Purification and characterization of an endogenous inhibitor of the sialyltransferase CMP-*N*-acetylneuraminate:lactosylceramide α 2,6-*N*-acetylneuraminyltransferase (EC 2.4.99.-)

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The presence in the 100 000 *g* supernatant of rat brain homogenate of an inhibitor of the sialyltransferase has been confirmed. It is also present in chicken and bovine brain and in other rat and bovine organs. The inhibitor has been purified, a preparation with a specific activity 130-fold higher than that of the original 100 000 *g* supernatant of brain being obtained. It runs as a single peak in polyacrylamide-gel electrophoresis; when run in the presence of SDS, two components appeared. The apparent M_r of these components were 14 800 and 22 400. The inhibitor has been characterized as a heat-stable protein of acidic nature. It has effect on the glycolipid and the glycoprotein sialyltransferase activities but has no effect on the galactosaminyltransferase activity.

INTRODUCTION

Information about the mechanisms that regulate the synthesis of the carbohydrate chain of gangliosides during development is rather scanty. There are reports that the rate of incorporation of labelled precursors changes with the age of the animals, in some cases even abruptly, as in the brain of the rat, which at 25–30 days of age has an incorporation of labelled precursors about one half of that at 15 days of age (Burton *et al.*, 1963; Rubiolo de Maccioni & Caputto, 1968). Another change worthy of consideration is that in the pattern of gangliosides which occurs when the animals pass from the embryonic to the adult state (Vanier *et al.*, 1971).

One of the possible mechanisms by which regulation can occur is the activity of inhibitors of the glycosyltransferases that catalyse each step of the synthesis of the glycosidic chain. The following inhibitors have been reported: an inhibitor of the sialyltransferase (Duffard & Caputto, 1972), one of glucosyl- and galactosyltransferase (Constantino-Ceccarini & Suzuki, 1978) and another of galactosaminyltransferase (Quiroga *et al.*, 1984). The possibility that these inhibitors play a role in the regulation of the ganglioside or glycoprotein synthesis elicits an interest in their study, but as yet there is no direct proof that they do play a role *in vivo*. In the present study we have purified, and have determined some of the properties of, the sialyltransferase inhibitor.

MATERIALS AND METHODS

Materials

Lactosylceramide, asialofetuin (Type I), CMP-*N*-acetylneuraminate, UDP-*N*-acetylglactosamine, bovine serum albumin, Sephadex G-25 (medium grade), Sephadex G-50 (medium grade), DEAE-Sephadex, chymotryp-

sin (Type II), trypsin (Type II), M_r standards (10 000–70 000), amino acid phenylthiohydantoin derivatives (standards) and Coomassie Brilliant Blue R were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). CMP- $[^3\text{H}]N$ -acetylneuraminate (18.9 Ci/mmol), UDP- $[^3\text{H}]N$ -acetylglactosamine (11 Ci/mmol) from New England Nuclear (Boston, MA, U.S.A.). G_{M3} ($\text{II}^3\text{NeuAc-LacCer}$) was generously given by Lc. Santiago Quiroga of the Universidad Nacional de Córdoba, Córdoba, Argentina. All other reagents were of the highest grade commercially available.

Sialyltransferase preparation

The brain (cerebellum excluded) from a 15-day-old albino rat was homogenized in 10 vol. of cold water with the use of a glass homogenizer with Teflon pestle and the homogenate was centrifuged at 1000 *g* for 10 min. A 6 ml portion of the supernatant fraction was then centrifuged at 100 000 *g* for 1 h. The pellet from the last centrifugation was resuspended in 3.6 ml of 140 mM-cacodylate/HCl buffer, pH 6.8. All the operations were carried out at 4 °C. The preparation contained approx. 200 μg of protein in 20 μl . The enzyme was prepared on the day of use.

Determination of the sialyltransferase

The sialyltransferase (CMP-*N*-acetylneuraminate:lactosylceramide α 2,6-*N*-acetylneuraminyltransferase; EC 2.4.99.-) was determined essentially as described by Landa *et al.* (1978). The incubation mixture contained, in a volume of 50 μl , 27 μM -CMP- $[^3\text{H}]N$ -acetylneuraminate (60 000 c.p.m./nmol), 200 μM -lactosylceramide, 15 mM-MnCl₂, 140 mM-cacodylate/HCl buffer, pH 6.8, 100 μg of Triton CF-54/Tween 80 (2:1, w/v) and 20 μl of enzyme preparation (approx. 200 μg of protein). For testing the inhibitor, a measured amount of inhibitor

Abbreviation used: G_{M3} , ganglioside G_{M3} ($\text{II}^3\text{NeuAc-LacCer}$) (nomenclature of Svennerholm, 1963).

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solution was added initially to the tube and dried in a desiccator under vacuum; the rest of the components of the system were then added and shaken vigorously. After 30 min of incubation at 37 °C, the reaction was stopped by adding 2 ml of 5% (w/v) trichloroacetic acid containing 0.5% (w/v) phosphotungstic acid. The precipitate was centrifuged down and, after elimination of the supernatant, the pellet was extracted with chloroform/methanol (2:1, v/v) in quantity enough to obtain a single liquid phase. The mixture was centrifuged at 1000 *g* for 30 min, and the lipid extract was transferred to a vial, dried, and then resuspended in toluene containing 2% (w/v) PPO (2,5-diphenyloxazole) in order to measure the radioactivity.

Transference of sialyl groups from CMP-*N*-acetylneuraminic acid to asialofetuin

In this study the enzyme activity was measured as described by Ng & Dain (1977). The tubes containing the inhibitor were treated as for sialyltransferase above, that is, the inhibitor was dried in the reaction tube and then resuspended in the incubation mixture.

Amino acid analysis

A sample of 70–100 µg of inhibitor protein was hydrolysed in 6 M-HCl containing 2% (v/v) phenol and 1% (v/v) 2-mercaptoethanol in an ampule sealed under N₂. The hydrolysis was carried out at 110 °C for 24 h. After hydrolysis the resulting mixture was dried under vacuum at 65 °C for 1 h. The amino acid phenylthiohydantoin derivatives were prepared by the method of Tarr (1977) and quantified by reverse-phase h.p.l.c. as described by Gates *et al.* (1979) on a column (4.6 mm × 250 mm) of Ultrasphere ODS (particle size 5 µm). The phenylthiohydantoin derivative of norleucine was used as an internal standard.

Other determinations

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Electrophoresis were carried out on a 12% (w/v) polyacrylamide gel without (Davis, 1964) or with SDS (Laemmli, 1970). The gels were stained for protein with Coomassie Brilliant Blue R (Weber & Osborn, 1969) and for carbohydrate by the periodic acid/Schiff method as outlined by Fairbanks *et al.* (1971). Galactosaminyltransferase activity was determined as described by Quiroga *et al.* (1984). Phosphorus was determined by the method of Bartlett (1959), as modified by Dodge & Phillips (1967). Pentoses were assayed by the orcinol reaction (Ashwell, 1957).

RESULTS

Inhibition of the sialyltransferase by extracts from different tissues

When the present study was commenced, there were two uncertainties that required clarification. One was whether we were dealing with non-specific inhibition by proteins or other components that were known to modify the level of activity according to the protein/detergent ratio present during transferase determination (Mårtensson *et al.*, 1974). The other point was whether brain was the best source of inhibitor. Table 1 shows that the 100000 *g* supernatant of rat and bovine brain had a higher inhibitory capacity than the 100000 *g* supernatant

Table 1. Distribution of the inhibitor of sialyltransferase in different animal tissues

The inhibitory preparation used in each case was the supernatant obtained after centrifuging, for 45 min at 100000 *g*, a cold-water homogenate of 30 mg of tissue. Values are the mean for two experiments each run in duplicate; in every case the difference between duplicates were less than 8% of the mean. Values are expressed as percentage inhibition of sialyltransferase activity, compared with the control activity determined with the same enzyme source but without the inhibitory preparation.

Enzyme source	Inhibitory preparation	Inhibition of sialyltransferase (%)
Rat* brain	Rat‡ brain	68
	Rat‡ liver	44
	Rat‡ kidney	31
	Chicken§ brain	59
	Bovine brain	45
	Bovine liver	13
	Bovine kidney	16
	Bovine spleen	16
Chicken† brain	Chicken§ brain	51
	Rat‡ brain	42

* 15 days old.

† 20 days old.

‡ 3 months old.

§ 20 days old.

Fig. 1. Effect of heating the inhibitor at different temperatures on its inhibitory capability

The inhibitor was a supernatant obtained after centrifuging, for 45 min at 100000 *g*, a cold-water homogenate of 3-month-old-rat brain. Non-heated (●), heated for 5 min at 70 °C (▲) or heated for 5 min at 100 °C (■) inhibitor preparation was used. The numbers on the abscissa indicate amounts (ml) of inhibitor preparation that were dried under vacuum in each tube before the activity assay (see the text). The data plotted are the means of duplicate determinations and are representative of experiments repeated five or more times.

Table 2. Purification of the inhibitor from rat brain

The data in this Table are from one representative experiment, which was repeated with similar results at least four times.

Fraction*	Protein (mg)	Activity (units)†	Specific activity (units/mg)
100 000 <i>g</i> Supernatant	313	250	0.8
Heated preparation	80	193	2.4
Sephadex G-50 peak I	4.2	46.5	11
DEAE-Sephadex			
Peak A	0.44	8.4	19
Peak B	0.09	10	111

* The original material was a homogenate of brain of 3-month-old rats.
† One unit of inhibitory activity was defined as the amount of inhibitor required to produce 50% inhibition in the standard test (see the text).

of liver and kidney obtained from the same amount of tissue of the respective species. These results answered both questions, since apparently both rat and bovine brains contain some specific factor that determines the inhibitory capacity and are the richest sources of this factor.

Heat-stability

Duffard & Caputto (1972) established the heat-stability of the sialyltransferase inhibitor. This was confirmed in the present work. Fig. 1 shows that heating the

100 000 *g* supernatant from rat brain for 5 min at 70 °C and 100 °C did not affect the inhibitory capability of the preparation. It is noteworthy that, with this inhibitor, we could not inhibit the enzymic activity by more than 65%.

Purification of the inhibitor

(a) Preparation of a stable powder. The inhibitor was obtained from the brain (cerebellum excluded) of albino 2–3-month-old rats homogenized in 5 vol. (w/v) of cold water. After 20–30 min, the homogenate was centrifuged at 100 000 *g* for 45 min at 4 °C, the supernatant was heated at 100 °C for 5 min and the precipitate formed during the heating process was eliminated by centrifugation at 100 000 *g* for 30 min. The supernatant of this centrifugation was freeze-dried and stored (heated preparation). The purification achieved by this process was around 3-fold with respect to the 100 000 *g* brain supernatant (Table 2). This preparation was kept at –15 °C, under which conditions it was found to be unaltered in its inhibitory capacity for at least 6 months.

(b) Purification on a column of Sephadex G-50. A 300 mg portion of the powder obtained as described above was suspended in 10 ml of water and centrifuged to eliminate visible particles. The clear supernatant was passed through a column (2.2 cm × 75 cm) of Sephadex G-50 equilibrated with water and eluted with water at a rate of 12 ml/h. The elution profile was monitored by A_{280} and by the inhibitory effect on the sialyltransferase, as shown in Fig. 2. The A_{280} shows two peaks; one was eluted at V_0 and the other at V_t . The first was probably a proteinaceous material with low specific activity as an inhibitor of the sialyltransferase, whereas the peak eluted at V_t is probably a mixture of nucleotides, as is indicated by the absorption spectrum (insert to Fig. 2) and by the presence of organic phosphorus and pentoses (results not

Fig. 2. Elution profile from a Sephadex G-50 column of the sialyltransferase inhibitor

A heated preparation of inhibitor (300 mg dissolved in 10 ml of water) was applied on to a column of Sephadex G-50 (2.2 cm × 75 cm) and eluted with water; 5 ml fractions were collected. ●, Inhibitory capacity; ▲, protein determined by A_{280} . The inset shows the absorption spectrum of peak II. The data plotted are representative of experiments repeated three or more times.

Fig. 3. Ion-exchange chromatography of the sialyltransferase inhibitor

Peak I (6 mg) eluted from the Sephadex G-50 column (Fig. 2) was applied to a column of DEAE-Sephadex (0.8 cm $\times$ 4.5 cm) equilibrated with 0.03 M-Tris/HCl buffer, pH 8. The elution was carried out with a continuous gradient (----) of 0.05 to 1 M-NaCl in 0.03 M-Tris/HCl buffer, pH 8; 1 ml fractions were collected, and, after being desalted, they were assayed for inhibitory capacity (●). A_{280} (▲) was recorded. The data plotted are representative of experiments repeated three or more times.

Fig. 4. Electrophoresis of the inhibitor

Lane 1, material eluted from a column of Sephadex G-50, peak I. The amount of protein loaded was 60 μ g. Lane 2, material eluted from a column of DEAE-Sephadex, peak A. The amount of protein loaded was 80 μ g. Lane 3, material eluted from a column of DEAE-Sephadex, peak B. The amount of protein loaded was 17 μ g. Electrophoresis was in a 12% (w/v) polyacrylamide gels in Tris/glycine buffer, pH 8.3, which were stained with Coomassie Brilliant Blue R.

shown). This peak shows considerable inhibition towards sialyltransferase, but we did not pursue its study because nucleotides often show glycosyltransferase-inhibitory activity (Eppler *et al.*, 1980; Bernacki, 1975). The peak of main interest is peak I, which emerged from the column with fractions 58–70. The apparent purification at this step is 13.7-fold (Table 2), but it is clear from the elution profile that the calculation on purification of the inhibitory capacity of this single peak was complicated because of the presence of another inhibitor and,

Fig. 5. Apparent M_r of the purified inhibitor

Determinations were performed on 12% (w/v) polyacrylamide gels with SDS. Protein markers (open circles) were: bovine serum albumin (M_r 66000); egg albumin (M_r 45000); porcine stomach-mucosa pepsin (M_r 34700); bovine pancreas trypsinogen (M_r 24000); bovine milk β -lactoglobulin (M_r 18400); egg lysozyme (M_r 14300). Components of the purified inhibitor preparation are shown as closed circles.

furthermore, because peak III (Fig. 2) corresponds to a previously undetected activator that is not the subject of the present paper.

(c) **Purification on a column of DEAE-Sephadex.** A column (0.8 cm $\times$ 4.5 cm) of DEAE-Sephadex was prepared and equilibrated with 0.03 M-Tris/HCl buffer, pH 8. A 6 mg portion of the material eluted from peak I (Fig. 2) was applied to the DEAE-Sephadex column and eluted with a continuous gradient of NaCl between 0.05 and 1 M in 0.03 M-Tris/HCl buffer, pH 8. The flow rate was 20 ml/h, and fractions of 1 ml each were collected. Determination of the inhibitory capacity in each fraction after desalting in columns of Sephadex G-25 showed that this activity was eluted as two peaks from the DEAE-Sephadex column (peaks A and B in Fig. 3). Peak B (purified preparation), which was eluted between 0.65 and 0.80 M-NaCl, had a specific activity, when related to protein, 5.8-fold higher than that of peak A and was consequently subjected to further studies. Its specific activity was 139-fold higher than that of the original 100 000 g brain supernatant (Table 2).

Electrophoretic analysis

A 17 μ g portion of material from peak B (Fig. 3) was subjected to electrophoresis in 12% (w/v) polyacrylamide gel without SDS. Stained with Coomassie

Table 3. Effect of chymotrypsin and trypsin on inhibitor activity

The inhibitor preparations were incubated with either chymotrypsin or trypsin (200 μ g of proteolytic enzyme/ml of 140 mM-cacodylate/HCl buffer, pH 6.8, for 14 h at 37 °C. The ratio of proteolytic enzyme to inhibitor was 1:10). At the end of the incubation period the mixture was inactivated at 100 °C for 5 min. The activity of the inhibitor was determined as described in the text. An uninhibited control tube to which heated proteolytic enzyme was added was considered as 100% activity. Values are the means for two experiments, each run in duplicate; in every case the differences between duplicates were less than 10% of the mean.

Inhibitor preparation	Inhibition of sialyltransferase activity (%)		
	Non-treated inhibitor	Inhibitor treated with:	
		Chymo-trypsin	Trypsin
Heated preparation	65.2	—	34.6
Sephadex G-50			
Peak I	53	19.2	25.1
Peak II	50.5	—	44.6
DEAE-Sephadex, peak B	52	20.3	24

Blue, the gel showed a single component (Fig. 4). Attempts were made to stain the gel with periodic acid/Schiff reagent, but the results were negative. When the same preparation was run on a similar gel but containing SDS, two components appeared that stained with similar intensity upon treatment with Coomassie Blue. As shown in Fig. 5, when these spots were run together with standard proteins of known M_r , they migrated to positions corresponding to M_r values of 14800 and 22400.

Effect of proteolytic enzymes on the inhibitor

Samples of inhibitor preparation in different states of purification showed a decrease in their inhibitor capacities when they were incubated with chymotrypsin and trypsin (Table 3). Peak II obtained from the column of Sephadex G-50 was not affected by incubation with trypsin, and this observation, together with the absorption spectrum (inset to Fig. 2) supports the hypothesis that this inhibitor is not a protein but possibly a mixture of nucleotides.

Amino acid composition

The average of two determinations of the amino acid composition of the purified preparation of inhibitor is shown in Table 4. There is a prevalence of the non-polar amino acids glycine, alanine, valine and leucine, followed by the acidic amino acids aspartic and glutamic acids. The percentage of basic amino acids is low: arginine and histidine were found, but lysine could not be detected. The hydroxylated amino acids threonine, serine and tyrosine account for 14% of the total amino acids analysed.

Table 4. Amino acid composition of the sialyltransferase inhibitor

The period of hydrolysis was 24 h under the conditions stated in the text. Results are the means for duplicate hydrolyses and determinations. The values were calculated on the basis of analysed amino acid residues. Tryptophan, cysteine and methionine were not determined.

Amino acids	Composition (mol/100 mol)
Asparagine/aspartic acid	13.1
Threonine	5.5
Serine	4.8
Glutamine/glutamic acid	11.2
Proline	1.8
Glycine	15.1
Alanine	14.7
Valine	14.9
Isoleucine	2.7
Leucine	7.6
Tyrosine	4.0
Phenylalanine	2.2
Histidine	0.3
Lysine	0.0
Arginine	1.5

The acid character of this preparation accords with the observation that the inhibitory activity was retained in the DEAE-Sephadex column (Fig. 3) and required 0.72 M-NaCl to be eluted. Also, in the gel-electrophoretic run, the bulk of the protein, presumably the inhibitor, showed an acidic behaviour. This amino acid analysis, however, should be considered tentative until it can be shown whether the two components obtained by electrophoresis in the presence of SDS are both parts of the inhibitor.

Kinetics of the transference in the presence of the inhibitor

Fig. 6(a) shows the kinetics of the transference in the presence of the purified preparation of inhibitor and variable amounts of CMP-[3 H]N-acetylneuraminate; the double-reciprocal plot shows that the apparent K_m in the absence and presence of the inhibitor was of the same value (0.150 mM), whereas the V_{max} values in the absence and presence of inhibitor were 0.59 and 0.34 nmol/h per mg of protein respectively, indicating that there is no competition between CMP-N-acetylneuraminate and the inhibitor for the enzyme.

Results of the experiments in the presence of purified inhibitor preparation and variable amounts of acceptor substrate are shown in Fig. 6(b). A double-reciprocal plot of these data produced curved lines indicative of a sigmoidal activity profile. The V_{max} values calculated from the three points of higher lactosylceramide concentrations were 17.5 and 11.4 nmol/h per mg of protein in the absence and presence of the inhibitor respectively. A replot of the data according to the Hill equation was performed, using the least-squares method, yielding straight lines (regression coefficient of 0.996 in both cases) with values for the Hill coefficient (h) of 1.17 and 1.13 and values for K of 2.63 and 2.88 mM in the absence and presence of the inhibitor respectively. This suggests

Fig. 6. Kinetics of sialyltransferase activity in the absence and presence of purified inhibitor

(a) Double-reciprocal plot as a function of the CMP-[^3H]N-acetylneuraminate concentration. Lactosylceramide concentration was 2 mM in each determination. ○, Without inhibitor; ●, 20 μg of purified preparation of inhibitor. The data plotted are the means of duplicate determinations and are representative of experiments repeated twice or more. (b) The data are plotted as velocity (v) versus lactosylceramide concentration ($[S]$) determined in the presence of 150 μM -CMP-[^3H]N-acetylneuraminate: ○, without inhibitor; ●, 20 μg of purified inhibitor. The inset shows the curves at low concentration of lactosylceramide. The data plotted are the means of duplicate determinations, which varied by less than 10% of the mean.

Table 5. Effect of the inhibitor on glycosyltransferase activity

The enzyme preparation was a pellet obtained after centrifuging, for 1 h at 100 000 g , a cold-water homogenate of 15-day-old-rat brain. Values are expressed as percentage of inhibition of glycosyltransferase activity compared with the control activity without inhibitory preparation. Results are the means for two experiments run in duplicate; in every case the difference between duplicates were less than 10% of the mean, except for the determinations of UDP- N -acetylglucosamine:G $_{M3}$ transferase. Abbreviations used: AF, asialofetuin; LC, lactosylceramide; IP, inhibitor preparation; HIP, heated inhibitor preparation; PIP, purified inhibitor preparation; NeuAc, N -acetylneuraminate; GalNAc, N -acetylgalactosamine.

Incubation system donor (concn. in μM)	Acceptor (concn. in μM)	IP (μg)	Inhibition (%)
CMP-[^3H]NeuAc (130)	AF (50)	HIP (270)	30
	AF (200)	HIP (270)	32
CMP-[^3H]NeuAc (27)	LC (200)	HIP (270)	49
	LC (200)	PIP (11)	45
CMP-[^3H]NeuAc (130)	AF (200)	PIP (11)	29
UDP-[^3H]GalNAc (5)	GM $_3$ (250)	PIP (11)	14
UDP-[^3H]GalNAc (40)	GM $_3$ (250)	PIP (11)	-1

that there is no competition between lactosylceramide and the inhibitor for the enzyme.

Specificity of the inhibitor

(a) **Activity in a system with asialofetuin.** Table 5 shows the results of testing the inhibitory capacity of a heated preparation from rat brain and the purified preparation when used in a system designed to transfer sialyl groups from CMP- N -acetylneuraminate to an asialofetuin preparation. The inhibitory capacity was observed with both preparations, and since the inhibitory activity produced the same relative effect with the heated and the

purified preparation on both substrates, it was tentatively inferred that both inhibitors are one and the same, since their relationship was not changed by 46-fold purification.

Breen & Regan (1986) have reported an endogenous inhibitor from rat brain which regulates the activity of sialyltransferase in the glycosylation of cellular surface proteins.

(b) **Effect on the galactosaminyltransferase activity.** The purified preparation had no inhibitory effect on the transference of galactosaminyl groups from UDP- N -acetylgalactosamine to G $_{M3}$. The same amount of purified

preparation had an inhibitory capability of 45 % towards sialyltransferase activity under standard conditions (Table 5).

DISCUSSION

The presence in the 100000 g supernatant fraction of rat brain of an inhibitor of the sialyltransferase was confirmed. The inhibitor has been purified and characterized as a protein. There is preliminary evidence that this inhibitor is also present in chicken and bovine brain and in other rat and bovine organs. In rat brain it has been shown by preincubation experiments that the inhibitor is not an enzyme or any other factor that may destroy either, or both, of the substrates (Duffard & Caputto, 1972). No experiment of this type was performed for the inhibitors of other sources.

The inhibitor considered in the present paper was taken from a single peak that was eluted from a DEAE-Sephadex column at a concentration of NaCl between 0.65 and 0.80 M; it ran as a single peak in polyacrylamide-gel electrophoresis without SDS. No indications of other proteins could be seen in the electrophoretogram. However, when run in the presence of SDS, two components appeared that were stained with similar intensity by Coomassie Blue. The apparent M_r of these components were 14800 and 22400. Whether these components are parts of the inhibitor or not has yet to be determined.

The proteinaceous nature of the inhibitor is indicated by its susceptibility to the action of the proteolytic enzymes chymotrypsin and trypsin and by the presence of amino acids in the purified preparation. The acidic nature of the inhibitor was shown by its behaviour on the DEAE-Sephadex column and in polyacrylamide-gel electrophoresis without SDS; this is in agreement with the prevalence of glutamic acid and aspartic acid over arginine and histidine found in the electrophoretically homogeneous preparation.

It has been shown that, during development, the regulation of the synthesis of gangliosides in brain produces changes in their pattern due to the prevalence of certain pathways over others (Suzuki, 1965; Rubiolo de Maccioni & Caputto, 1968; Vanier *et al.*, 1971; Yusuf *et al.*, 1977; Irwin *et al.*, 1980). The situation is certainly very complex, owing to the cellular diversity of the brain, and it will be necessary to know more about the pattern of gangliosides in each type of cells and to correlate this distribution with that of the different inhibitors known before reaching a definite conclusion. It is reasonable, however, to assume that the sialyltransferase inhibitor

plays a role in the regulation of the synthesis of gangliosides and sialoglycoproteins *in vivo*.

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