McDonald, T. J., Dupre, J., Tatemoto, K., Greenberg, G. R., Radziuk, J., & Mutt, V. (1985) *Diabetes 34*, 192-196.
Ottlecz, A., Snyder, G. D., & McCann, S. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 9861-9865.
Rökaeus, A. (1987) *Trends Neurosci. 10*, 158-164.
Servin, A. L., Amiranoff, B., Rouyer-Fessard, C., Tatemoto, K., & Laburthe, M. (1987) *Biochem. Biophys. Res. Commun. 144*, 298-306.
Silvestre, R. A., Miralles, P., Monge, L., Villanueva, M. L., & Marco, J. (1987) *Life Sci. 40*, 1829-1833.
Tatemoto, K., Rökaeus, A., Jörnvall, H., Mc Donald, T. J., & Mutt, V. (1983) *FEBS Lett. 164*, 124-128.
Voisin, T., Couvineau, A., Rouyer-Fessard, C., & Laburthe, M. (1991) *J. Biol. Chem. 266*, 10762-10767.

Fuc-GM1 Ganglioside Mimics the Receptor Function of GM1 for Cholera Toxin†

M. Masserini,*,‡ E. Freire,§ P. Palestini,‡ E. Calappi,‡ and G. Tettamanti‡

Department of Medical Chemistry and Biochemistry, University of Milano, Milano, Italy, and Biocalorimetry Center, Johns Hopkins University, Baltimore, Maryland 21218

Received May 24, 1991; Revised Manuscript Received December 5, 1991

ABSTRACT: The ability of Fuc-GM1 ganglioside to mimic the receptor function of GM1 for cholera toxin (CT) has been investigated. For this purpose, rat glioma C6 cultured cells were enriched with Fuc-GM1 and the responsiveness to CT was compared with that of cells enriched with GM1 ganglioside. Fuc-GM1 was taken up by cells as rapidly and to the same extent as GM1. When comparable amounts of ganglioside were associated, the cells enriched with Fuc-GM1 bound the same amount of ^{125}I-CT as did cells enriched with GM1. Under conditions in which GM1- and Fuc-GM1-enriched cells bound comparable amounts of CT, the Fuc-GM1-treated cells accumulated virtually the same amount of cyclic AMP as did GM1-treated cells, and activation of adenylate cyclase was also similar. The lag time preceding the CT-induced cAMP accumulation was the same in Fuc-GM1- and GM1-enriched cells. High-sensitivity isothermal titration calorimetry (ITC) experiments showed that the association constants of CT with Fuc-GM1 or GM1 ganglioside were comparable (4×10^7 M^{-1} and 1.9×10^7 M^{-1}, respectively, at 25 °C). Also, the association constants of the B-subunit pentamer with Fuc-GM1 or GM1 ganglioside were comparable (about 3×10^7 M^{-1} and 7×10^7 M^{-1}, respectively, at 25 °C).

Cholera toxin (CT)[1] is composed of two subunits, A and B, the latter being composed of five identical subunits arranged in a pentameric ring. When CT interacts with the host cells it binds through the B-subunit pentamer to its surface receptor. A lag period then follows during which the A subunit penetrates the plasma membrane and splits into peptides A1 and A2; the A1 fragment catalyzes the NAD^+-dependent ADP-ribosylation of the regulatory protein G, which irreversibly binds to, and activates, adenylate cyclase (Holmgren, 1981; Fishman, 1982). GM1 ganglioside has been indicated as the receptor for the toxin (Cuatrecasas, 1973). The ability of GM1 to be taken up by GM1-deficient cells and to sensitize the cells to cholera toxin has further confirmed the ability of GM1 to function as a toxin receptor [for a review about the topic, see Van Heyningen (1983)]. Although other naturally occurring gangliosides have shown the ability to bind CT with high specificity (Nakamura et al., 1987), the cellular responses following this interaction have so far been described only in the case of GM1 ganglioside. In recent years the presence of fucosylated gangliosides, including Fuc-GM1, in normal and transformed cells has been reported and is the focus of increased research (Hakomori, 1989; Chigorno et al., 1982; Ariga et al., 1987). With the present investigation we studied the ability of Fuc-GM1 to function as receptor for CT. The receptor function of Fuc-GM1 has been investigated by testing the ability to elicit cAMP accumulation after functional incorporation into the receptor-deficient rat glioma C6 cells (Fishman, 1980; Fishman et al., 1980) and subsequent exposure to CT, compared with the same cellular system enriched with GM1 ganglioside. The association of CT and its B-subunit pentamer with GM1 and Fuc-GM1 gangliosides has also been directly measured by high-sensitivity isothermal titration calorimetry (ITC), thus providing the overall energetics for the ganglioside–toxin interaction.

Materials and Methods

Reagents and Other Products. Commercial chemicals were of the purest quality available; solvents were distilled and water was doubly distilled in a glass apparatus. Silica gel thin-layer plates (HPTLC, Kieselgel 60) were from Merck (Darmstadt, FRG); solutions for C6 glioma cell culture and washing were from Flow Laboratories (Irwine, U.K.). *N*-Acetylneuraminic acid (NeuAc), bovine serum albumin, cholera toxin, and B-subunit pentamer were purchased from Sigma Chemical Co. (St. Louis, MO). For comparison, CT purified as described by Tomasi et al. (1979), with the phosphocellulose chromatography modification introduced by Mekalanos et al. (1979), was utilized for some experiments and was a kind gift of Dr. Maurizio Tomasi (National Institutes of Health, Rome, Italy).

† Supported by grants from the National Institutes of Health (NS-24520 and RR-04328) and from Consiglio Nazionale delle Ricerche (CNR, Rome, 90.01494.CT04).

* Corresponding author; present address Via Saldini 50, 20133 Milano, Italy.
‡ University of Milano.
§ Johns Hopkins University.

[1] Abbreviations: DMEM, Dulbecco's modification of Eagle's medium; PBS, Dulbecco's phosphate-buffered saline solution without calcium and magnesium; FCS, fetal calf serum; CT, cholera toxin; cAMP, adenosine 3′,5′-(cyclic)monophosphate; DEPC, dielaidoylphosphatidylcholine.

0006-2960/92/0431-2422$03.00/0

Dielaidoylphosphatidylcholine (DEPC) was from Avanti Polar Lipids (Birmingham, AL). ^{125}I-CT was prepared as described (Cuatrecasas, 1973).

Ganglioside Preparation. Gangliosides GM1 and GD1a were prepared from calf brain and Fuc-GM1 from pig brain, according to Tettamanti et al. (1973). Identification and structural analysis of GM1 and GD1a were assessed as described by Sonnino et al. (1984). Identification and structural analysis of Fuc-GM1 were assessed as in the original method of Ghidoni et al. (1976). Gangliosides were tritium-labeled at the sphingosine moiety following the method of Ghidoni et al., (1982). The specific radioactivity was 1.08 Ci/mmol and the radioactive purity was better than 99%. The purity of gangliosides was assessed by HPLC (Johnson et al., 1990) and by high-performance thin-layer chromatography (HPTLC), loading different ganglioside amounts and using four different solvent systems (Ghidoni et al., 1976): (A) propanol/water, 7:3 by volume; (B) chloroform/methanol/0.5 M NH_3, 70:35:3 by volume; (C) propanol/32% NH_3/water, 6:2:1 by volume; and (D) butanol/pyridine/water, 6:2:1 by volume. After Ehrlich spray staining and densitometric (or radiochromatographic) scanning of the plate as described by Chigorno et al. (1982), with the improvements introduced by Sonnino et al. (1986), the final purity of all gangliosides was found to be better than 99.5% with regard to the oligosaccharide portion. In particular, the GM1 content in the purified Fuc-GM1 and the Fuc-GM1 content in the purified GM1 were less than 0.2%.

Cell Culture Conditions. C6 cells were propagated as described by Henneberry et al. (1975) in Dulbecco's modification of Eagle's Medium (DMEM) supplemented with 5% fetal calf serum (FCS). Subcultures were made on 3-cm (diameter) culture dishes. Cells used for experiments were at 80–90% of confluency (200–300 μg of cell protein/dish; about 9×10^5 cells/dish). Harvesting, rupture, and homogenization of cells were carried out as described by Chigorno et al. (1986). Cell viability was assessed by the trypan blue absorption method (Phillips, 1973). The morphology of the cultured cells and the efficiency of the homogenization procedure were examined using a phase-contrast microscope.

Treatment of C6 Cells with GM1 or Fuc-GM1. Aliquots (2 mL) of ^{3}H-ganglioside-containing cell culture medium (without FCS; about 10^6 dpm/mL) were added to each dish, and the dishes were incubated at 37 °C for up to 8 h. At the end of incubation, the cells were washed three times with 2 mL (each time) of PBS buffer (0.05 M, pH 7.4) in order to remove unbound ganglioside and then maintained at 37 °C for 30 min with 2 mL of DMEM containing 5% FCS to remove the ganglioside molecules weakly adhering to the cells. The cell preparations obtained after FCS treatment contained the "serum-stable" fraction of associated ganglioside (Schwarzmann et al., 1983; Chigorno et al., 1985). At the end of the treatment, cells were harvested and pelleted by centrifugation (1000*g*, 10 min).

Assessment of the Amount of Ganglioside Associated with Cells. The associated radioactivity was counted after overnight treatment with 1 M NaOH of a sample of cells (1 mL/mg of cell protein). Gangliosides were extracted from cells, according to Tettamanti et al. (1973). Volatile radioactivity and lipid-bound radioactivity were determined as described by Trinchera et al. (1988). Individual gangliosides were separated by high-performance thin-layer chromatography.

Thin-Layer Chromatography of Gangliosides. Plates were developed at room temperature with the solvent system chloroform/methanol/0.2% aqueous $CaCl_2$, 50:42:11 by volume. Ganglioside spots were made visible by autoradiography and quantified by radiochromatoscanning of the plate and by densitometry after staining (Ghidoni et al., 1986).

Treatment of Cells with Cholera Toxin and Cyclic AMP Assay. Essentially, the procedure described by Masserini et al. (1990) was followed. An established amount of CT in 2 mL of DMEM (without FCS) was added to the cells. The DMEM solution contained bovine serum albumin (0.01%) and 3-isobutyl-1-methylxanthine (0.2 mM) as a phosphodiesterase inhibitor (Fishman et al., 1980). After an established period of incubation, cAMP was extracted and assayed as described (Masserini et al., 1990). Adenylate cyclase activity was assayed by measuring the amount of cAMP formed upon incubation of 20 μg (as protein) of cell homogenate for 10 min at 37 °C, under the experimental conditions described by Fishman and Atikkan (1980).

Binding of ^{125}I-CT. The cells containing the serum-stable form of associated ganglioside were incubated at 20 °C for 10 min with 20–50 pmol of ^{125}I-CT ($2–4 \times 10^4$ cpm/pmol) [in the presence of increasing amounts of unlabeled toxin (up to 1 μM)] in 2 mL of DMEM buffered with Hepes at pH 7.4 and containing 0.1% BSA. The medium was removed and cells were washed three times with 2 mL of ice-cold phosphate-buffered saline, scraped, and counted in a β-counter. L-Fucose for competition experiments of toxin binding to Fuc-GM1-enriched cells was used as described (Cuatrecases, 1973). The amount of bound CT was evaluated from the amount of unlabeled toxin producing 50% displacement of labeled toxin.

Preparation of Samples for Calorimetric Experiments. Unilamellar vesicles containing 2% (molar) Fuc-GM1 or GM1 were utilized. To this purpose, mixtures of DEPC and gangliosides in the established proportion were dried from chloroform/methanol solutions and lyophilized. After resuspension and vortexing in 10 mM phosphate buffer, pH 7.4, containing 50 mM KCl, the lipid dispersions were extruded through 1000-Å pore size filters (Nucleopore, Pleasanton, CA) using a N_2-operated extruder (Lipoprep, Ottawa, Canada). The vesicles were diluted to a final concentration of 32 μM ganglioside and were used to load the calorimetric cell. CT was dialyzed overnight against the same buffer, the protein content was assayed and the concentration adjusted to 35 μM, and this solution was used for injection into the ganglioside-containing cells.

Isothermal Titration Calorimetry (ITC). The ITC experiments were performed with an Omega (Microcal, Amherst, MA) calorimeter. The binding constants for the association of GM1 or Fuc-GM1 to CT or to B-subunit pentamer were estimated by measuring the concentration dependence of the heat of reaction of the toxin with vesicle-embedded gangliosides at 25.003 °C. Cholera toxin was injected in a stepwise fashion until complete binding saturation was reached. Analysis of the concentration dependence of the reaction heats were performed as described before (Schon & Freire, 1989) using software developed in our laboratories.

Other Assays. Protein content of solubilized cell pellets was determined according to Peterson (1977) with bovine serum albumin as the reference standard. Lipid-bound sialic acid was determined according to Svennerholm (1957) and phospholipid concentration according to Marinetti (1962).

Results

Association of Ganglioside with Cells. Cell viability, after incubation with GM1 or Fuc-GM1, was good for the duration of all experiments. C6 cells were incubated in the presence of 5 μM GM1 or Fuc-GM1 ganglioside for increasing times and then assayed for serum-stable associated ganglioside. The

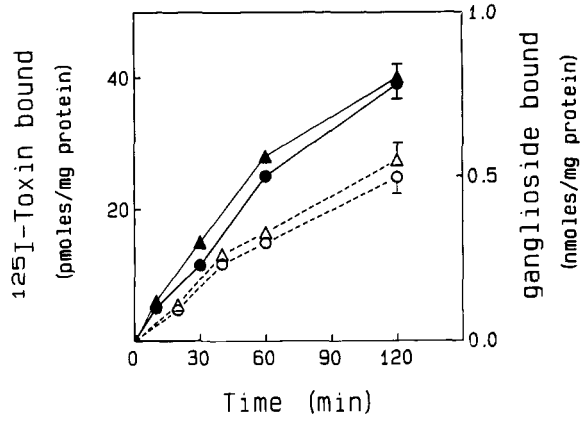

FIGURE 1: Time course of ^{3}H-GM1 and ^{3}H-Fuc-GM1 association (open symbols) with C6 glioma cells following incubation with 5 μM ganglioside, and amounts of ^{125}I-cholera toxin specifically bound (closed symbols) by cells previously incubated with ganglioside for the indicated time. Circles, Fuc-GM1; triangles, GM1.

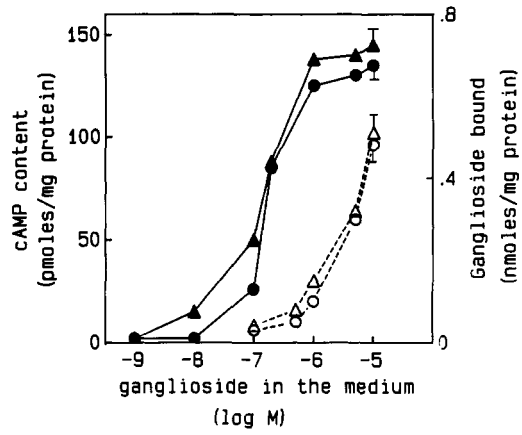

FIGURE 2: Effect of ganglioside concentration on ^{3}H-GM1 and ^{3}H-Fuc-GM1 association (open symbols) with C6 glioma cells and subsequent cholera toxin-induced cAMP accumulation (closed symbols) (10 μg of toxin/dish; 40-min incubation). Circles, Fuc-GM1; triangles, GM1.

time course of the association, up to 2 h of incubation, is reported in Figure 1. As shown, the rate of ganglioside association with cells was virtually the same for GM1 and Fuc-GM1 and led to the association of about 0.3 nmol of glycolipid/mg of protein after 1 h of incubation. This experiment was repeated and ^{125}I-CT binding was simultaneously measured. Over time, the amount of toxin bound increased at the same rate for both gangliosides (Figure 1). In another experiment, cells were incubated for 1 h with GM1 or Fuc-GM1 and washed with FCS. ^{125}I-CT binding was then measured using toxin which had been preincubated with 300 ng/mL Fuc-GM1 or GM1, respectively, at 20 °C for 30 min. In either case 75% inhibition of binding was found with respect to controls. Preincubation of ^{125}I-CT with free fucose (10 mg/mL), under identical experimental conditions, led to about 30% inhibition of binding to Fuc-GM1-enriched cells.

Analysis of Cell-Associated Radioactivity. At incubation times up to 1 h, almost all (99%) of the cell-bound radioactivity was recovered in the aqueous phase of the lipid extract. Less than 5% of the radioactivity present in the aqueous phase was found to be volatile and diffusible (possibly ^3H_2O). Radiochromatoscanning and autoradiography of the TLC of the aqueous phase showed the presence of a single radioactive peak having the same R_f as the tritiated ganglioside present in the incubation mixture.

Endogenous Ganglioside and cAMP Content of C6 Cells. The endogenous GM1 and Fuc-GM1 content of C6 cells is 2 and 0.6 pmol/10^6 cells, respectively. The average cAMP content of C6 cells grown in the presence of FCS and not treated with exogenous gangliosides was 2 pmol/mg of protein. This value did not change either by incubating the cells in the absence of FCS or in the presence of gangliosides.

C6 cells were incubated for 1 h with amounts of GM1 or Fuc-GM1 ranging from 10^{-9} to 10^{-5} M, washed with FCS, and then exposed to CT (10 μg/plate) for 40 min, and the cAMP content was assayed. The cells treated with Fuc-GM1 accumulated the same cAMP amount as GM1-treated cells (Figure 2). The amount of GM1 and Fuc-GM1 associated with cells was the same, suggesting that the two gangliosides have similar partitioning into the cells (Figure 2). This experiment was repeated and ^{125}I-CT binding was simultaneously measured. As shown in Figure 3, for a given amount of toxin bound, cAMP accumulation in GM1- or Fuc-GM1-treated cells was comparable.

The time course of cAMP accumulation in cells incubated for 1 h in the presence of 5 μM ganglioside, washed with FCS,

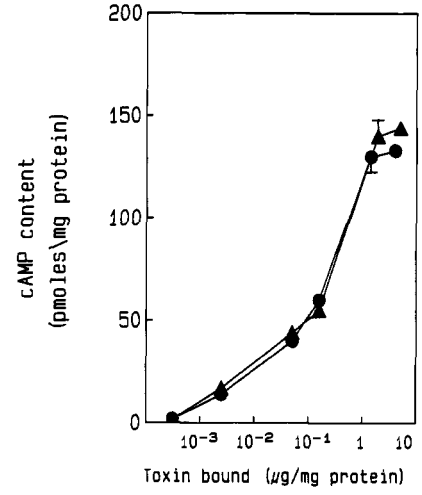

FIGURE 3: Cholera toxin bound and cAMP accumulation in C6 glioma cells previously incubated for 1 h with GM1 or Fuc-GM1 at different concentrations and then incubated in the presence of varying amounts of CT (up to 10 μg/dish; 40-min incubation). ●, Fuc-GM1; ▲, GM1.

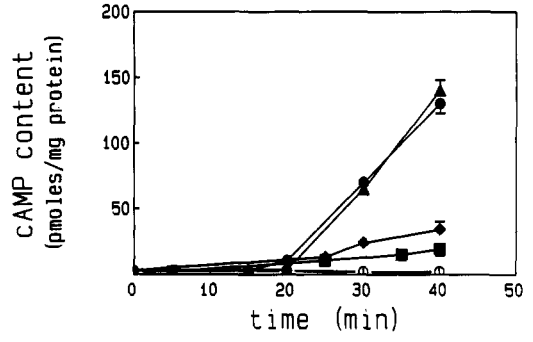

FIGURE 4: Time course of CT-induced cAMP accumulation in C6 glioma cells previously incubated for 1 h with 5 μM GM1 or Fuc-GM1 ganglioside and subsequently incubated with CT (10 μg/dish). ●, Fuc-GM1; ▲, GM1; ■, cells not incubated with exogenous ganglioside and incubated with CT; ○, cells incubated with exogenous ganglioside and not exposed to CT; ◆, cells incubated with GD1a ganglioside and exposed to CT.

and exposed to 10 μg of CT/dish is reported in Figure 4. No significant difference between cells treated with Fuc-GM1 or GM1 was found in the amount of cAMP accumulated after a given time nor in the duration of the lag time preceding a detectable accumulation of cyclic AMP. Control experiments

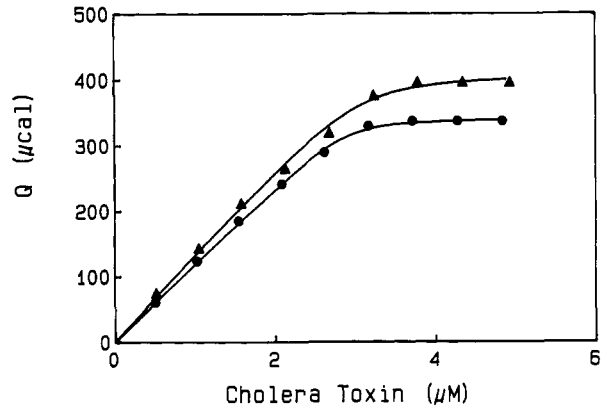

FIGURE 5: Calorimetric binding isotherm of CT to GM1 or Fuc-GM1 ganglioside. The cumulative heat, Q, is plotted versus the total concentration of CT. The solid lines are the theoretical curves calculated with the parameters reported in Table I. ●, Fuc-GM1; ▲, GM1.

were also carried out with cells enriched with comparable amounts of GD1a ganglioside and are also reported in the same figure.

Adenylate Cyclase Activity. Adenylate cyclase activity of cells containing the serum-stable form of associated GM1 or Fuc-GM1 (obtained upon incubation for 1 h in the presence of 5×10^{-6} M ganglioside) and incubated with 10 μg of CT/dish for 60 min was 50.3 or 43.2 pmol min^{-1} (mg of protein)$^{-1}$, respectively. Activity in control cells (not enriched with exogenous gangliosides) was 17.5 pmol min^{-1} (mg of protein)$^{-1}$.

ITC Studies of CT or B Subunit Binding to GM1 and Fuc-GM1 Gangliosides. A sequence of injections (10 μL each) of CT into vesicular dispersions containing GM1 or Fuc-GM1 gangliosides, at 8-min intervals, was performed. The association of CT with ganglioside-containing vesicles is an exothermic process decreasing with the number of successive injections as expected for a system exhibiting saturation. Under the present experimental conditions, is completed after 7 or 6 injections using GM1 or Fuc-GM1, respectively. The raw data were corrected for heats of dilution (obtained by injecting CT or B-subunit pentamer into buffer alone or into buffer containing DEPC vesicles without gangliosides).

Figure 5 shows the cumulative reaction heats as a function of the total concentration of CT, for both GM1 and Fuc-GM1. The experimental data were fitted by nonlinear least squares analysis in order to obtain the best values for the enthalpy of association and the association constant for GM1 and Fuc-GM1. The glycolipid molecules were assumed to equally partition into the outer and inner layers of the vesicles. The solid lines in the figure represent the theoretical curves calculated with the best set of fitted parameters. According to this analysis, the experimental data are consistent with a CT/ganglioside binding enthalpy of -97 ± 7 kcal/mol of GM1 and -86 ± 6 kcal/mol for Fuc-GM1. The association constants were very similar in both cases, 1.9×10^7 M^{-1} for GM1 and 4×10^7 M^{-1} for Fuc-GM1 (Table I). Additional experiments using the B-subunit pentamer also yielded similar association constants (7×10^7 M^{-1} and 3×10^7 M^{-1} for GM1 and Fuc-GM1, respectively) (Table I).

Control experiments were performed using vesicles containing GD1a ganglioside: under identical experimental conditions the amount of heat released was insignificant (<1 μcal/injection) compared to injection heats of the order of 100 μcal for GM1 or FucGM1. These experiments demonstrate that the heat effects observed upon addition of CT to vesicles containing GM1 or FucGM1 are due to the specific binding of the toxin to its receptor.

Table I: Thermodynamic Parameters for the Interaction of CT or CT B Subunit with GM1 or Fuc-GM1 Ganglioside at 25 °C

ganglioside	ligand	ΔH (kcal/mol)	ΔS [cal/(K·mol)]	association constant (M^{-1})
GM1	CT	-97 ± 7	-292 ± 10	1.9 ± 10^7
Fuc-GM1	CT	-86 ± 6	-254 ± 9	3.4×10^7
GM1	B subunit	-124 ± 8	-380 ± 15	7.3×10^7
FucGM1	B subunit	-117 ± 7	-358 ± 13	3.3×10^7

Discussion

The results obtained in the present investigation show that GM1 and Fuc-GM1 ganglioside are taken up by the receptor-deficient C6 cells essentially in the same fashion. The strong binding of GM1 with the [^{125}I]iodotoxin is also exhibited by Fuc-GM1 ganglioside. cAMP accumulation and adenylate cyclase activation were similar in cells treated with Fuc-GM1 or GM1 and exposed to CT. The lag period preceding CT-induced cAMP accumulation and the rate of cAMP accumulation in cells enriched with Fuc-GM1 or GM1 are also comparable. In order to measure the association constant of Fuc-GM1 to CT we chose ultrasensitive isothermal titration calorimetry since the validity of this technique to determine the energetics of the binding of CT to GM1 ganglioside or GM1 oligosaccharide has been demonstrated before, and binding constants determined calorimetrically are similar to those determined using radiolabeled toxin and other techniques (Schon & Freire, 1989; Straume & Freire, 1990; van Heyningen, 1983). The ITC data herein obtained with gangliosides inserted in membraneous model systems, that is, under ideal conditions, show that the affinity and the stoichiometry of the toxin or its B-subunit pentamer for Fuc-GM1 are comparable to those for GM1 ganglioside. All this body of results suggests that Fuc-GM1 completely mimics the ability of GM1 ganglioside in binding CT and in sensitizing cells to CT. In other words, Fuc-GM1 functions as receptor for cholera toxin.

It has been established that the binding of CT to GM1 involves the glycolipid oligosaccharide portion and particularly the sialic acid and the terminal galactose moieties. The present investigation shows that the presence of a fucose residue, α-glycosidically linked to the 2-position of the terminal galactose of GM1, still allows CT to exert its action and suggests that the fucosyl residue may be involved in the binding. Of course, this problem needs and deserves further investigation.

Fuc-GM1 has been characterized in several normal and tumor cells (Ohashi & Yamakawa, 1977; Macher et al., 1979; Suzuki et al., 1975; Holmes & Hakomori, 1982; Ghidoni et al., 1976). An investigation in order to critically evaluate its presence in intestinal mucosa could be appropriate, since at this level it could coparticipate in the undoubted receptor role of GM1 for CT in vivo. Moreover, CT and CT B-subunit pentamer have been employed as tools to investigate the functional implications of GM1, for instance, in the regulation of cell growth (Spiegel, 1988, 1989). The data obtained from the present investigation offer the possibility to investigate also the functional implications of Fuc-GM1 ganglioside. For instance, the change in fucosylation, commonly observed in transformed cells (Hakomori, 1989), and the observation that Fuc-GM1 could be present in cells sensitive to CT B-subunit pentamer (Pessina et al., 1989) suggest an interesting opportunity.

References

Ariga, T., Kobayashi, K., Kuroda, Y., Yu, R. K., Suzuki, M.,

Kitagawa, H., Inagaki, F., & Miyatake, T. (1987) *J. Biol. Chem. 262*, 14146–14153.
Chigorno, V., Sonnino, S., Ghidoni, R., & Tettamanti, G. (1982) *Neurochem. Int. 4*, 397–403.
Chigorno, V., Pitto, M., Cardace, G., Acquotti, D., Kirschner, G., Sonnino, S., Ghidoni, G., & Tettamanti, G. (1985) *Glycoconjugate J. 2*, 279–291.
Chigorno, V., Cardace, G., Pitto, M., Sonnino, S., Ghidoni, R., & Tettamanti, G. (1986) *Anal. Biochem. 153*, 283–294.
Cuatrecasas, P. (1973) *Biochemistry 12*, 3547–3558.
Fishman, P. H. (1980) *J. Membr. Biol. 54*, 61–72.
Fishman, P. H. (1982) *J. Membr. Biol. 69*, 85–97.
Fishman, P. H., & Atikkan, E. E. (1980) *J. Membr. Biol. 54*, 51–60.
Fishman, P. H., Pacusza, T., Hom, B., & Moss, J. (1980) *J. Biol. Chem. 255*, 7657–7664.
Ghidoni, R., Sonnino, S., Tettamanti, G., Wiegandt, H., & Zambotti, V. (1976) *J. Neurochem. 27*, 511–515.
Ghidoni, R., Trinchera, M., Venerando, B., Fiorilli, A., Sonnino, S., & Tettamanti, G. (1986) *Biochem. J. 237*, 147–155.
Hakomori, S.-I. (1989) in *Gangliosides and cancer* (Oettgen, H., Ed.) pp 57–68, VCH Press Inc., Weinheim, Germany, and New York.
Henneberry, R. C., Fishman, P. H., & Freese, E. (1975) *Cell 5*, 1–9.
Holmes, E. H., Hakomori, S.-I. (1982) *J. Biol. Chem. 257*, 7698–7703.
Holmgren, J. (1981) *Nature 292*, 413–417.
Johnson, S. W., Masserini, M., & Alhadeff, J. A. (1990) *Anal. Biochem. 189*, 209–212.
Macher, P. A., Pacuszka, T., Mullin, B. R., Sweeley, C. C., Brady, R. O., & Fishman, P. H. (1979) *Biochim. Biophys. Acta 588*, 35–43.
Marinetti, G. V. (1962) *J. Lipid Res. 3*, 1–20.
Masserini, M., Palestini, P., Pitto, M., Chigorno, V., Tomasi, M., & Tettamanti, G. (1990) *Biochem. J. 271*, 107–111.
Mekalanos, J. J., Collier, R. J., & Romig, W. R. (1979) *J. Biol. Chem. 254*, 5855–5861.
Nakamura, K., Suzuki, M., Inagaki, F., Yamakawa, T., & Suzuki, A. (1987) *J. Biochem. (Tokyo) 101*, 825–835.
Ohashi, M., & Yamakawa, T. (1977) *J. Biochem. (Tokyo) 81*, 1675–1690.
Pessina, A., Mineo, E., Masserini, M., Neri, M. G., & Cocuzza, C. E. (1989) *Biochim. Biophys. Acta 1013*, 206–211.
Peterson, G. L. (1977) *Anal. Biochem. 83*, 346–356.
Phillips, H. J. (1973) in *Tissue culture methods and applications* (Kruse, P. F., & Patterson, M. K., Eds.) pp 167–212, Academic Press, New York.
Schon, A., & Freire, E. (1989) *Biochemistry 28*, 5019–5024.
Schwarzmann, G., Hoffmann-Bleihauer, P., Shubert, J., Sandhoff, K., & Marsh, D. (1983) *Biochemistry 22*, 5041–5048.
Sonnino, S., Ghidoni, R., Gazzotti, G., Kirschner, G., Galli, G., Tettamanti, G. (1984) *J. Lipid Res. 258*, 620–629.
Sonnino, S., Acquotti, D., Riboni, L., Giuliani, A., Kirschner, G., & Tettamanti, G. (1986) *Chem. Phys. Lipids 42*, 3–26.
Spiegel, S. (1988) in *New trends in ganglioside research* (Ledeen, Hogan, & Tettamanti, Eds.) pp 405–421, Liviana Press, Padova, Italy.
Spiegel, S. (1989) in *Gangliosides and cancer* (Oettgen, H., Ed.) pp 19–29, VCH Press Inc., Weinheim, Germany, and New York.
Suzuki, A., Ishizuka, I., & Yamakawa, T. (1975) *J. Biochem. (Tokyo) 78*, 947–94.
Svennerholm, L. (1957) *Biochim. Biophys. Acta 13*, 856–858.
Tettamanti, G., Bonali, F., Marchesini, S., & Zambotti, V. (1973) *Biochim. Biophys. Acta 296*, 160–170.
Tomasi, M., Battistini, A., Araco, A., Roda, L. G., & D'Agnolo, G. (1979) *Eur. J. Biochem. 93*, 621–627.
Trinchera, M., Wiesmann, U., Pitto, M., Acquotti, D., & Ghidoni, R. (1988) *Biochem. J. 252*, 375–379.
Van Heyningen, S. (1983) in *Membrane receptors* (Kleinzeller, A., & Richard, B., Eds.) pp 445–471, Academic Press, New York.
Wiseman, T., Williston, S., Brandts, J. F., & Lin, L.-N. (1989) *Anal. Biochem. 179*, 131–137.