STUDIES ON RETINAL GANGLIOSIDE METABOLISM

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Inborn errors in ganglioside metabolism produce large changes in cellular functions, but as yet the biochemical role of gangliosides has not been established. Several models have been used to elucidate their role and some encouraging data have been reported. Thus, theoretical as well as biochemical studies have implicated gangliosides in the functioning of specialized plasma membranes (1). Nevertheless, the complexity of the nervous tissue restricts the understanding of their precise functional role.

We have chosen a relatively simple model, the retina, which is morphologically connected to the brain and which derives embryologically from the same origin. Retina presents a simpler morphological organization than the central nervous system and a more restricted functional activity. On the other hand, it can be isolated with only minor damage and can be stimulated with its natural stimulus: light, at a physiological level. To assess the biochemical role of gangliosides in retina, we investigated the ontogenic behaviour of the biosynthetic and catabolic enzymes of the gangliosides as well as the effect of physiological stimulus (light) on the ganglioside metabolism.

MATERIALS AND METHODS

These studies utilized chicken retinas of various ages.

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The procedure of intravitreal injection of \( N \)-acetyl [D-mannosamine-U-\( ^{14} \)C] and corresponding analyses were published elsewhere (2). Neuraminidase assays were already reported (3).

Enzymatic and biochemical determination of UDP-glucose:ceramide glucosyltransferase and UDP-galactose:glucosylceramide galactosyltransferase were performed as described previously (4,5).

The assay of cytidine-5'-monophospho-\( N \)-acetyl neuraminic acid galactosyl glucosyltransferase sialyltransferase was performed as follows: the incubation mixture of 100 \( \mu \)l final volume contained 25 nmoles of lactosylceramide, 0.3 mg of Triton CF-54, 100 \( \mu \)moles of cacodylate-HCl pH 6.35, 100 nmoles of labelled CMP-sialic acid (sialic-4-\( ^{14} \)C : 1 mCi/\( \mu \)mole) and 0.3 to 0.6 mg protein of retinal homogenates. After 90 min of incubation at 37\(^{\circ} \)C, the reaction was stopped by addition of 2 ml chloroform-methanol (2:1 v/v). The products were isolated by chromatography of the lipidic material on a small Sephadex G25 superfine column (1 g, \( \phi \) 1 cm). Elution was performed first with 2 x 2.5 ml chloroform-methanol-water (60:30:4.5 v/v/v) and then with 2 ml chloroform-methanol (2:1 v/v). CMP-sialic acid was recovered in the 2 x 2.5 ml methanol-water (1:1 v/v) fraction. Thin-layer chromatography was performed on DC-Fertigplatten Kieselgel 60 from Merck in a Camag sandwich chamber. The chromatographic mixture consisted of chloroform-methanol-0.25 % KCl (60:35:8 v/v/v). The chromatography lasted for 4-5 h. The labelled compounds were scanned on the TL Scanner II LB 2723 from Berthold (Germany). A parallel determination of the radioactivity of the spots was obtained using a liquid scintillation spectrometer with the Kieselgel dispersed in a liquid phosphor blend (10 ml of 0.4 % omnifluor in toluol). Densitometry on Vernon densitometer was performed after spraying with resorcinol (6,7). Identification of the synthetized compounds was confirmed by cochromatography with standard gangliosides.

RESULTS

The ontogenetic behaviors of UDP-glucose:ceramide glucosyltransferase (CGluT) and UDP-galactose:glucosylceramide galactosyltransferase (CGLuGalT) activities in chicken retina are shown in Fig. 1. Both enzymes involved in the primary steps of the synthesis of the gangliosides showed a rapid increase in activity from 8-day-old embryos to a maximum for 10-11 day-old embryos. CGluT activity then decreased until 4 days after hatching followed by a second maximum at 10 days. The activity then slowly decreased until adulthood. CGLuGalT in contrast had its maximum at the 11th day of embryonic life and decreased toward its value at adulthood, where it attained 20 \% of maximal activity.

Both enzymes showed their highest activity during the embryonic