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Postnatal changes in dolichol-pathway enzyme activities in cerebral cortex neurons

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Neuronal perikarya were isolated from rat cerebral cortex at different stages of postnatal development. Membranes sedimenting at 100000 **g** were obtained from these neurons to study several glycosyltransferases of the dolichol pathway. Enzyme activities from stages before and during synapse formation were compared (days 5 and 15 respectively). Dolichyl diphosphate (Dol-*P*-*P*) *N*-acetylglucosamine, dolichyl phosphate mannose and dolichyl phosphate glucose synthases and the enzymes catalysing Dol-*P*-*P*-$GlcNAc_2Man_9Glc_3$ formation were higher at day 15 of postnatal development. The glycosyl transfer of the latter compound to endogenous protein(s) as well as to a dinitrophenyl-heptapeptide was also measured. The activity was higher at day 15. Furthermore, the activity of dolichyl phosphate mannose synthase was also measured during the time when the number of synapses ceased to increase (day 36) and in the adult stage. The activity of dolichyl phosphate mannose synthase was higher at day 36 than at day 15, and declined in the adult stage. From these results it may be concluded that there is an increase in the glycosylation of asparagine-type glycoproteins during synapse formation in the neurons of the cerebral cortex.

A large proportion of membrane-bound and secretory molecules are glycoproteins. In the mammalian brain about 85–90% of these glycoproteins are of the asparagine type (Margolis & Margolis, 1979). Structural studies have revealed that proteins with *N*-glycosidically linked oligosaccharides are present in microsomal and synaptosomal brain fractions (Krusius *et al.*, 1978).

Glycosylation of asparagine-type glycoproteins takes place via sugar-lipid intermediates. The sugars are added step by step to a dolichol derivative. The resulting oligosaccharide is then transferred 'en bloc' to the asparagine residue of a protein (Parodi *et al.*, 1972; Behrens *et al.*, 1973). The oligosaccharide contains nine mannose residues, two *N*-acetylglucosamine residues and one to three glucose residues. Processing of the carbohydrate moiety subsequently takes place by removal of glucose followed by several other changes that lead to the formation of the various asparagine-linked oligosaccharides (Parodi & Leloir, 1979).

Other work focused attention on the biosynthetic mechanism of asparagine-type glycoproteins in the central nervous system. Behrens *et al.* (1971*b*) found evidence for the formation of dolichol-linked monosaccharides in brain tissues. Further biochemical characterization of some sugar-lipid intermediates and asparagine-type glycopeptides was more recently carried out by Waechter & Sher (1979).

Variations in the activity of Dol-*P*-Glc synthase during the prenatal development of chicken brain were reported by Breckenridge & Wolfe (1973).

Whole brain was used in the above-mentioned studies. This implied the presence of complex anatomical structures. Moreover, the preparations obtained contain several cell types, i.e. neurons, glial cells, blood cells and capillaries. These difficulties are increased by the myriad of cellular events occurring in the different brain structures at a given developmental stage.

In mammals, the cerebral cortex develops during the first few weeks after birth. During this period, the cells have reached their final position in the cortex and have ceased to divide. It is during this stage that we can place the most important event of neuronal differentiation, synaptogenesis.

Synapse formation in the rat cerebral cortex has been described in the work of Aghajanian & Bloom (1967) and takes place during the first month of

Abbreviations used: Dol-*P*-*P*-Glc oligosaccharide, dolichyl diphosphate glucose$_{1-3}$-mannose$_9$-*N*-acetylglucosamine$_2$; Dol-*P*, dolichyl phosphate.

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postnatal development. A preparation enriched in neuronal perikarya can be easily obtained by the procedure of Sellinger *et al.* (1971). Therefore it is possible to study problems that arise in glycoprotein synthesis in one developing cell type, the neuron, and one important cellular event, synapse formation.

In the present paper we describe variations in the activities of five enzymes involved in the dolichol pathway of the neuronal perikarya during synaptogenesis.

Materials and methods

Chemicals

The specific activities of the sugar nucleotides used were 227 Ci/mol, 268 Ci/mol and 300 Ci/mol for GDP-[^{14}C]Man, UDP-[^{14}C]Glc and UDP-[^{14}C]-GlcNAc respectively. The heptapeptide composition was dinitrophenyl-Ala-Leu-Glu-Asp-Ala-Thr-Arg and synthesized by the method of Ronin *et al.* (1978). It was a gift from Dr. Clara Pena, Department of Biological Chemistry, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires.

Membrane preparation

The procedure to obtain neuronal perikaryal membranes from the cerebral cortices of Wistar albino rats was essentially that described by Sellinger *et al.* (1971). Cerebral cortices were minced and pooled for suspension on a solution containing 7.5% poly(vinylpyrrolidin-2-one), 12 mM-$CaCl_2$ and 1% bovine serum albumin. The suspension was layered on top of a discontinuous 1.0 M-/1.75 M-sucrose gradient. Centrifugation was carried out for 30 min at 50000*g* on an SW 25.2 Spinco rotor. All the procedures were carried out at 4°C. The neuronal pellet was suspended in 0.25 M-sucrose and homogenized on a Dounce-type homogenizer with a tight-fitted pestle. The homogenate was centrifuged at 7000*g* on the SS 34 Sorval rotor for 10 min. The supernatant fluid was stored on ice and the procedure was repeated once with the pellet. Both supernatant fluids were then pooled and centrifuged for 60 min at 100000*g* on a R-65 Spinco rotor. The membranes obtained in the pellet were stored at −70°C to be used as enzyme source after resuspension in 0.25 M-sucrose. All the measurements of the glycosyltransferase activity were made using this preparation as enzyme source. There was no apparent significant loss of glycosyltransferase activity for up to 1 month of storage at−70°C.

Assays for the synthesis of dolichol-linked saccharides

In the incubation mixture 0.5 μmol of Mg^{2+}–EDTA and 0.5 μmol of $MgCl_2$ were added first, with or without addition of 5 μg of Dol-*P*. The tubes were dried under reduced pressure and the following components were added in a final volume of 50 μl: 50000–100000 c.p.m. of the corresponding ^{14}C-labelled sugar nucleotides, 0.1 M-Tris/maleate buffer (pH 7.8), 0.1 M-mercaptoethanol, 35–330 μg of membrane protein and 0.1–0.05% Triton X-100. In the experiments to study enzyme activity as a function of time, portions were taken at different intervals from tubes containing 6-fold the amount of the reaction mixtures. After the incubation at 30°C the products were isolated by adding chloroform/methanol/4 mM-$MgCl_2$ (3:2:1, by vol.) to the incubation mixtures. The suspension was centrifuged and the organic phase was separated and dried under N_2. Subsequently a toluene-based scintillation solvent (New England Nuclear) was added. The samples were counted on a Packard Scintillation spectrometer.

Assay for protein glycosylation

With endogenous acceptor. The substrate of the reaction, Dol-*P*-*P*-[^{14}C]Glc oligosaccharide, was synthesized by using liver microsomes as previously described (Parodi *et al.*, 1972). Paper chromatography of the oligosaccharide obtained by mild acid hydrolysis (pH 2 at 100°C for 15 min) of the lipid-bound oligosaccharide revealed a clear predominance of the $Glc_3Man_9GlcNAc_2$ oligosaccharide variety. This was determined by paper chromatography of the product with propanol/nitromethane/water (5:2:4, by vol.) as solvent, by the method of Staneloni *et al.* (1980*a*). The Dol-*P*-*P*-Glc oligosaccharide:protein oligosaccharide transferase (hereinafter called the 'oligosaccharide transferase') was measured as follows. The incubation mixture contained about 3000 c.p.m. of Dol-*P*-*P*-[^{14}C]Glc oligosaccharide labelled in the glucose moiety (previously dried under N_2), 71 mM-Tris/maleate buffer (pH 7.6), 71 mM-mercaptoethanol, 8 mM-$MnCl_2$ and membrane preparation. The optimal concentration of protein in Triton X-100 was variable, depending on the membrane preparation used. The mixtures were incubated at 30°C and stopped with 10% cold trichloroacetic acid. The samples were heated and treated with diethyl ether and methanol as described previously (Idoyaga Vargas & Carminatti, 1977).

Exogenous acceptor. The standard assay for the transfer of the lipid oligosaccharide to the exogenous acceptor contained about 10000 c.p.m. of Dol-*P*-*P*-[^{14}C]Glc oligosaccharide, previously dried under N_2, 1.1 mM-dinitrophenyl-heptapeptide, 100 mM-mercaptoethanol, 50 mM-Tris/HCl buffer (pH 7.4), 10 mM-$MnCl_2$, 0.1% Triton X-100 and about 150 μg of membrane proteins. The reaction was stopped by the addition of 150 μl of cold ethanol and the mixture was stored overnight at −15°C. After low-speed centrifugation the resulting super-

natant fluid was passed through a Bio-Gel P-2 column. The column was equilibrated with pyridine/acetate buffer (0.1 M, pH 5). The compounds obtained in the void volume, as determined with Blue Dextran as a marker, were then submitted to paper electrophoresis as described by Ronin *et al.* (1978).

Results

Five enzyme activities of the dolichol pathway were studied by using membrane preparations from day-5 and day-15 neuronal perikarya. According to Aghajanian & Bloom (1967) the neurons from day-5 cerebral cortices do not form synapses detectable by the phosphotungstic acid-staining technique. On the other hand, at day 15, the number of synapses amount to little less than half the maximum.

In all cases the optimal detergent concentration was determined before measurement of transferase activities. In addition, linearity conditions with respect to the amount of protein concentration and enzyme activity were established. The enzyme assay was performed as indicated in the Materials and methods section, with and/or without the addition of exogenous acceptors.

The amount of membrane protein was approximately equal when the activities of day-5- and day-15-neuronal enzyme preparations were compared.

Activity of Dol-P-P-GlcNAc synthase

This synthase activity was not detectable in the absence of exogenous Dol-*P*. Furthermore, the amount of membrane protein added to detect activity in the presence of liver Dol-*P* was about five times higher than the amount required for the measurement of Dol-*P*-Man and Dol-*P*-Glc synthase activities. Thus, despite this relatively high protein concentration, the endogenous lipid receptor concentration was not high enough to allow the formation of measurable amounts of glycosylated product. Fig. 1(*a*) illustrates the variations of enzyme activity in the presence of Dol-*P* as a function of time. Product formation was found to be linear in both membrane preparations up to 6 min of incubation time. Activity was about 2.5 times higher in the enzyme preparations from day-15 neurons.

Activity of Dol-P-Man synthase

The addition of GDP-[^{14}C]Man to membrane preparations leads to the formation of various amounts of Dol-*P*-Man. As shown in Fig. 1(*b*), enzyme activity is linear up to 5 min of incubation for the day-15 membrane preparation. Dol-*P*-Man synthase activity in the presence of exogenous Dol-*P* was found to be about 3-fold higher in day-15- than in day-5-neuronal membranes. As mentioned before, the protein concentration was about 5-fold lower

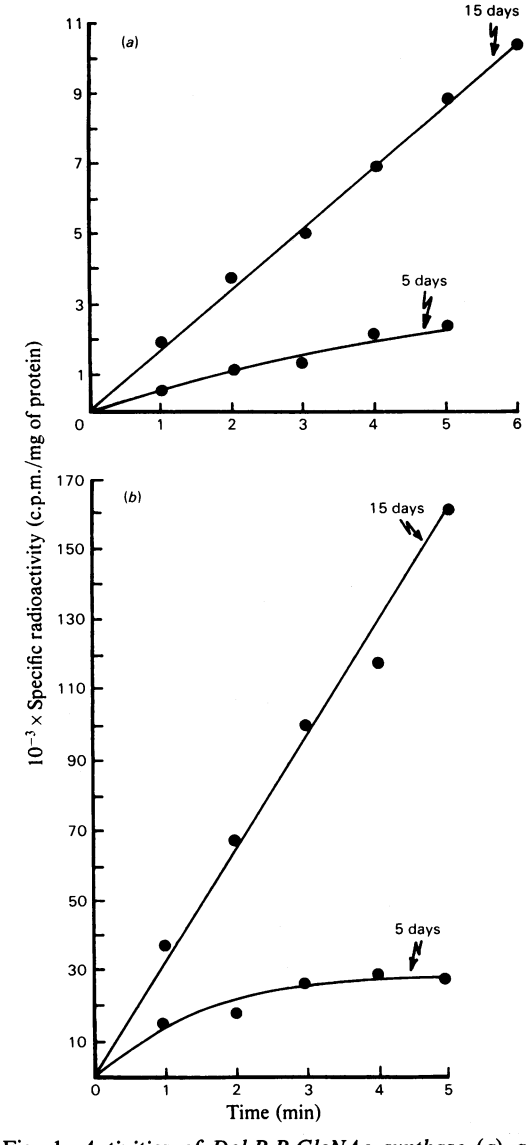

Fig. 1. *Activities of Dol-P-P-GlcNAc synthase* (*a*) *and Dol-P-Man synthase* (*b*) *from day-15 and day-5 neuronal perikaryal membranes in the presence of exogenous Dol-P*
The substrate was UDP-[^{14}C]GlcNAc (*a*) or GDP-[^{14}C]Man (*b*). For details see the Materials and methods section.

than the one used in the experiment shown in Fig. 1(*a*). Therefore, the specific activity of Dol-*P*-Man synthase is higher than that of the *N*-acetylglucosamine dolichol-linked synthases. The above comparison was made in the presence of exogenous Dol-*P*. In addition, for Dol-*P*-Man synthase the activity in the presence of Dol-*P* is 15–20 times higher than when using endogenous acceptor.

Activity of Dol-P-Glc synthase and measurement of the formation of the Dol-P-P-Glc oligosaccharide

Two different approaches were undertaken to measure the transfer of glucose from UDP-[^{14}C]Glc to Dol-*P*. The reaction was carried out either in the presence of liver Dol-*P* or only with endogenous acceptor. Detergent was used in the assay with Dol-*P*. As shown in Fig. 2(*a*), in the presence of exogenous Dol-*P* the activity of Dol-*P*-Glc synthase at day 15 was linear up to 2min of incubation. Furthermore, this activity was found to be about 4–6-fold higher than that of the day-5 membrane preparation. Fig. 2(*b*) shows the activity of Dol-*P*-Glc synthase as a function of time in the absence of exogenous Dol-*P*. In this case, however, the amount of membrane protein used in the assay was about 11–12 times higher than that of the experiment of Fig. 2(*a*). It can be seen that synthase activity proceeds linearly for both the day-15 and day-5 membrane preparations. The synthase activity of the membranes obtained from neurons undergoing synaptogenesis (day 15) was higher than that of day-5 membranes. The difference between day-15 and day-5 membranes was more pronounced than for the experiments shown in Fig. 2(*a*).

Developmental variations in the activity of this enzyme have been previously described in whole chicken brain (Breckenridge & Wolfe, 1973). However, there is at present no information regarding the enzyme that glucosylates the Dol-*P*-*P*-oligosaccharide in developing tissues. This problem was approached by studying glucose transfer to endogenous Dol-*P*-*P*-oligosaccharide from the membranes of both age groups using UDP-[^{14}C]Glc. The results are shown in Fig. 3(*a*). These curves are, however, the result of two enzyme activities, i.e. (1) glucose transfer from UDP-Glc to Dol-*P* and (2) glucose transfer from Dol-*P*-Glc to Dol-*P*-*P*-Glc oligosaccharide and the level of endogenous acceptors. An approx. 12-fold difference in activity was observed in favour of the transferases of the day-15 group.

The oligosaccharides synthesized by day-15 and day-5 neuronal perikaryal membranes were compared after mild acid hydrolysis of the product extractable with chloroform/methanol/water (10:10:3, by vol.). When run on paper chromatography with butanol/pyridine/water (4:3:4, by vol.) for 15 days, both compounds have similar R_F values and behave like liver Dol-*P*-*P*-Glc oligosaccharide having three glucose residues. Thus, at least under these conditions, the oligosaccharide synthesized in both age groups seems to fulfil the requirement for optimal transfer to protein, as previously described (Staneloni *et al.*, 1980*b*).

Activity of Dol-P-P-Glc oligosaccharide:protein oligosaccharide transferase

Proportionality between the oligosaccharide transferase activity and protein concentration is shown in Fig. 3(*b*). Each point represents the highest value obtained with various concentrations of detergent for a given protein concentration. As can be seen in Fig. 3(*b*), activity increased progressively in proportion to the amount of membrane protein up to 200μg. The donor substrate used was Dol-*P*-*P*-Glc oligosaccharide, possessing three glucoses. The acceptor was an endogenous membrane protein(s).

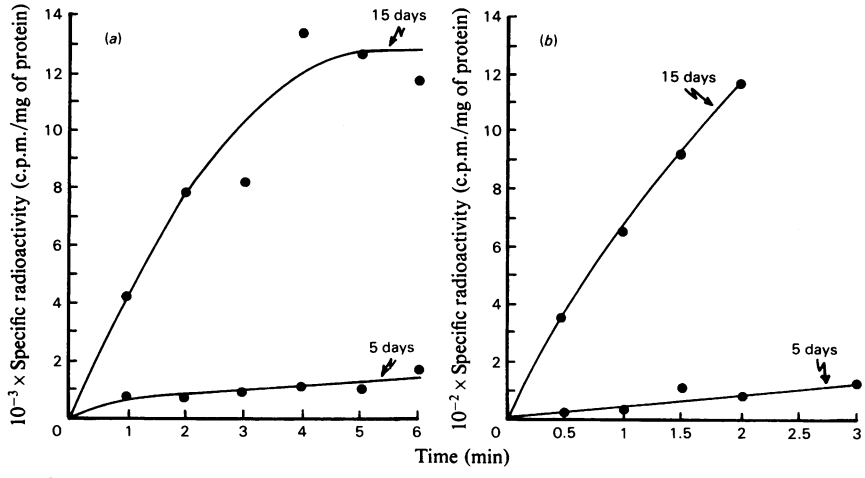

Fig. 2. *Activity of Dol-P-Glc synthase from day-15 and day-5 neuronal perikaryal membranes in the presence of exogenous Dol-P (a) and with endogenous lipid acceptor (b)*

The substrate was UDP-[^{14}C]Glc. For details see the Materials and methods section.

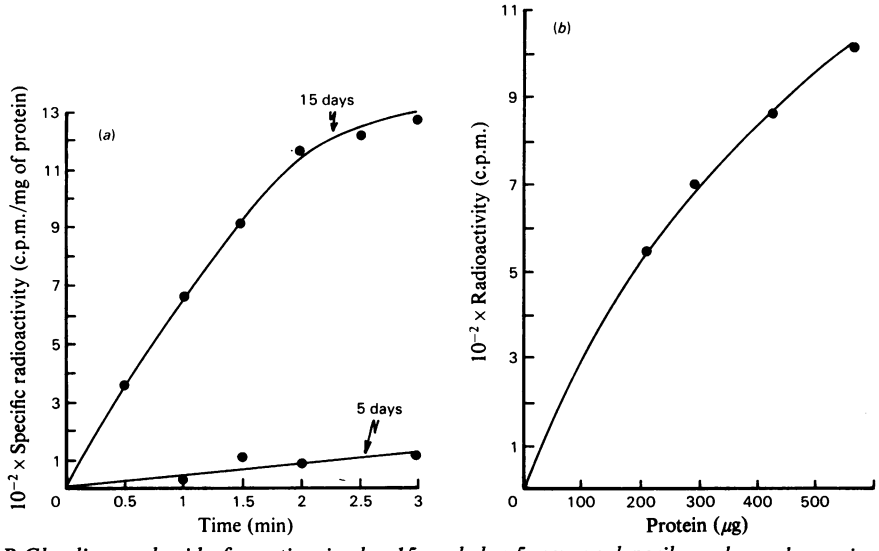

Fig. 3. *Dol-P-P-Glc-oligosaccharide formation in day-15 and day-5 neuronal perikaryal membrane in the absence of exogenous lipid acceptor (a) and activity of Dol-P-P-Glc-oligosaccharide:protein oligosaccharide transferase (day 15) as a function of protein (b)*

The substrate was UDP-[^{14}C]Glc (*a*) or Dol-*P*-*P*-[^{14}C]Glc-oligosaccharide (*b*). For details see the Materials and methods section.

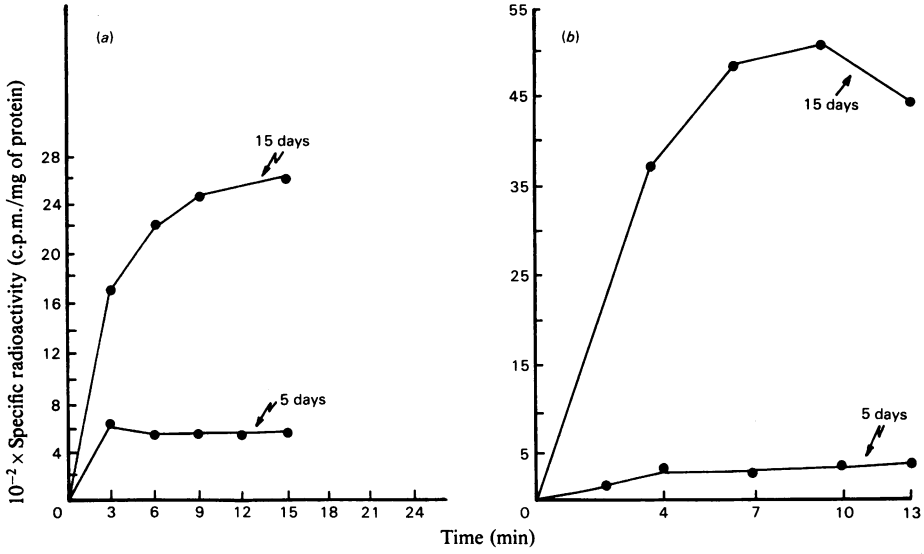

Fig. 4. *Activity of Dol-P-P-Glc oligosaccharide:protein oligosaccharide transferase from day-15 and day-5 neuronal perikaryal membranes*

(*a*) shows oligosaccharide transfer to endogenous protein acceptor; (*b*) shows oligosaccharide transfer to exogenous acceptor (dinitrophenyl-heptapeptide).

Comparison of the rate of protein glycosylation was then carried out. The results are shown in Fig. 4(*a*); the enzymes from day-15 neurons were able to transfer the oligosaccharide to protein at a higher rate than that of day-5-neuronal oligosaccharide transferase.

It may be argued that the amount of endogenous protein acceptor might be rate limiting in the day-5-neuronal preparation. To rule out this possibility, a dinitrophenylated synthetic heptapeptide was used as exogenous acceptor. This peptide has been used by Ronin *et al.* (1978) to study protein

glycosylation in microsomes from the thyroid gland. The reaction was carried out under conditions similar to those described in the legend to Fig. 4(*a*) in the presence of 1.1 mM exogenous acceptor. The results shown in Fig. 4(*b*) demonstrate that day-15 neuronal membranes glycosylate the dinitrophenylated heptapeptide at about 10 times the rate of those of day 5.

The reaction product was analysed after stopping the incubation with ethanol. The ethanol-soluble labelled compounds were passed through a Bio-Gel P-2 column. The radioactive compounds that eluted in the void volume were run on paper electrophoresis with 5% (v/v) formic acid. Fig. 5 shows the synthetic labelled compound differed markedly from the uncharged substrate, which remained at the origin. The radioactive product differed in its electrophoretic properties from Dol-*P*-*P*-[^{14}C]Glc oligosaccharide. That is, it migrated like a charged compound. In conclusion, the radioactive product may be the glycosylated heptapeptide as shown by Ronin *et al.* (1978).

It therefore seems that the rate of protein glycosylation is higher in the neuronal perikaryal membranes from day-15 rat cerebral cortices.

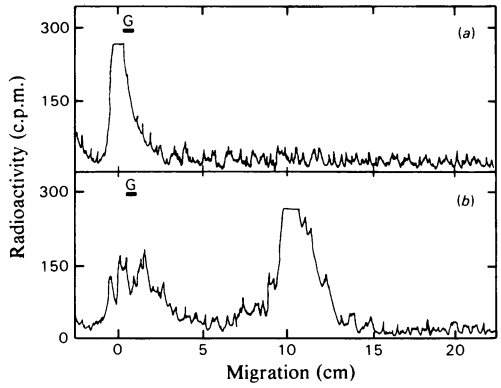

Fig. 5. *Electrophoretogram of an ethanol/water-soluble compound obtained by incubation of Dol-P-P-*[^{14}C]*Glc oligosaccharide with day-15 neuronal perikaryal membranes in the presence of exogenous dinitrophenyl-heptapeptide*

(*a*) Control (non-incubated); (*b*) incubated at 30°C for 10 min. G indicates the position of marker glucose.

Dol-P-Man synthase activity during the period comprising the onset and termination of synaptogenesis

In the previous series of experiments, enzyme activities were measured up to day 15 of neuronal postnatal development. According to Aghajanian & Bloom (1967), the number of synaptic junctions increases up to day 26 and does not change appreciably thereafter. Therefore, it was interesting to determine the variations in Dol-*P*-Man synthase during the period covering the process of synaptogenesis measuring enzyme activity beyond day 15.

A mannosyltransferase activity in the presence of exogenous Dol-*P* proceeds linearly with time in all the age groups examined (see Fig. 1*b*). The changes in enzyme activity for the period of synaptogenesis and the adult stage are shown in Table 1. From the comparison of the values of specific activities it can be concluded that there is a progressive increase of the transferase taking place throughout the whole period of synapse formation. In the adult stage the activity declines.

Effect of detergent and of sonication on Dol-P-Glc synthase and on the formation of Dol-P-P-Glc oligosaccharide in mixed preparations from day-5 and day-15 microsomes

The marked difference in the activities found between day-5 and day-15-neuronal enzymes suggested the possibility of the presence of activator(s) or inhibitor(s) in the enzyme preparation. To examine this problem the following approach was undertaken. First, the membranes from both age groups were mixed and then submitted to either sonication or detergent treatment. This treatment is expected to disrupt the membranes. This procedure should allow the interactions of the putative factor(s) with the enzymes involved in the reactions from either membrane fraction. The results obtained are shown in Table 2. When untreated vesicles from

Table 1. *Dol-P-Man synthase activity as a function of age in microsomes from neuronal perikarya*
Assay conditions were as described in the Materials and methods section.

Postnatal age	Total radioactivity (c.p.m.)*	Protein (μg)	Specific radioactivity (c.p.m./mg of protein)
Day 5	550	32.8	16700
Day 15	3620	37.0	97800
Day 36	5990	38.8	154400
Adult	870	17.0	51760

* Total radioactivity found in the organic phase obtained from 50 μl of the standard incubation mixture.

Table 2. *Effect of detergent and of sonication on the formation of sugar-lipid intermediates in mixed preparations from day-5 and day-15 neuronal membranes*

Assay conditions were as described in the Materials and methods section.

Membrane treatment	Protein (μg)		Formation of:		
	Day 5	Day 15	Dol-*P*-*P*-[^{14}C]Glc oligosaccharide (c.p.m.)	Dol-*P*-[^{14}C]Glc (c.p.m.)	Additions
Sonication	283	—	142	241	—
	660	—	364	482	—
	—	302	1134	1311	—
	330	302	1375	1355	—
	660	302	1322	1405	—
Detergent	80	—		129	Dol-*P*
	—	68	—	1023	Dol-*P*
	80	68	—	1146	Dol-*P*
None	315	—	103	75	—
	—	210	475	438	—
	315	210	559	578	—

both age groups were mixed in the absence of Dol-*P*, they yielded almost additive activity values. These results indicated that there is no apparent soluble factor released by the neuronal vesicles under our experimental conditions. On the other hand, the results after sonication or detergent treatment were similar to those obtained with untreated membranes. That is, the enzyme activities from the mixed membranes were approximately equal to the sum of the activities of each age group measured separately. These results are not compatible with the concept of membranous components affecting enzyme activity.

Discussion

The results obtained in this work demonstrate variations in the activities of several glycosyl-transferring enzymes of the dolichol pathway during the postnatal development of the rat cerebral cortex. The activities of Dol-*P*-Man, Dol-*P*-Glc, Dol-*P*-*P*-GlcNAc synthases and the oligosaccharide transferase together with the enzymes catalysing the formation of Dol-*P*-*P*-Glc oligosaccharide were higher at day 15 than at day 5 after birth.

Our findings indicate an increase in the glycosylation of asparagine-linked oligosaccharides during synaptogenesis. The activity of most of the enzymes involved in the initiation and elongation of the oligosaccharide chain has been measured. In all cases a striking correlation was found. The activities were relatively low before the onset of synaptogenesis (day 5). However, at a time when about half of the maximum number of synapses have been formed (day 15), all the enzyme activities studied increased between 2- and 10-fold.

Enzymes catalysing initial reactions on a given pathway and exhibiting relatively low activities can be rate-limiting. The initial step in dolichol glycosylation is catalysed by Dol-*P*-*P*-GlcNAc synthase. At day 5 this enzyme exhibited a markedly low activity compared with the other synthases. This situation was not modified despite the increase in enzyme activity observed at day 15. Thus Dol-*P*-*P*-GlcNAc synthase may play a regulatory role in the dolichol cycle. However, this hypothesis remains to be elucidated. In this regard results reported by Kean (1980) favour this possibility.

The activity of Dol-*P*-*P*-GlcNAc synthase has been reported in whole rat brain (Behrens *et al.*, 1971*b*), calf brain (Waechter & Harford, 1977) and calf white- and grey-matter membranes (Harford *et al.*, 1979). No clear localization of the enzyme within any given cellular type of the nervous system was achieved in any of these cases. Furthermore, developmental variations of activity were not studied. Our results demonstrate an increase in Dol-*P*-*P*-GlcNAc synthase during synapse formation.

It has been reported that mannose can be transferred to a dolichyl-linked saccharide either directly from GDP-Man or through Dol-*P*-Man (Chapman *et al.*, 1979, 1980). Our results demonstrate an increased activity of the enzyme catalysing the formation of Dol-*P*-Man at day 15 compared with day-5 neuronal perikaryal membranes.

Transference of glucose to the dolichol-linked oligosaccharide appears to be a necessary step for protein glycosylation (Staneloni *et al.*, 1980*b*). It has been established previously that this transfer occurs via Dol-*P*-Glc (Behrens *et al.*, 1971*a*). Our results indicate a higher activity of Dol-*P*-Glc synthase at day 15 than at day 5. In addition, the results show a similar increase in the formation of Dol-*P*-*P*-Glc oligosaccharide from UDP-Glc at day 15. This is compatible with a higher amount of glucosylation of

endogenous lipid oligosaccharide during synaptogenesis.

The increase in activities of the sugar lipid synthases could be due to (*a*) synthesis of enzymes *de novo* or (*b*) the presence of enzyme activator(s) or inhibitor(s). Our results do not support the second alternative. For, when day-5 and day-15 membranes were mixed, no marked change in overall activity was observed. Thus dolichol-pathway regulation by synthesis of the sugar-transferring enzymes *de novo* remains a valid alternative to be explored.

Dol-*P*-Man synthase was chosen to study further the relationship between enzyme activity and synapse formation. It was observed that the activity increased progressively up to day 30, levelling-off thereafter. This provided strong evidence for a temporal correlation between dolichol glycosylation and synaptogenesis. In contrast with these results, Harford & Waechter (1980) found a sharp variation of mannosyltransferase in pig brain white-matter membranes. They observed a rapid increase followed by a sharp fall in the activity taking place during the second week of postnatal development. It is noteworthy to mention that white-matter membranes are mainly associated with the oligodendroglial cells.

It has been proposed that the concentration of endogenous Dol-*P* may be the rate-limiting step in the dolichol pathway (Hemming, 1977). Circumstantial evidence has been provided to support this concept (Harford & Waechter, 1980). However, more recent experiments showed that after hepatic depression of dolichol synthesis by cholesterol feeding, the rate of [^{3}H]mannose incorporation into liver and plasma glycoproteins was unchanged (James & Kandutsch, 1980). The possible regulatory role of Dol-*P* cannot be excluded by our present results. Nevertheless, the addition of exogenous Dol-*P* in our experiments should minimize the possible effect of variations in the concentration of endogenous lipid acceptor(s) on the reaction rate. Thus it may be concluded that the higher amounts of sugar-lipid formation found during synaptogenesis in neurons are at least partially related to increased rates of enzyme activity. The possible exception might be Dol-*P*-*P*-Glc oligosaccharide formation. Because no exogenous Dol-*P* was added, the influence of endogenous lipid acceptors cannot be ruled out.

The last step in the dolichol pathway is the glycosylation of membrane-bound protein acceptors. An important finding of the present work is the increase in protein glycosylation in day-15 neuronal membranes. This phenomenon occurred regardless of the presence of endogenous protein acceptors or exogenously supplied synthetic heptapeptides.

It has been previously shown by experiments carried out *in vivo* that the same type of membrane preparations used to measure the oligosaccharide transferase exhibit the highest incorporation of radioactive amino acids into protein during the second week of postnatal development (Sellinger *et al.*, 1973). In addition, cerebral cortex neurons showed pronounced changes in the morphology of the rough endoplasmic reticulum in this period (Caley & Maxwell, 1968). Enlargement of the cisternae and increased numbers of membrane-bound ribosomes were features commonly observed in these developing neurons. Taken together, all these results indicate that there is an increase in the glycosylation of asparagine-type glycoproteins in cerebral cortex neurons undergoing synaptogenesis. This is compatible with the results obtained by Margolis *et al.* (1976). They found that glycoproteins containing mannose and glucosamine increased progressively between day 1 and day 30 of brain postnatal development. However, a causal relationship has yet to be established between the changes in lipid and protein glycosylation and synaptogenesis in the cerebral cortex.

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