Glycoconjugate Journal (1998) **15:** 233–241

Identification, expression and tissue distribution of cytidine 5′-monophosphate *N*-acetylneuraminic acid synthetase activity in the rat

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We report the postnatal developmental profiles of *N*-acetylneuraminic acid cytidylyltransferase (EC 2.7.7.43) (CMP-Neu5Ac synthetase) in different rat tissues. This enzyme, which catalyses the activation of NeuAc to CMP-Neu5Ac, was detected in brain, kidney, heart, spleen, liver, stomach, intestine, lung, thymus, prostate and urinary bladder but not in skeletal muscle. Comparative analysis of the different specific activity profiles obtained shows that the expression of CMP Neu5Ac synthetase is tissue-dependent and does not seem to be embryologically determined. Changes in the level of sialylation during development were also found to be intimately related to variations in the expression of this enzyme, at least in brain, heart, kidney, stomach, intestine and lung.

***Keywords*: *N*-acetylneuraminic acid cytidylyltransferase, rat**

***Abbreviations*: NeuAc or sialic acid, *N*-acetyl-D-neuraminic acid; CTP, Cytidine 5′-triphosphate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PSA, polysialic acid; N-CAM, Neural cell adhesion molecules; CMP-Neu5Ac, cytidine 5′-monophospho-*N*-acetyl neuraminic acid and DTT, dithiothreitol**

Introduction

Sialic acids (NeuAc) are important acidic sugar constituents of different biological macromolecules. In vertebrates, they are attached to glycolipids (gangliosides) and glycoproteins (sialoglycoproteins) as terminal non-reducing sugar residues. Although their functional significance still remains obscure, the negative charge that they confer to gangliosides and sialoglycoproteins plays an important role in receptor-mediated signal transduction [1] as well as direct cell–cell and cell–extracellular matrix interactions [2], probably via steric and/or charge effects at the cell surface [3]. Moreover, it is not only the presence or absence of sialic acid that determines specific functions; the degree of sialylation also plays an important biological role in both molecular degradation and development and differentiation processes. Thus, regulated addition of NeuAc residues to polysialic acid (PSA), a linear homopolymer composed of α(2-8) linked NeuAc residues and attached to N-CAM (neural cell adhesion molecules) sialoproteins, is known to affect a variety of cell–cell interactions that are important in the path-finding activity and target innervation of axons [4–7], the development of skeletal muscle [8], kidney formation [9], and the migration of neural precursors in the brain [10]. Furthermore, numerous studies have reported changes in ganglioside expression (through variations in the number of sialic acid residues) during embryonic differentiation and ageing [2, 11].

A deeper knowledge of sialic acid metabolism and its regulation is important to understand the significance of NeuAc in the signal transduction and cell interaction involved in development and differentiation processes.

The incorporation of sialic acid to sialoglycoconjugates (gangliosides and glycoproteins) occurs through the action of sialylglycosyltransferases. Although these enzymes show very high substrate specificity, which generally differs for each acceptor, all of them use CMP-Neu5Ac as a source of sialic acid [12–15]. Therefore, CMP-Neu5Ac synthesis plays an important role in later incorporation into carbohydrate structures and, during biological development, modifications in the levels of synthesis are to be expected.

NeuAc cytidylyltransferase (EC 2.7.7.43) (CMP-Neu5Ac synthetase), the protein responsible for CMP-Neu5Ac biosynthesis, has been reported in several eukaryotic as well as in prokaryotic organisms [16–21]. In mammals, this enzyme has been found in different tissues: hog submaxillary glands [22], rat spleen [20], frog liver [19], calf brain [23],

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mouse kidney [24], and rat colon [25]. More recently, we have purified and characterized CMP-Neu5Ac synthetase from rat liver, and have confirmed the important role played by this protein in the regulation of sialic acid metabolism [26]. Now, using a rapid partial purification step and specific antibodies contained against this enzyme, we analyse the presence and levels of CMP-Neu5Ac synthetase in different developing rat tissues. The results show that the levels of this enzyme are closely coordinated with the changing levels of sialylation that occur during the development of different tissues.

Materials and methods

Chemicals

N-acetylneuraminic acid (NeuAc), resorcinol, dithiothreitol (DTT), protamine sulfate and Reactive Brown-10 Agarose were supplied by Sigma Chemical Co. (St Louis, MO, USA). Sephadex G-25 (PD-10), Sephacryl S-200, Blue Sepharose Cl-6B and molecular weight markers were purchased from Pharmacia Fine Chemicals (Sweden). Cytidine 5′-triphosphate (CTP) was from Boehringer Mannheim (Mannheim, Germany). All other products were of analytical quality.

Animals

Wistar rats of different ages (newborns, 8–9 days, and 1, 2 and 3 months old) fed *ad libitum* on a commercial diet were used to obtain the different extracts of CMP-Neu5Ac synthetase. In total purification of this enzyme, male Wistar rats of 105–115 g were used.

Analysis of CMP-Neu5Ac synthetase

Partial purification of CMP-Neu5Ac synthetase from rat tissues of different ages

All procedures were carried out at 4 °C unless otherwise indicated. Since more than 85% of this enzyme is associated with nuclei [20, 24–26], we analysed the presence of CMP-Neu5Ac synthetase in this subcellular fraction.

Step 1: Preparation of nuclear fractions. Rats were killed by decapitation and the different tissues were rapidly removed and chilled on ice. Tissues were minced with scissors, washed twice with 0.25 M sucrose in 50 mM Tris-HCl, pH 7, resuspended in nine volumes (w/v) of the same buffer and homogenized with a motor driven glass/Teflon Potter-Elvejheim type (B. Braun, Melsungen, AG), (15 strokes at 1500 rev min^{-1}). The homogenate was filtered through gauze and centrifuged at $700 \times \mathbf{g}$ for 10 min. The supernatant was discarded after checking for any CMP-Neu5Ac synthetase activity present and the pellet (fraction rich in nuclei) was washed twice in the above buffer

Step 2: Collection of nuclear extracts. The washed pellet from step 1 was homogenized in three volumes (w/v) of 20 mM Tris-HCl buffer, pH 7.0, containing 1% mercaptoethanol and 1.0 M KCl, using a Polytron homogenizer (Kinematica, Switzerland) at a setting of 6 (four 20 s bursts with 60 s intervals). The homogenate was centrifuged for 20 min at $35\,000 \times \mathbf{g}$, and the precipitate was discarded. The nucleic acids present in this crude supernatant extract were then precipitated by the addition of 0.1% (w/v) protamine sulfate and removed by centrifugation at $35\,000 \times \mathbf{g}$ for 20 min.

Step 3: Ammonium sulfate fractionation. The proteins present in the supernatant obtained from step 2 were precipitated with ammonium sulfate. The fraction precipitating between 35–60% (containing all the CMP-Neu5Ac synthetase activity) was collected by centrifugation at $35\,000 \times \mathbf{g}$ for 20 min.

Step 4: Desalting. The precipitate obtained from the above centrifugation was resuspended in 50 mM Tris-HCl buffer, pH 7.0 containing 25 mM $MgCl_2$ and 1 mM DTT and was passed through a Sephadex G-25 (PD-10) column equilibrated with the same buffer. The eluate thus obtained was used both for quantification of CMP-Neu5Ac synthetase activity and for immunochemical analyses.

Enzymatic assay

CMP-Neu5Ac synthetase was assayed by a modification of the method of Kean and Roseman [22], according to Rodríguez-Aparicio *et al.* [26]. The assay mixture contained in a final volume of 250 µl: 0.18 M Tris HCl, pH 8.0; 10 mM $MgCl_2$; 3mM CTP (previously adjusted to pH 7.0 with potassium bicarbonate); 5 mM NeuAc; 5 mM DTT and 15 µl of protein extract. Incubations were carried out in a water bath at 37 °C for 30 min. After incubation, the reactions were stopped by adding 50 µl of $NaBH_4$ (100 µg ml^{-1}) (which reduces the excess of NeuAc) and the solutions were then incubated for 15 min at 20 °C. Later, $NaBH_4$ was destroyed by adding three drops of glacial acetic acid and keeping the mixture at room temperature for 15 min. The CMP-Neu5Ac synthesized was measured by the resorcinol method [27].

The results obtained correspond to statistical treatment of the values obtained after analysis of three independent samples from each rat tissue of the same age.

One unit of enzyme was defined as the amount of enzyme that synthesizes 1 µmol of CMP-Neu5Ac per min at 37 °C under the assay conditions. Specific activity was expressed as U mg^{-1} of protein.

Protein was determined by the Bradford method [28] using serum albumin as standard.

Immunochemical analysis

Purification of CMP-Neu5Ac synthetase to apparent homogeneity

To purify CMP-Neu5Ac synthetase to apparent homogeneity, livers from male Wistar rats of 105–115 g were used.

The purification process has been previously described by Rodríguez-Aparicio *et al.* [26].

Antiserum production against CMP-Neu5Ac synthetase protein

Rabbit polyclonal antibodies were prepared against CMP-Neu5Ac synthetase protein from rat liver by immunizing the animals with pure enzyme (128 μg per dose) as previously described [29–32]. The serum thus obtained was then partially purified by a passage through a Protein-A-Sepharose column according to a previously described method [33].

Immunodetection of CMP-Neu5Ac synthetase protein

Nuclear proteins from different partially purified tissue extracts were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions [34] and transferred electrophoretically to nitrocellulose membranes. Correct transferase was checked by immersing the membranes in Rouge Ponceau solution [35]. After filter blocking, immunoblot detections were carried out using the partially purified polyclonal antibodies diluted 1:5000 (previously determined by titration of the purified serum) as described elsewhere [31].

Results and discussion

Analysis of CMP-Neu5Ac synthetase from rat tissues

The presence and amounts of CMP-Neu5Ac synthetase from different rat tissues were analysed by enzyme activity assays and by immunoblot of samples partially purified from each organ studied. Since this protein is located in the nuclear fraction [18, 24–26], the enzyme was partially purified by isolation of this cellular fraction (see Materials and methods). During the process, 90–95% of CMP-Neu5Ac synthetase enzyme was obtained. Immunoblot analysis, using polyclonal antibodies against the CMP-Neu5Ac synthetase from rat liver (see Materials and methods), permitted the detection of an amount of protein equivalent to 0.1 U of enzyme. The specificity of these antibodies was checked by immunochemical analysis and by coupling to CN-Br-activated Sepharose 4B. Using these coupled antibodies, we have been able to immunopurify CMP-Neu5Ac synthetase from rat brain and liver tissues (manuscript in preparation).

CMP-Neu5Ac synthetase from brain

Brain tissue afforded the greatest amount of information about the relationship between sialylation and the development process. Moreover, the brain contains about 20-fold higher amounts of gangliosides than extraneural tissues [2, 36] and indeed neural cell adhesion molecules (N-CAM) were originally reported in neural structures [37, 38]. The extent of sialylation of these structures has been suggested to regulate overall neural functions, such as cell acquisition and migration, fibre outgrowth, and synapse formation, indices of the increasing developmental age of postnatal tissue [2, 39, 40]. Therefore, brain sialylation levels must become adapted to each developmental stage and modifications in the cerebral synthesis of activated sialic acid (CMP-Neu5Ac) by the action of CMP-Neu5Ac synthetase are to be expected.

To relate the sialyl-developmental regulation process to the appearance of CMP-Neu5Ac synthetase in the brain, we analysed the enzymatic activity present in brain extracts from rats of different ages (new-born, 8–9-, 30-, 60- and 90-days-old). As shown in Figure 1, the amount of enzyme increased substantially during the early days of life of the animals, reaching a maximum (5 U mg^{-1}) at 8–9 days of age. After this time, activity levels slowly decreased to 3 U mg^{-1} after 90 days of age (Figure 1). When the presence of this protein was analysed by immunoblot, we observed that the different activities were proportional to the spot intensity of CMP-Neu5Ac synthetase at each of the ages assayed (Figure 2). Moreover, as shown in Figure 2, antibodies raised against rat liver CMP-Neu5Ac synthetase showed a very specific reaction against brain protein. Even when partially purified extract was used, we obtained a single band of antigen-antibody reaction with a similar molecular mass (58 kDa) to that previously reported [26].

The high CMP-Neu5Ac synthetase activity recorded at 8–9 days after birth coincided with extensive fibre outgrowth [41] and preceded the accumulation of gangliosides and glycoproteins containing NeuAc previously reported for this period of brain development [41, 42]. Additionally, the decrease in activity observed by us in extracts from rats of 30-, 60- and 90-days-old (Figures 1 and 2) is consistent with the loss of expression of N-CAM sialylation observed after the formation of stable synapses [43–46]. These observations show that the amount of CMP-Neu5Ac synthetase is related to brain sialylation levels and suggest that this enzyme may affect brain development.

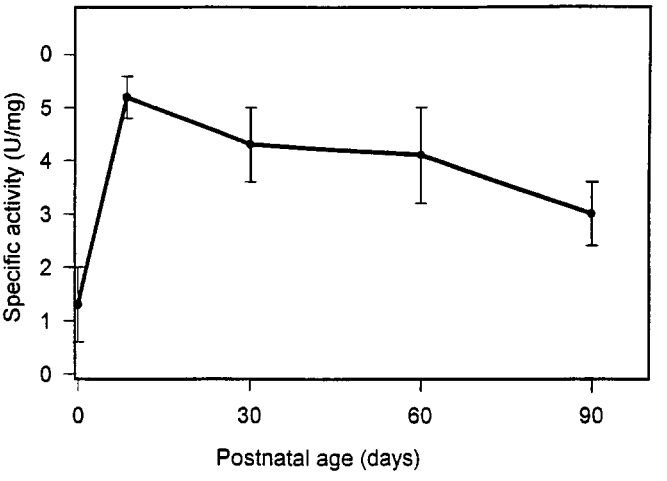

Figure 1. CMP-Neu5Ac synthetase activity from rat brain. Values are given as means ± SEM (n = 3).

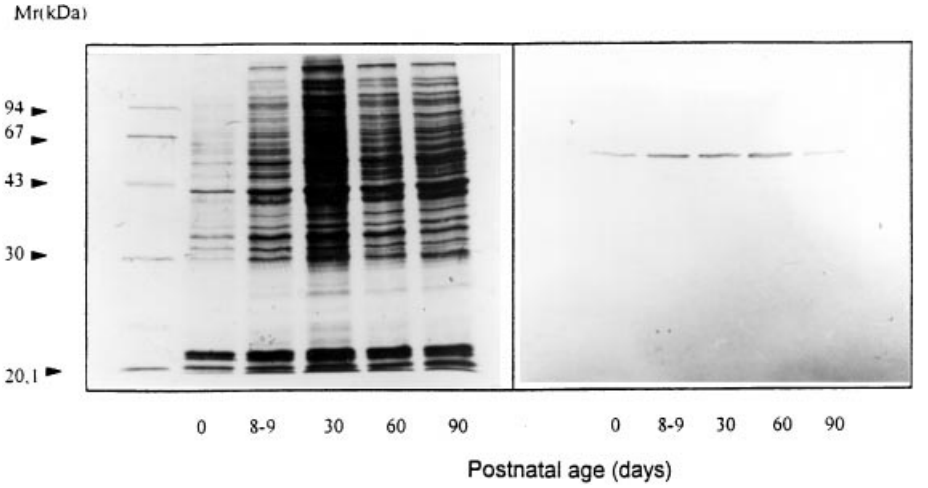

Figure 2. SDS.PAGE immunoblot analysis of CMP-Neu5Ac synthetase from rat brain extracts. (A) SDS-PAGE stained with Coomassie-Blue R-250. (B) Immunoblot analysed with anti-CMP-Neu5Ac synthetase from rat liver as described in Materials and methods. Each sample contained 70 μg of protein. St: Molecular weight markers (phosphorylase b, M_r 94 000; bovine serum albumin, M_r 67 000; ovalbumin, M_r 43 000; carbonic anhydrase, M_r 30 000; soybean trypsin inhibitor, M_r 20 100; and α-lactalbumin, M_r 14 000).

CMP-Neu5Ac synthetase from kidney

Analysis of CMP-Neu5Ac synthetase in kidney did not reveal the presence of the enzyme in new-born rat extracts, either when activity was assayed or when immunoblot was used (Figure 3). However, enzyme activity was detected in 8–9-day-old animal extracts and these levels increased to reach a maximum at 30 days of age (9.2 U mg^{-1}). Thereafter, activity decreased to 3.2 U mg^{-1} at 90 days of age.

The absence of CMP-Neu5Ac synthetase in the kidneys of new-born rats indicates that the level of this enzyme was below the detection level of this enzyme was below the detection level of our systems, since a minimal amount of activity is required for the sialylation maintenance of general mammalian structures (basal sialylation process). The increase observed after the birth of the animals suggests a reactivation of the sialylation process.

The expression of polysialic acid units in the kidney is developmentally regulated and their disappearance is concomitant with the postnatal maturation of this organ [47]. As in the brain, this observation suggests a possible role for sialic acids in cell–cell contact-mediated renal differentiation processes, as has been described between mesenchymal and uretic band-cells [48].

If CMP-Neu5Ac synthetase is indeed related to the developmental process, the delay observed in maximum CMP-Neu5Ac synthetase activity in kidney (rats between 8–9 and 30 postnatal days) (Figure 3) with respect to brain enzyme levels (rats of about 8–9 days old) (Figure 1) may be a consequence of the long period required for nephron formation, which continues even into postnatal life [49]. The decreases observed as from 30 days old (Figure 3) may be related to the decrease in PSA expression described when the basic structures of the nephrons are formed [47, 48].

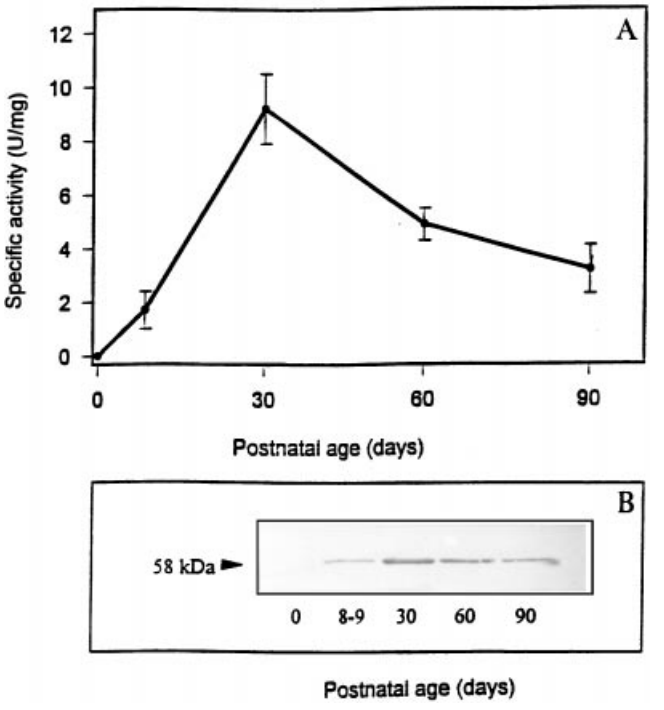

Figure 3. CMP-Neu5Ac synthetase from rat kidney. (A) Activity and (B) SDS-PAGE immunoblot analysis (each sample contained 48 μg of protein).

CMP-Neu5Ac synthetase from heart

CMP-Neu5Ac synthetase was detected in heart extracts from all ages of animals analysed (Figure 4). However, a markedly lower level of specific activity (5–10-fold) was observed with respect to brain and kidney was observed

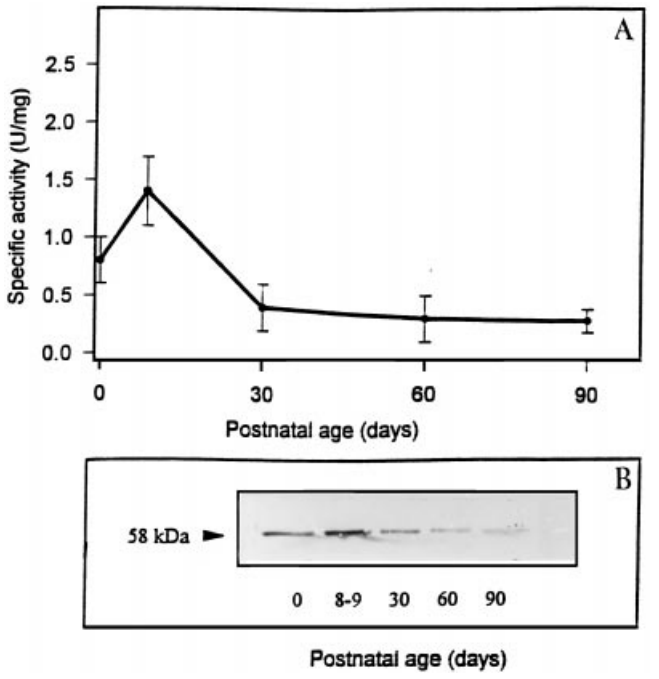

Figure 4. CMP-Neu5Ac synthetase from rat heart. (A) Activity and (B) SDS-PAGE immunoblot analysis (each sample contained 330 μg of protein).

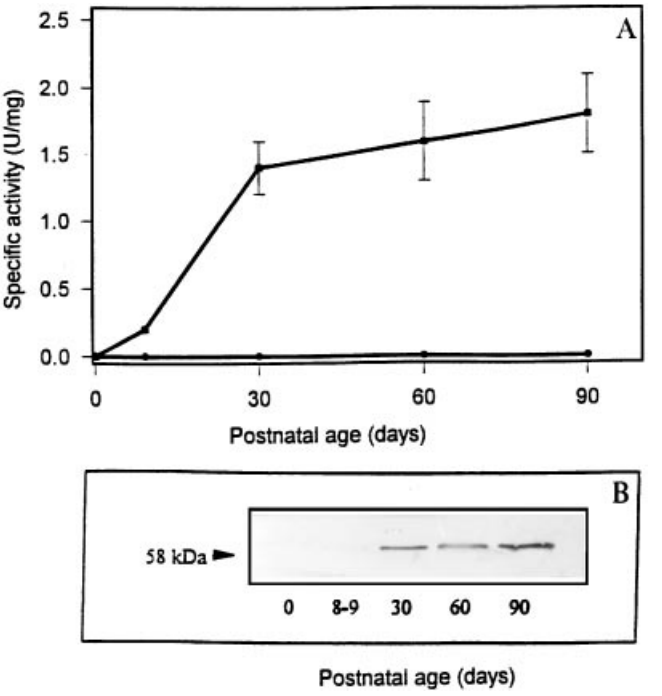

Figure 5. CMP-Neu5Ac synthetase from rat spleen (■) and skeletal muscle (●). (A) Specific activities. (B) SDS-PAGE immunoblot analysis of spleen extracts (each sample contained 120 μg of protein).

(Figures 1 and 3). Moreover, for detection by immunoanalysis it was necessary to concentrate the protein extracts 5-fold (Figure 4). As shown in Figure 4, the highest enzyme activity was recorded in animals of 8–9 days of age (1.5 U mg^{-1}). After this age, a substantial decrease was detected, levels thereafter stabilizing at a minimum in extracts from rats of 30 days of age (0.3 U mg^{-1}). These results indicate that, at least after birth, the rat heart undergoes a loss of sialylation levels, minimal values being reached after 30 days of age.

Although during the embryonic formation of this organ a significantly stronger degree of polysialylation has been detected, the relatively low degree of polysialylation recorded when the epicordial layer has been completed [50] could be related to the low CMP-Neu5Ac synthetase levels observed by us and suggests that this enzyme may also be related to the sialylation developmental process of the heart. Additionally, the decrease in CMP-Neu5Ac synthetase activity detected at 8–9 days after birth is concurrent with this suggestion since Lackie *et al.* [49] have reported that in rat heart polysialylated forms predominate until a week after birth.

CMP-Neu5Ac synthetase from spleen

Spleen extracts were also analysed for CMP-Neu5Ac synthetase activity. As shown in Figure 5, no activity was detected in extracts from newborn animals and very low levels (0.2 U mg^{-1}) were recorded in samples from 8–9-day-old rats. After this time, activity increased to 1.5 U mg^{-1} in rats of 30 days of age, this value persisting in rats of 60–90 days of age. This activity pattern was corroborated by immunoblot analysis (Figure 5). The concentrated samples (two-fold) specifically showed the presence of this protein and its proportionality to the activity recorded.

The CMP-Neu5Ac synthetase levels shown by rat spleen extracts indicate that after the early weeks of life the sialylation process becomes stabilized. The minimum CMP-Neu5Ac synthetase levels required by this organ (basal sialylation process) are higher than those of the heart (Figure 4). Specific sialylation processes, such as those undergone by erythrocyte surface glycoproteins, could be related to these differences. The lack of information about the significance of sialylation in spleen development does not allow us, in this case, to relate the results on CMP-Neu5Ac synthetase to the differentiation process of this organ.

CMP-Neu5Ac synthetase from skeletal muscle

Skeletal muscle extracts from rats of different ages did not exhibit detectable CMP-Neu5Ac synthetase either according to activity analysis (Figure 5) or immunoblotting (data not shown). However, a low level of this enzyme (lower than our detection systems) must be necessary for maintaining minimal amounts of activated sialic acid for sialylation of the different structures.

Regarding the possible relationship between CMP-Neu5Ac synthetase activity and the developmental process, other authors have detected the presence of polysialylated N-CAM during the embryogenesis of skeletal muscle [51–54]. However, after birth a rapid decrease in N-CAM levels has been observed [55], reaching a minimum during the early days of life. These findings suggest that after birth the sialylation of N-CAM from skeletal muscle is minimal and hence a reduction in CMP-Neu5Ac synthetase levels would be expected, as observed by us here.

CMP-Neu5Ac synthetase from liver

The liver is the only tissue in which CMP-Neu5Ac synthetase has been purified and characterized [26]. Using this protein source, we purified the enzyme to obtain the polyclonal antibodies employed in the present work (see Materials and methods). Analysis of CMP-Neu5Ac synthetase activity in liver extracts from rats of different ages revealed its presence in all of them (Figure 6). Moreover, extracts from newborns showed a high level of enzymatic activity (5.5 U mg^{-1}) that increased with the age of the rats, reaching a value of 8.7 U mg^{-1} at 90 days of age. Similar results were obtained with immunoblot analysis (Figure 6).

The profile of CMP-Neu5Ac synthetase levels shown by liver tissues does not allow us to relate the protein to the development of this organ. However, the functional diversity present in the liver could account for the high CMP-Neu5Ac synthetase levels detected by us, even during ageing.

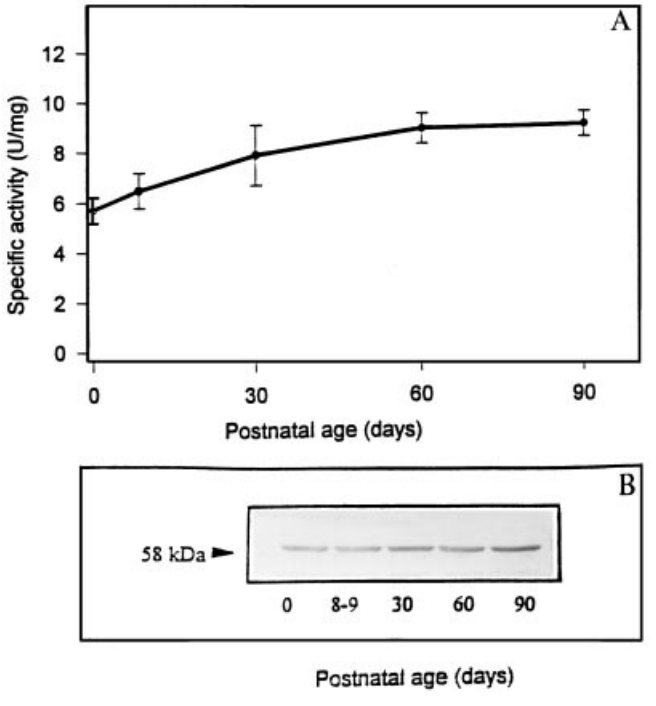

Figure 6. CMP-Neu5Ac synthetase from rat liver. (A) Activity and (B) SDS-PAGE immunoblot analysis (each sample contained 45 μg of protein).

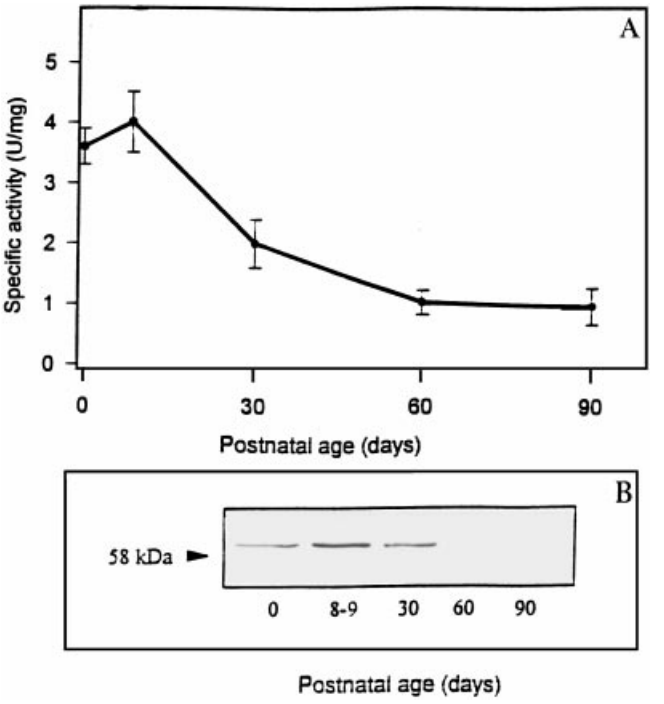

Figure 7. CMP-Neu5Ac synthetase from rat lung. (A) Activity and (B) SDS-PAGE immunoblot analysis (each sample contained 55 μg of protein).

CMP-Neu5Ac synthetase from lung

Analysis of lung extracts revealed that this tissue also expresses CMP-Neu5Ac synthetase activity. As shown in Figure 7, enzymatic activity was higher in newborns and 8–9-day-old rats. After this period, its activity decreased (50% in lung extracts from rats of 30 days old), reaching minimal values in animals of 60 and 90 days old (25% of specific activity). The maximum CMP-Neu5Ac synthetase specific activity detected by us in newborns and 8–9-day-old rats coincides with the moment when the respiratory tract begins its physiological functions. This observation suggests the existence of adaptive mechanisms where variations in CMP-Neu5Ac synthetase levels would play an important role in the process of development-related sialylation. The presence in the respiratory tract of structures with a high degree of sialylation, which have been detected until early postnatal rat life [49], supports this hypothesis.

CMP-Neu5Ac synthetase from testicle and ovary

Analysis of CMP-Neu5Ac synthetase in gonadal structures revealed the presence of the enzyme in extracts from animals of 30, 60 and 90 days old but not in rats of 8–9 days old (Figure 8). Testicle samples exhibited higher CMP-Neu5Ac synthetase levels in rats of 30 days of age (6 U mg^{-1}).

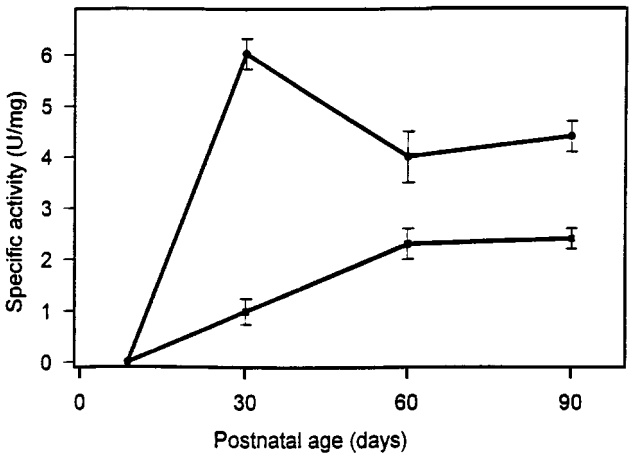

Figure 8. CMP-Neu5Ac synthetase activity from rat testicle (●) and ovary (■).

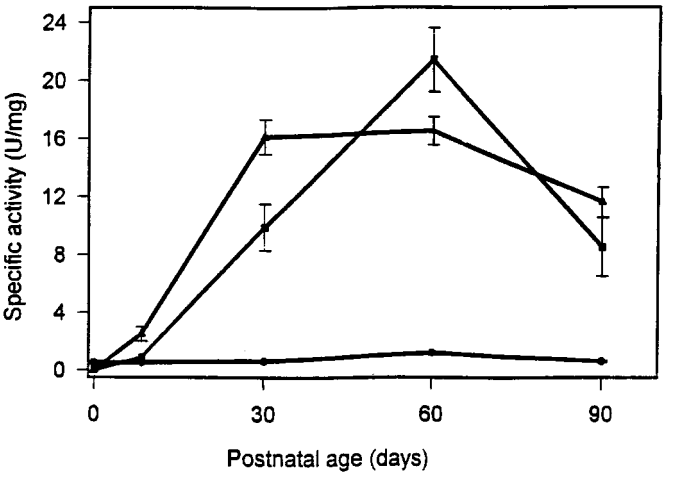

Figure 9. CMP-Neu5Ac synthetase activity from rat stomach (●), and small (▲) and large (■) intestine.

Extracts from animals of 60 and 90 days old showed less activity (4 U mg^{-1}). In ovary samples, maximum specific activity was observed in rats of 60 and 90 days of age, although the enzyme levels in this organ (2.3 U mg^{-1}) were lower than those found in testicle samples (Figure 8). These results indicate that CMP-Neu5Ac synthetase levels from testicle and ovary increase with ageing, suggesting that the sialylation level is higher in rats of 30 and 60 days of age, respectively.

CMP-Neu5Ac synthetase from stomach and intestine

Analysis of the presence of CMP-Neu5Ac synthetase activity in the tubular digestive tract was analysed in stomach and portions of both the small and large intestine. The enzyme was observed in all these structures and was seen to be age-dependent (Figure 9). Its activity was not detected in extracts from newborns and minimum values were recorded in 8–9-day-old rats. After this time, activity increased, reaching maximum levels in animals of 30–60 days of age. The variation in CMP-Neu5Ac synthetase activity during postnatal growth suggests the existence of modifications in the sialylation level of the digestive tract that could be a consequence of the animals' adaptation to a different diet. Comparative analysis of these enzymatic activities (Figure 9) revealed that the specific activity of the stomach was smaller (one order of magnitude) than the other intestinal portions tested. This observation is consistent with the low sialylation level observed in the epithelial lining of the rat stomach as compared with those of the other structures of the digestive tract [55] and links the activity of CMP-Neu5Ac synthetase to the different degrees sialylation prevailing at different times of development.

CMP-Neu5Ac synthetase from other tissues

CMP-Neu5Ac synthetase activity was also analysed in thymus, prostate and the urinary bladder. The thymus showed CMP-Neu5Ac synthetase activity only in tissue extracts from rats of 90 days of age (0.9 U mg^{-1} protein). As shown in Figure 10, no activity was detected in extracts from newborns, 8–9, 30- and 60-day-old animals. Similar results were obtained when the presence of this protein was analysed by immunoblot (data not shown).

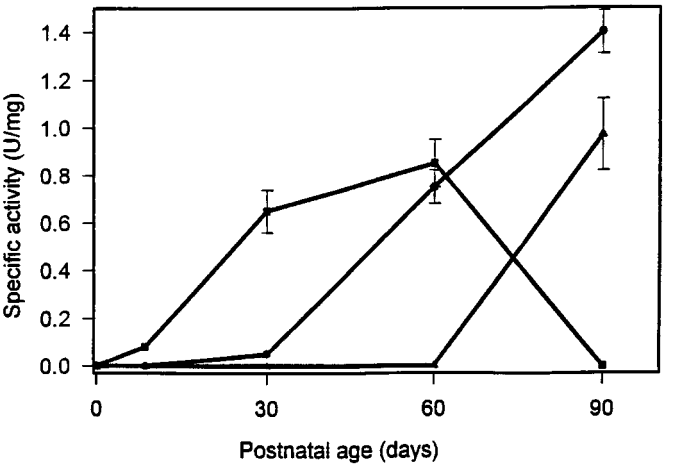

Figure 10. CMP-Neu5Ac synthetase from rat thymus (▲), prostate (●) and urinary bladder (■).

In the prostate gland, analysis of CMP-Neu5Ac synthetase activity revealed the absence of this enzyme in extracts from newborns and 8–9-day-old animals. Low activity was recorded in extracts from rats of 30 days of age but this increased linearly in ensuing assays (60 and 90 days old) (Figure 10). This specific activity profile suggests a possible relationship between prostate CMP-Neu5Ac synthetase activity and the molecular sexual maturation process.

With respect to the urinary bladder, CMP-Neu5Ac synthetase activity was detected in 8–9-day-old rat extracts and its levels increased to reach a maximum at 60 days of

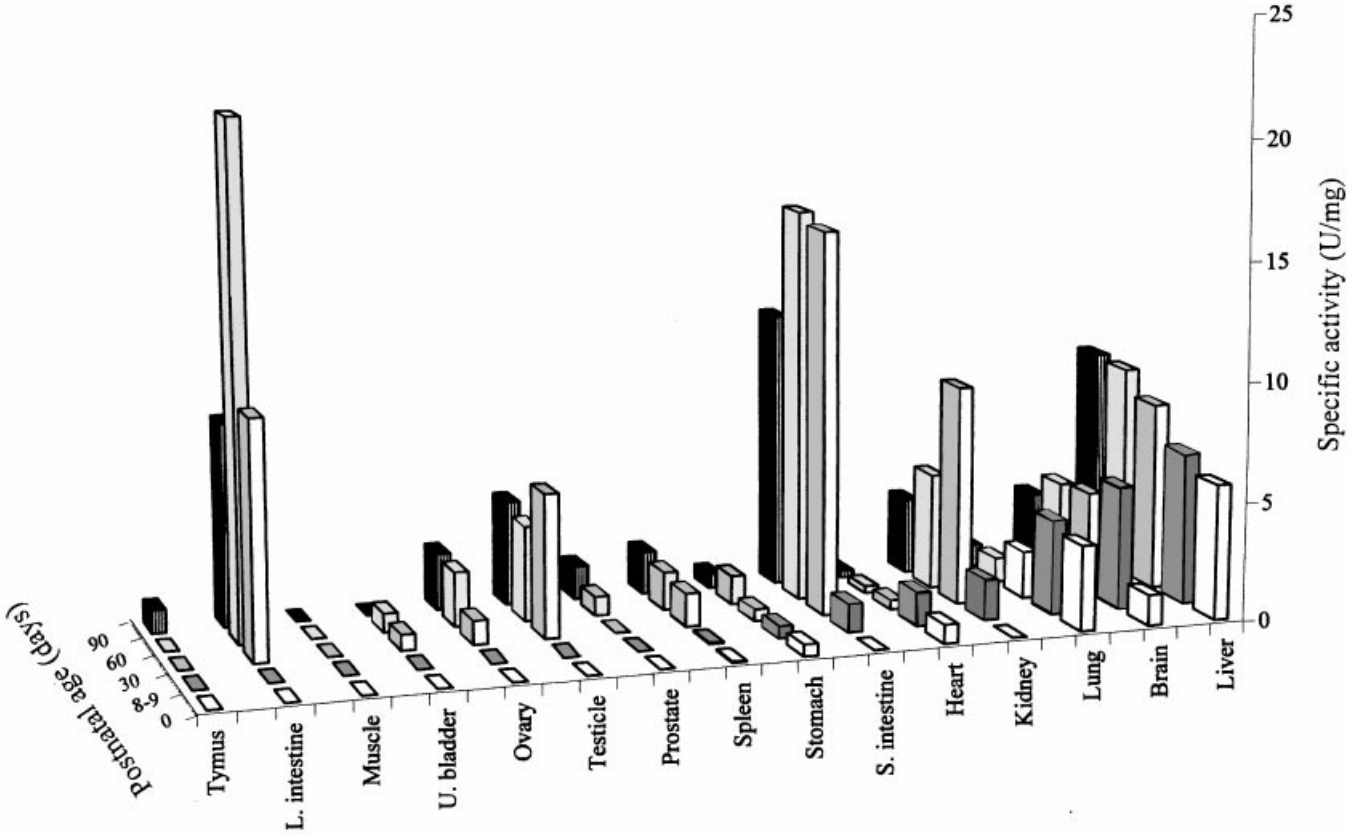

Figure 11. Comparative analysis of the CMP-Neu5Ac synthetase from different rat tissues.

age (0.8 U mg^{-1}). However, extracts from new-borns and 90-day-old animals did not show the presence of this enzyme (Figure 10). Although the bladder is derived from the large intestine, our results show that the CMP-Neu5Ac synthetase activity of this organ is different (20-fold less in the bladder). This observation suggests a different kind of behaviour as regards the degree of sialylation of molecular structures.

Like the digestive tract, liver and lung (see above), the organs studied in this part of the work are endo-mesodermic derived structures. However, the significant differences observed in both the enzyme levels and the activity profile of CMP-Neu5Ac synthetase suggest that the sialylation process is tissue-dependent and would not be determined embryologically. Comparative analysis of all the results obtained in this work support such a hypothesis. As shown in Figure 11, different patterns and levels of activity were obtained from each tissue analysed.

Finally, the results reported in this work suggest that the expression of CMP-Neu5Ac synthetase and the changes in the levels of sialylation during development are intimately related, at least in brain, heart, kidney, stomach, intestine, and lung tissues. This in turn suggests that the enzyme would participate in the developmental process. Accordingly, the analysis and quantification of CMP-Neu5Ac synthetase could be applied in clinical and anatomical tests to establish the time of development of each tissue and, consequently, of each organism. Also, the design of different mechanisms that would permit specific regulation of the activity of CMP-Neu5Ac synthetase.

Acknowledgements

We are gratefully indebted to R. Sánchez Barbero and R. Ordás for their participation in the elaboration of this manuscript. This work was supported by grants from the 'Dirección General de Investigación Científica y Técnica' (PB 93-0296).

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