explain opiate tolerance and precipitated withdrawal.

Although it seems possible that an interaction with adenylate cyclase represents one of the initial events in the action of opiates, little is known about the subsequent biochemical events resulting from opiate agonist action. We have found in this study that culture of N4TG1 cells in the presence of opiates produces profound effects on biosynthesis of both membrane glycosphingolipid and glycoprotein. The effects are stereospecific, dose-dependent, and naloxone-reversible, indicating that they are mediated by opiate receptors.

MATERIALS AND METHODS

Cell Culture Conditions. Mouse neuroblastoma cell line N4TG1 (obtained from A. Gilman, University of Virginia, Charlottesville, VA), glioblastoma cell line G26-20 (obtained from S. E. Pfeiffer, University of Connecticut, Farmington, CT), and human skin fibroblasts (derived from control stocks maintained in this laboratory) were cultured on 100-mm Falcon plastic dishes in modified Eagle's medium supplemented with 10% fetal calf serum as described (9). Stock solutions of levorphanol, morphine sulfate, dextrorphan, naloxone, and [DAla^{2}-D-Leu^{5}]enkephalin were made up freshly for each experiment in 50% ethanol/deionized water and added to culture media in amounts not exceeding 20 μl/10 ml of medium. Cultures were double-labeled with either a combination of [^{3}H]GlcN and [^{14}C]Gal or [^{3}H]Gal and [^{14}C]GlcN in order to monitor complex carbohydrate biosynthesis, [^{3}H]leucine for following protein synthesis, and [*methyl*-^{14}C]dThd for DNA synthesis. After 24 hr, the cells were harvested mechanically with a rubber policeman, sonicated briefly for 10 sec at power setting 4 with a model W-185 sonifier (Heat Systems, Inc., Plainview, NJ), and subjected to various extraction procedures.

Glycosphingolipid Analysis. The sonicated suspension containing approximately 10^{7} cells in 1.2 ml of water was extracted with 6.0 ml of chloroform/methanol, 2:1 (vol/vol), for 30 min, with care taken to vortex the biphasic system at frequent intervals. The mixture was centrifuged at 600 × g for 5 min and the upper phase (containing sialoglycosphingolipids), interphase (a tight pad of insoluble protein, glycoprotein, and proteoglycan), and lower phase were carefully separated. The protein pellet was washed with chloroform/methanol, 2:1 (vol/vol), and used for gel electrophoretic studies.

The upper phase was dried and applied to a column (2.0 × 0.5 cm) of Sephadex G-25 in chloroform/methanol/water, 120:60:9 (vol/vol), to remove labeled sugars and other contaminants (10). The 10 ml of eluant was dried and subjected to thin-layer chromatography on Analtech Silica Gel G plates in

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Abbreviations: G_{D1a}, AcNeu-Gal-GalNAc[AcNeu]Gal-Glc-ceramide; G_{M2}, GalNAc-[AcNeu]Gal-Glc-ceramide; G_{M3}, AcNeu-Gal-Glc-ceramide; G_{M1}, Gal-GalNAc[AcNeu]Gal-Glc-ceramide.

the solvent system chloroform/methanol/0.02% aqueous calcium chloride, 60:40:9 (vol/vol). Plates were stained with resorcinol (11), and bands corresponding to previously characterized human brain ganglioside standards were eluted and their radioactivity was determined by liquid scintillation counting. For quantitative analyses the plates were stained with iodine vapor and the sugars were analyzed by gas/liquid chromatography (12).

The lower phase was fractionated into neutral lipids, glycolipids, and phospholipids on a 2.0 × 0.5 cm column of silicic acid (Unisil; Clarkson Chemical Company, Inc., Williamsport, PA) as described (9, 13) and the individual lipid areas were scraped for liquid scintillation counting or analysis by gas/liquid chromatography (9, 13). Phospholipids were separated by two-dimensional thin-layer chromatography as described (14) and their radioactivity was determined by liquid scintillation counting.

Polyacrylamide Gel Electrophoresis. Insoluble membrane fractions were solubilized in 1% sodium dodecyl sulfate (15). An aliquot (10%) was removed for direct liquid scintillation counting and further aliquots (20 μl) were applied to either 7.5 or 5% polyacrylamide slab cells with a stacking gel, according to the method of Grossfeld and Shooter (15). Protein bands were stained with Coomassie blue or periodic acid-Schiff according to Steck and Yu (16) and the gel was sliced to facilitate determination of the level of radioactivity in each protein band.

Protein, Proteoglycan, and Nucleic Acid Synthesis. Soluble media proteins were precipitated four times with cold 10% trichloroacetic acid and an aliquot was subjected to liquid scintillation counting. Soluble media proteoglycans were precipitated with cetylpyridinium chloride as described (17) and cellular nucleic acids were precipitated with trichloroacetic acid. The precipitates were solubilized and aliquots were removed for liquid scintillation counting (17).

Drugs. Morphine sulfate, levorphanol, dextrorphan, and naloxone were obtained from B. Wainer (University of Chicago). [DAla^2,DLeu^5]Enkephalin was a kind gift from S. Wilkinson (Burroughs-Wellcome Co., Beckenham, England).

RESULTS

Initial observations indicated that the sialoglycosphingolipid composition of mouse neuroblastoma N4TG1 cells consisted mainly of G_{M2} (GalNAc-[AcNeu]Gal-Glc-ceramide) and G_{D1a} (AcNeu-Gal-GalNAc-[AcNeu]Gal-Glc-ceramide) gangliosides of the mammalian central nervous system together with G_{M3} and G_{M1} (Gal-GalNAc[AcNeu]Gal-Glc-ceramide) (and possibly G_{D3}, G_{D1b}, or G_{T1a}) as minor components (Table 1).

The effect of culturing N4TG1 cells in the presence of various opiate agonists or antagonists or the enkephalin analogue [DAla^2,DLeu^5]enkephalin on ganglioside synthesis is illustrated in Table 2. We utilized the enkephalin analogue [DAla^2,DLeu^5]enkephalin throughout this study because of its greatly increased stability compared to [Leu^5]- or [Met^5]enkephalin. Morphine, levorphanol, and [DAla^2,DLeu^5]enkephalin at concentrations of 0.1–1 μM were potent inhibitors of total ganglioside synthesis. These actions could be partially reversed by adding naloxone (5 μM) simultaneously with the opiate. However, the most complete reversal was obtained by preincubating cells for 60 min with naloxone before adding the opiate or [DAla^2,DLeu^5]enkephalin. Naloxone by itself or dextrorphan (1 μM) had no inhibitory effect on total ganglioside synthesis.

Further analysis of the effects of the above agents revealed that inhibition of synthesis of a number of individual gangliosides occurred, including G_{M2}, G_{M1}, and G_{D1a} (Table 3), and these effects were reversed by naloxone. Both morphine (in the concentration range 10^{-9} to 10^{-6} M) and [DAla^2,DLeu^5]enkephalin (in the concentration range 10^{-10} to 10^{-6} M) gave a dose-dependent inhibition of both G_{D1a} and G_{M2} biosynthesis that was naloxone-reversible (Fig. 1). In the case of G_{D1a}, the morphine concentration for 50% inhibition was 7.5×10^{-8} M and for [DAla^2,DLeu^5]enkephalin it was 5×10^{-7} M. For G_{M2}, these concentrations were 10^{-7} M for morphine and 5×10^{-7} M for [DAla^2,DLeu^5]enkephalin. Naloxone alone or dextrorphan had no effect. Furthermore, it was observed that the opiates and

Table 1. Sialoglycosphingolipid composition of mouse neuroblastoma cell line N4TG1

Glycolipid	Concentration, nmol/mg protein	[^{3}H]GlcN incorporation, cpm/mg protein
G_{M3}	25	1,550
G_{M2}	65	16,000
G_{M1}	20	2,500
G_{D1a}*	105	16,000
G_T†	10	2,750

* Possibly includes some G_{D3}.
† Structure not conclusively established; could be G_{D1b}, G_{T1a}, G_{T1b}, or an as yet uncharacterized ganglioside.

Table 2. Effect of opiates and enkephalin on incorporation of [^{3}H]GlcN into cellular sialoglycosphingolipids and glycoproteins of N4TG1 cells

Addition	Sialoglycosphingolipids (total gangliosides), cpm/10^7 cells	Glycoproteins, cpm/10^7 cells
None	11,900	16,700
Morphine (1 μM)	4,470	5,590
Morphine (0.1 μM)	6,340	8,060
Morphine (0.1 μM) + naloxone (5 μM)	8,000	13,720
Morphine (0.1 μM) + naloxone (5 μM), 1-hr preincubation	10,300	17,500
Levorphanol (1 μM)	4,340	11,000
[DAla^2,DLeu^5]-Enkephalin (1 μM)	3,230	10,000
[DAla^2,DLeu^5]-Enkephalin (0.1 μM)	3,330	13,370
[DAla^2,DLeu^5]-Enkephalin (0.1 μM) + naloxone (5 μM), 1-hr preincubation	7,000	16,350

Table 3. Effect of opiates and enkephalin on cell surface glycolipid synthesis in N4TG1 cells

Narcotic added*	% of control†			
	G_{D1a}	G_{M2}	G_{M3}	GL-4†
Levorphanol	50	13	94	122
Levorphanol + naloxone	81	62	90	80
Naloxone	89	70	90	125
Dextrorphan	120	94	89	85
[DAla^2,DLeu^5]Enkephalin	21	22	90	130
[DAla^2,DLeu^5]Enkephalin + naloxone	32	43	70	120

* All narcotics were added at 1 μM in culture medium. Naloxone was added at the same time as the opiate or enkephalin. Cells were cultured in the presence of drugs and isotope for 24 hr.
† Control (no narcotic) values (cpm of [^{3}H]GlcN incorporated per 10^7 cells): G_{D1a}, 7600; G_{M2}, 5400; G_{M3}, 2400; GL-4, 1250.

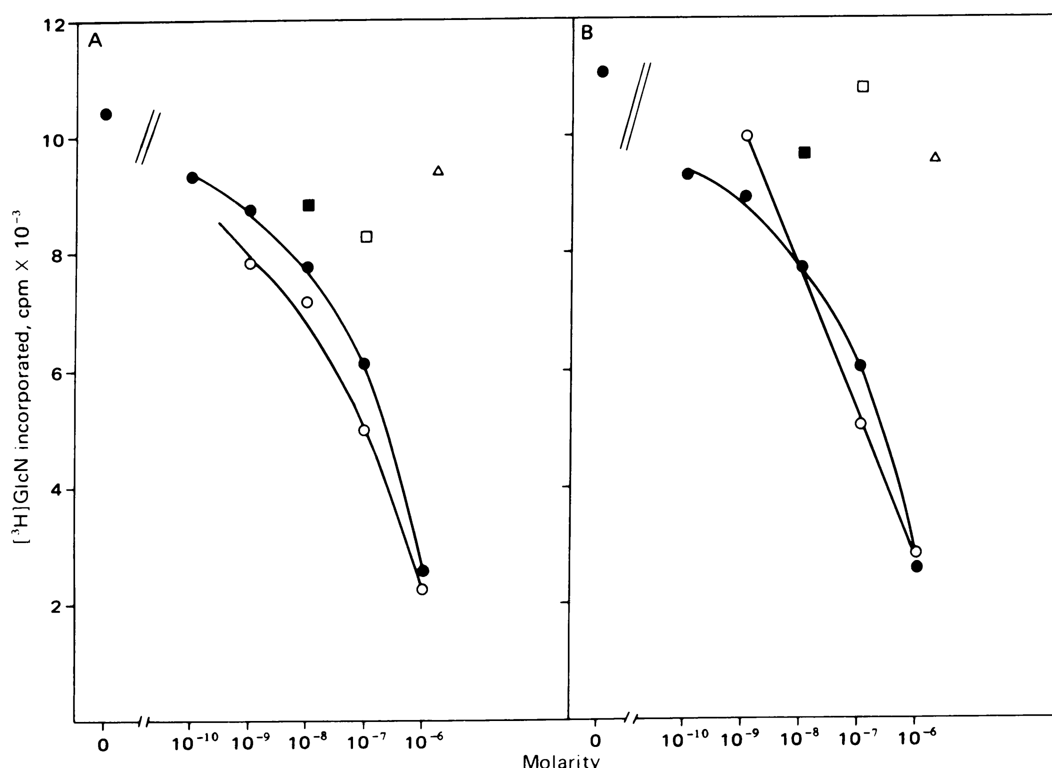

FIG. 1. Inhibition of 24-hr incorporation of [^{3}H]GlcN into gangliosides of mouse neuroblastoma N4TG1 cells by different concentrations of morphine (○) and [DAla^2,DLeu^5]enkephalin (●). (*A*) G_{D1a} ganglioside. (*B*) G_{M2} ganglioside. The effects of enkephalin and morphine were reversible by the coaddition of 2 μM naloxone (■ and □, respectively). Naloxone (△) and dextrorphan alone had no effect; levorphanol was as potent as morphine.

[DAla^2,DLeu^5]enkephalin had little effect on the synthesis of neutral glycosphingolipids (e.g., GL-4) or phospholipids; in fact, phospholipid synthesis was somewhat stimulated in a general, nonstereospecific fashion (Table 4).

Proteins insolubilized by the Folch partition-extraction procedure were affected in a manner similar to that observed for sialoglycosphingolipids (Tables 2 and 4). Polyacrylamide gel electrophoresis revealed that the effect was widespread among all the major membrane glycoproteins (Fig. 2) although bands 7 and 8 appeared to be the most affected and high molecular weight material (presumably proteoglycans, which do not enter the gel) showed normal specific activity. The gel scan shows that the effects on membrane glycoproteins were stereospecific and naloxone-reversible.

Table 4. Effect of opiates and enkephalin on synthesis of other complex carbohydrates in N4TG1 cells

	% of control†			
	Cell membrane		Secreted	
Narcotic added*	Glycoprotein	Phosphatidylcholine	Glycoprotein	Proteoglycans
Levorphanol	31	118	95	130
Levorphanol + naloxone	56	180	115	125
Naloxone	85	190	130	110
Dextrorphan	100	140	125	135
[DAla^2,DLeu^5]Enkephalin	26	140	83	125
[DAla^2,DLeu^5]Enkephalin + naloxone	50	100	81	130

* All narcotics were added at 1 μM in cultured medium. Naloxone was added at the same time as the opiate or enkephalin. Cells were cultured in the presence of isotope or drug for 24 hr.

† Control (no narcotic) values (cpm of [^{3}H]GlcN incorporated per 10^7 cells): cell membrane glycoprotein, 47,850; cell membrane phosphatidylcholine, 12,000; secreted glycoprotein, 113,040; secreted proteoglycans, 1260.

The major glycosaminoglycans and proteoglycans secreted by N4TG1 cells are hyaluronic acid, chondroitin-6-sulfate, and heparan sulfate as reported for other mouse neuroblastoma cells (18). Synthesis and secretion of proteoglycans by N4TG1 cells was not inhibited by opiates or [DAla^2,DLeu^5]enkephalin. In fact, a nonstereospecific 10–35% stimulation was commonly observed. A similar observation was made with regard to [^{3}H]GlcN or [^{14}C]Gal-labeled soluble glycoproteins, although in this case both levorphanol and enkephalin gave a slight (5–20%) inhibition, which is well below the 50–80% inhibition observed for membrane glycoproteins and sialoglycosphingolipids. This normal or somewhat enhanced labeling of other complex carbohydrates such as proteoglycans was taken as arguing against the possibility that opiates and enkephalins were acting at the level of either cellular uptake of isotopes or nucleotide sugar metabolism.

Studies on [*methyl*-^{14}C]dThd and [^{3}H]leucine incorporation into DNA and protein, respectively, showed no appreciable effect of opiate or enkephalin treatment (Table 5). This was confirmed by cell counts and estimates of total cell protein.

DISCUSSION

To date, little is known about the biochemical events mediating opiate action and the development of opiate addiction. Some studies using NG108-15 hybrid cells (also referred to as 108CC15 cells) have implicated the enzyme adenylate cyclase in both these phenomena (5–8). However, cyclic AMP is involved in a plethora of cellular functions, and nothing is known about which of these might be particularly relevant to opiate action. Apart from the studies on adenylate cyclase in neuro-

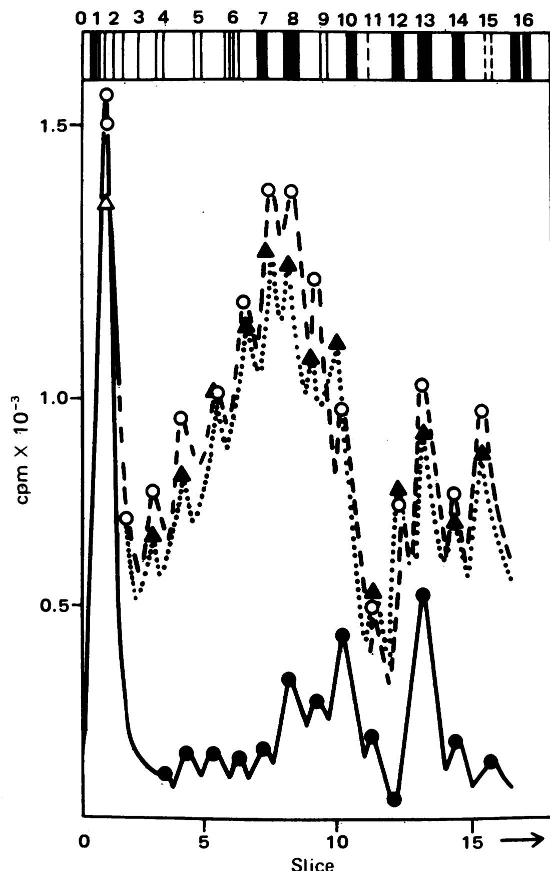

FIG. 2. Radioactivity scan of a 7.5% sodium dodecyl sulfate/polyacrylamide gel of N4TG1 cells after exposure to [^{3}H]GlcN and normal tissue culture medium (△), medium containing 1 μM levorphanol (●), or medium containing levorphanol and 2 μM naloxone (○) for 24 hr. Dextrorphan (1 μM) and naloxone (2 μM) alone had no effect.

blastoma–glial hybrids, previous studies on the cellular effects of opiates have either been carried out in cell lines, such as NB2a, which lack opiate receptors or have involved changes in phospholipid metabolism in cell-free extracts of whole brain (19). The discovery of stereospecific enkephalin binding sites in N4TG1 cells has permitted us to study specific effects of opiates on various cell surface components.

Levorphanol, morphine, and [DAla^2,DLeu^5]enkephalin all produced a profound, naloxone-reversible inhibition of both [^{3}H]glucosamine and [^{14}C]galactose incorporation into gangliosides G_{D1a}, G_{M1}, and G_{M2} (together with other minor gangliosides as yet unidentified) and a number of cell membrane-associated glycoproteins. The synthesis of G_{M3} ganglioside, nonglycosylated proteins, and nucleic acids was not inhibited, and, in fact, in certain cases was stimulated in a nonstereospecific fashion. We attribute this latter observation to some non-opiate receptor-mediated phenomenon because none of the effects was naloxone-reversible and similar effects can be observed in non-opiate receptor-containing lines such as mouse oligodendroglioma G26-20 and human skin fibroblasts (unpublished observations). Initial observations, however, have shown that specific effects similar to those seen in N4TG1 cells are also seen in the opiate receptor-bearing clone NG108-15.

Table 5. Effect of opiates and enkephalin on incorporation of [^{3}H]leucine and [methyl-^{14}C]dThd into cellular protein and DNA of N4TG1 cells

	Incorporation, cpm/10^7 cells	
Addition*	Protein synthesis ([^{14}C]Leu)	DNA synthesis ([^{14}C]dThd)
None	307,000	24,300
Morphine (1 μM)	265,000	20,300
Morphine (0.1 μM)	285,000	21,800
Morphine (0.1 μM) + naloxone (5 μM)	318,000	25,500
Levorphanol (1 μM)	254,000	20,700
Enkephalin (1 μM)	240,300	20,050
Enkephalin (0.1 μM)	252,000	20,800
Enkephalin (0.1 μM) + naloxone (5 μM)	271,000	22,000

* All drugs were added at 1 μM in culture medium. Naloxone was added at the same time as the opiate or enkephalin. Cells were cultured in the presence of drug and isotope for 24 hr.

It is curious to note that morphine and [DAla^2,DLeu^5]enkephalin appeared to be of similar potency in inhibiting N4TG1 ganglioside synthesis, because the enkephalin analogue has a considerably higher affinity than morphine for the N4TG1 cell opiate receptor (2). However, because of the duration of culture (24 hr), the relative rates of breakdown of the two agents must be considered. Moreover, the cellular events being monitored are considerably removed from the initial ligand–receptor interaction. The stereospecificity and naloxone reversibility observed certainly suggest opiate receptor-mediated events.

One possible explanation for the present observations might be that they are mediated by a glycosyltransferase (or transferases) that is regulated by fluctuations in cellular levels of cyclic AMP. It is well known that viral transformation of mouse 3T3 cells with simian virus 40 or polyoma results in decreased cyclic AMP levels and a fairly specific suppression of G_{M3}:*N*-acetylgalactosaminyltransferase (20). However, it is difficult at present to decrease cyclic AMP levels pharmacologically and one must therefore look for evidence that increased cyclic AMP concentrations stimulate transferase activities. In this context, it is well known that dibutyryl cyclic AMP (and butyrate itself) will activate sialotransferase (21) and we have observed an overall stimulation of G_{M2}, G_{M1}, and G_{D1a} ganglioside synthesis by dibutyryl cyclic AMP (2 mM for 24 hr) in N4TG1 cells (unpublished observations). It has also been observed that neuroblastoma cells treated with 1 mM dibutyryl cyclic AMP undergo morphological differentiation and show enhanced glycosylation of a number of secreted proteins (22).

Many recent observations have suggested a function for membrane sialoglycosphingolipids as receptors for cholera toxin, thyrotropin, and interferon and in control of cellular calcium regulation, cell–cell interaction, ion transport, and cell surface charge density (23–28). Clearly, the profound changes that take place in the membrane composition of glycoconjugates on exposure to opiates may subserve many of the changes associated with neuronal cell membranes after acute exposure to opiates or associated tolerance phenomena after chronic exposure to such drugs. In this context it will be important to determine if the opiate-induced cell surface changes reported here are a consequence of cyclic nucleotide changes or whether, in fact, they may be responsible for the observed alterations in adenylate cyclase activity. Our data, when taken together with previous studies [such as those that indicate that neurotransmitters such as norepinephrine, which increase cyclic AMP levels in cells (29), also stimulate incorporation of [^{3}H]GlcN into sialoglycosphingolipids (30)], suggest that many of the longer-term effects of neurotransmitters as well as their agonists and antagonists can be explained by cyclic AMP-mediated changes in cell surface complex carbohydrates.

We acknowledge the excellent technical assistance of Ms. Gwendolyn Schoon, Mr. Stewart M. Kernes, and Mr. John Y. Oh and the helpful, stimulating discussions with Dr. Bruce Wainer, Departments of Pathology and Pediatrics, University of Chicago. This work was supported in part by U.S. Public Health Service Grants HD-06426, HD-04583, and HD-09402, U.S. Public Health Service Research Career Development Award NS-00029 (to G.D.), and the Frenkel Fund of the University of Chicago.

1. Klee, W. A. & Nirenberg, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3474–3477.
2. Miller, R. J., Chang, K.-J., Cooper, B. & Cuatrecasas, P. (1978) *J. Biol. Chem.* **253**, 531–538.
3. Minna, J., Nelson, P., Peacock, J., Glazer, D. & Nirenberg, M. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 234–239.
4. Dawson, G., Kernes, S. M., Miller, R. J. & Wainer, B. (1978) *J. Biol. Chem.* **253**, 7999–8001.
5. Sharma, S. K., Klee, W. A. & Nirenberg, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3092–3096.
6. Brandt, M., Fischer, K., Moroder, L., Wunsch, E. & Hamprecht, B. (1976) *FEBS Lett.* **68**, 38–40.
7. Klee, W. A. & Nirenberg, M. (1976) *Nature (London)* **263**, 609–611.
8. Hamprecht, B. (1977) *Int. Rev. Cytol.* **48**, 99–170.
9. Dawson, G., Matalon, R. & Dorfman, A. (1972) *J. Biol. Chem.* **247**, 5944–5950.
10. Dawson, G. & Stoolmiller, A. C. (1976) *J. Neurochem.* **26**, 225–226.
11. Svennerholm, L. (1957) *Biochim. Biophys. Acta* **24**, 604–611.
12. Dawson, G. (1976) in *Lipid Chromatographic Analysis*, ed. Marinetti, G. V. (Dekker, New York), pp. 663–700.
13. Vance, D. E. & Sweeley, C. C. (1967) *J. Lipid Res.* **8**, 621–630.
14. Rouser, G., Kritchevsky, G. & Yamamoto, A. (1976) in *Lipid Chromatographic Analysis*, ed. Marinetti, G. V. (Dekker, New York), pp. 713–776.
15. Grossfeld, R. M. & Shooter, E. M. (1971) *J. Neurochem.* **18**, 2265–2277.
16. Steck, T. L. & Yu, J. (1973) *J. Supramol. Struct.* **1**, 220–232.
17. Dorfman, A. & Ho, P.-L. (1970) *Proc. Natl. Acad. Sci. USA* **66**, 495–499.
18. Stoolmiller, A. C., Dawson, G. & Dorfman, A. (1973) in *Tissue Culture of the Nervous System*, ed. Sato, G. (Plenum, New York), pp. 247–281.
19. Natsuki, R., Hitzemann, R. & Loh, H. (1978) *Mol. Pharmacol.* **14**, 448–453.
20. Cumar, F. A., Brady, R. O., Kolodny, E. H., McFarland, V. W. & Mora, P. T. (1970) *Proc. Natl. Acad. Sci. USA* **67**, 757–764.
21. Fishman, P. H., Bradley, R. M. & Henneberry, R. C. (1976) *Arch. Biochem. Biophys.* **172**, 618–625.
22. Truding, R., Shelanski, M. L. & Morell, P. (1975) *J. Biol. Chem.* **250**, 9348–9354.
23. Cuatrecasas, P. (1973) *Biochemistry* **12**, 3547–3581.
24. Moss, J., Fishman, P. H., Manganiello, V. C., Vaughan, M. & Brady, R. O. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1034–1037.
25. Mullin, B. R., Pacuszka, T., Lee, G., Kohn, L. D., Brady, R. O. & Fishman, P. H. (1978) *Science* **199**, 77–79.
26. Hansson, C. G., Karlsson, K.-A. & Samuelsson, B. E. (1978) *J. Biochem. (Tokyo)* **83**, 813–819.
27. Roseman, S. (1970) *Chem. Phys. Lipids* **5**, 270–297.
28. Karlsson, K.-A. (1976) in *Structure of Biological Membranes*, eds. Abrahamsson, S. & Pascher, I. (Plenum, New York), pp. 245–274.
29. Shubert, D., Tarikas, H. & LaCorbiere, M. (1976) *Science* **192**, 471–474.
30. Stoolmiller, A. C., Dawson, G. & Kemp, S. F. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1226.