GANGLIOSIDES OF WHOLE RETINA AND ROD OUTER SEGMENTS

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1. Introduction

Few studies have been done on the gangliosides of both the whole retina and the outer segments of the rod photoreceptor cells. Lowry et al. [1] suggested that gangliosides may be present in the rod outer segments (ROS). The presence of gangliosides in the whole retina of the ox and calf has been reported by Kostic et al. [2] and Handa and Burton [3]. Very recently Holm et al. established the structure of the major gangliosides from the whole retina of three mammalian species: human, ox and rabbit [4].

We report here a qualitative and quantitative analysis of the gangliosides from the whole retina of two mammals (calf and rat) and a bird (chicken). We also report an analysis of the gangliosides of the calf ROS. The ganglioside composition of the chicken retina is entirely different from that of the two mammals; the ganglioside distribution of the calf ROS is similar to the whole retina.

2. Materials and methods

2.1. *Chemicals*

All organic solvents (saturated in nitrogen) were of analytical grade. Other chemicals were of the highest available commercial purity. Pre-coated silica gel plates, type F_{254}, were obtained from merck AG, Darmstadt, W. Germany.

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2.2. *Retina*

For each experiment on whole retinas, 54 retinas from adult Wistar rats, 3–5 Hubbard chicken retinas (10 weeks-old) and 2 calf retinas were used. Rats and chickens were decapitated and eyes removed. The calf eyes were obtained from a local slaughterhouse within 2 min of the death of the animal. The retinas were dissected immediately after the removal of the eyes and placed in chloroform–methanol (2:1) v/v). For the preparation of ROS, 40 calf eyes were taken and the retinas removed also immediately after the death of the animal, were placed in 37% (w/v) sucrose. ROS were prepared as previously described [5] and all the other steps performed in ambiant light.

2.3. *Isolation and analysis of gangliosides*

Total lipids of either the whole retina or the ROS were extracted in chloroform–methanol mixtures and gangliosides partitioned into upper aqueous phases [6, 7].

The pooled upper phases were dialysed 5 times for 3 hr each, against distilled water at 4°C. The dialysate was lyophilised and then dissolved in a small volume (3–5 ml) of $CHCL_3$–CH_3OH (2:1 v/v). After 2 hr the liquid was centrifuged to remove undissolved non-lipid material [8] and the supernatant evaporated to dryness under N_2. The dry lipid material was hydrolysed in 2.5 ml of 0.4 M KOH in methanol at 37°C for 1 hr. The hydrolysate was again dialysed as before, lyophilised and dissolved in a known small volume of chloroform–methanol (2:1 v/v).

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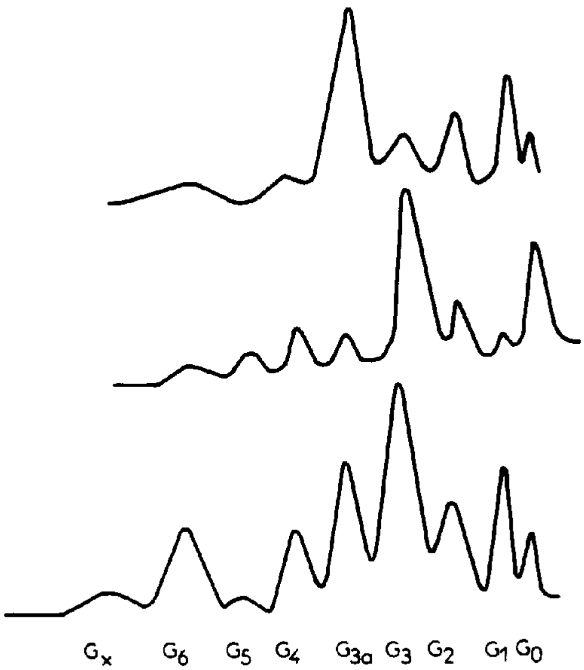

Fig. 1. Densitometric scanning of chromatogram of retinal gangliosides: Top, calf retina; Centre, references gangliosides obtained from porcine brain to which were added GM_2 and GM_3 obtained from Tay-Sachs diseased human brains and rat spleen, respectively; Bottom, chicken retina.

The gangliosides were quantitated by the resorcinol method [9,10] and separated by thin layer chromatography [11] using about 10 μg of total *N*-acetylneuraminic acid (NANA). The resorcinol reagent spray [12] was used to locate the gangliosides which were identified by running markers in parallel. The relative distribution was calculated from profiles obtained by reflective densitometry using a Vernon densitometer (fig. 1). Other determinations made were protein according to Lowry et al. [13] and cholesterol according to Grigaut [14].

3. Results and discussion

The amount of gangliosides in chicken, rat and calf is given in table 1. The NANA ganglioside content per retina in the chicken is smaller than in the calf and higher than in the rat. Compared to the rat the calf retina contains 39 times more NANA and the chicken 5.5 times as much. These ratios approximately represent the proportion of the fresh weight of the calf retina to the rat (31 times) and chicken to rat (5.5 times). The amounts of NANA in nmole per g wet weight are of the same magnitude in the three species.

When the concentration of NANA is expressed per mg of protein, the three species are remarkably similar, each with values of about 6.5 nmole NANA/mg protein. The ganglioside concentration in the calf retina is similar to the values reported for the ox [3,4], and the NANA level in chicken retina is comparable to that of the rabbit [4]. In all three species studied here the concentration of NANA in the retina (0.5 μmole/g wet weight) is about 5 times lower than in whole brain [15,16]. However it is well known that gangliosides are effectively only in the gray matter. If the ganglioside content of the retina is expressed per μg of cholesterol, a higher value is obtained for the

Table 1
Concentration of ganglioside NANA in chicken, rat and calf whole retinas, and calf ROS.

	Per retina	Per g wet weight	Per mg protein	Per μg cholesterol
Chicken retina*	34.1 ± 1.7	399 ± 16	6.8 ± 0.5	0.10 ± 0.01
Rat retina**	6.0 ± 1.2	483 ± 39	5.9 ± 0.9	0.23 ± 0.05
Calf retina**	233 ± 10	579 ± 34	6.5 ± 0.8	0.18 ± 0.03
Calf ROS***	2.6 ± 0.2	270 ± 32	2.9 ± 0.5	0.075 ± 0.007

Results are expressed in nmole.
*Means ± S.D. of 4 experiments
**Means ± S.D. of 5 experiments.
***Means ± S.D. of 3 experiments.

Table 2
Distribution of gangliosides in chicken, rat and calf whole retinas, and in calf ROS.

Gangliosides I II	Chicken retina*	Rat retina**	Calf retina**	Calf ROS***
G_x †	2.4 ± 0.4	–	–	–
G_6 – GM_3	16.5 ± 1.1	6.1 ± 0.8	1.0 ± 0.4	4.2 ± 1.1
G_5 – GM_2	0.7 ± 0.3	–	–	–
G_4 – GM_1	8.5 ± 0.4	2.7 ± 0.7	3.3 ± 0.3	3.2 ± 0.8
G_{3A} – GD_3	15.2 ± 0.5	36.5 ± 3.4	44.6 ± 3.3	52.5 ± 3.7
G_3 – GD_{1A}	30.9 ± 1.8	11.8 ± 1.9	13.7 ± 2.0	7.1 ± 1.2
G_2 – GD_{1B}	8.4 ± 0.4	17.7 ± 2.2	14.9 ± 1.1	14.6 ± 0.6
G_1 – GT_1	12.8 ± 0.3	15.3 ± 1.7	16.2 ± 1.7	13.4 ± 0.8
G_0 – GQ_1	4.6 ± 0.3	9.9 ± 1.5	6.3 ± 0.5	6.0 ± 0.9

Results are expressed as percentage of total NANA recovered.

I: Korey and Gonatas nomenclature (1963) [22].
II: Svennerholm nomenclature (1963) [23].
† Unidentified ganglioside; this spot runs over the G_6 – GM_3.
* Means ± S.D. of 4 experiments.
** Means ± S.D. of 5 experiments.
*** Means ± S.D. of 3 experiments.

rat than the calf, which is in turn higher than the chicken; these differences reflect the different amounts of cholesterol in each of the three species.

The retinal ganglioside pattern for the three species is summarised in table 2. The distribution in the two mammals is similar but an entirely different pattern is found in the chicken. In calf and rat retinas the disialosyllactosyl ceramide, GD_3, is at the highest concentration; it accounts for 37–45% of the total. In both species GD_{1A}, GD_{1B} and GT_1 each accounts for 12–18% of the total gangliosides. The major differences between the rat and calf retinas are in the two minor gangliosides, GQ_1 and GM_3; the rat (fig. 2) contains nearly twice as much of the former and 6 times more of the latter.

The most striking difference between the chicken and the two mammals in the ganglioside distribution, concerns the major ganglioside. In the chicken it is the disialosyltetraglycosyl ceramide GD_{1A}, which accounts for 31% of the total gangliosides, while GD_3 accounts for only 15%. Other notable differences concern the other disialosyltetraglycosyl ceramide, GD_{1B}, which is at a lower amount, and the higher value of the monosialosyl-*N*-tetraglycosyl ceramide, GM_1, and the monosialosyllactosyl ceramide, GM_3.

On the thin layer chromatogram (fig. 2) of the gangliosides from the chicken retina, 8 bands migrated parallel to the reference compounds. An additional spot, named G_x (2.4% of the total) which migrated farther, than the GM_3 standard, was revealed with the resorcinol spray. We were also able to detect in the chicken retina the monosialosyl-*N*-triglycosyl ceramide, GM_2 (0.7%). This was not seen in the other two species.

Previous studies on the ganglioside distribution of ox, calf and rabbit retinas [2, 3, 17] have yielded imprecise figures as a solvent mixture was employed which does not separate GM_1 and GD_3. Recent work by Holm et al. [4] used another solvent mixture for the analysis of the ganglioside pattern in human, ox and rabbit retina. Their results for the distribution of GD_3, GD_{1A}, GD_{1B} and GT_1 are of the same order of magnitude as ours on the calf and rat. They succeeded in separating a fraction from GD_3 which migrated as GM_1 (3–5%). Although their GM_3 value is higher than ours, they do not mention the tetrasialoganglioside, GQ_1. The three major brain gangliosides, GD_{1A}, GD_{1B} and GT_1 [7, 18] are present in the retina of the three species studied by Holm et al. [4].

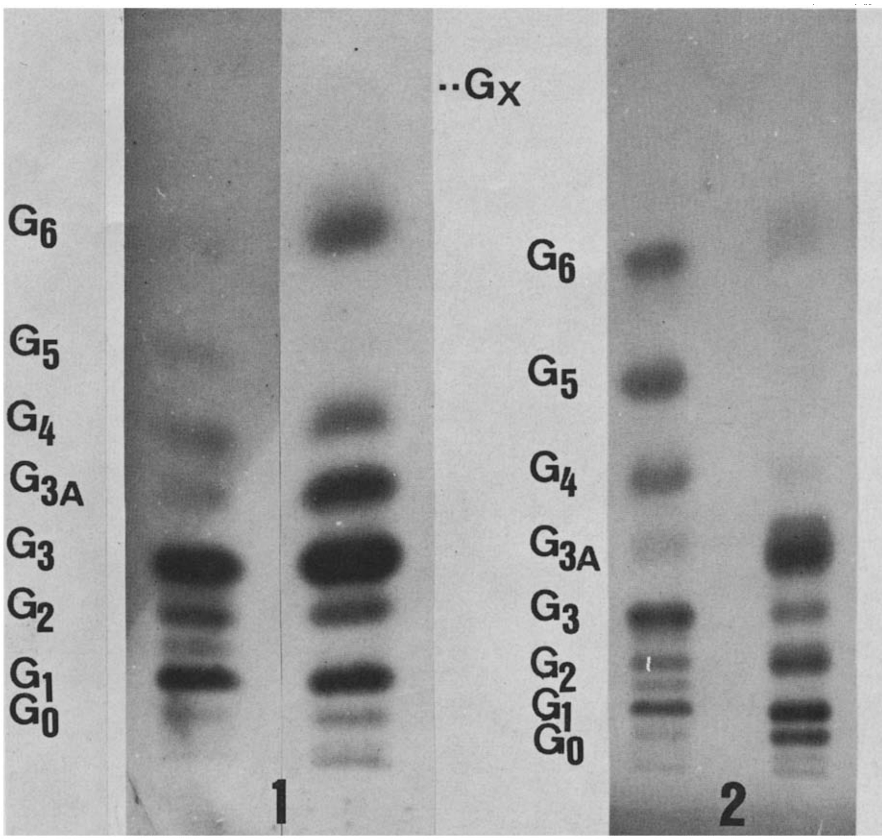

Fig. 2. Thin layer chromatography of ganglioside extracts of retina: 1) Chicken retina with markers on the left side; 2) Rat retina with markers on the left side.

Very few tissues appear to contain a very high amount of GD_3 [19,20].

The results concerning ganglioside NANA in calf ROS are expressed in the same units as for the whole retina (table 1). The first general observation is that the NANA content per retina is very low (about 2.5 nmole) and represents only 1% of the whole retina. However, when the results are expressed per g of ROS wet weight, per mg ROS protein or per μg ROS cholesterol, the ganglioside NANA concentration is about 50% of that of the whole retina when the latter is measured by the same units.

There are few results in the literature about gangliosides in the ROS. Early work, which was mainly concerned with the histochemical localisation in the different layers of the retina, seemed to indicate that gangliosides were present in the ROS [1]. More recently gangliosides were identified in the frog photoreceptors [21] by chemical analysis.

The same gangliosides are present in calf ROS as in the whole calf retina (table 2). The most important ganglioside is GD_3, which accounted for more than 50% of the total NANA. Relative to the total ganglioside content GD_{1A} is only half as abundant, and GM_3 four times as abundant in the ROS compared to whole calf retina. Only two gangliosides were reported to be present in frog photoreceptors [21]. The major one was GD_{1B} and the minor GT_1. This rather important discrepancy may be related to the morphological and functional differences between the outer segments of the two species; the frog retina contains green and red rods as well as cones.

Although the retina is derived embryologically from the central nervous system, the ganglioside concentration is only 20% of that in the brain. Three of the four major gangliosides of the mammalian brain are also present in high amounts in rat and calf retina.

The ganglioside pattern of the chicken retina is very different to that of mammals. This may be related to the difference in the chromoproteins in the outer segments; iodopsin in cones as well as rhodopsin in rods, in the chicken. Finally, it would be of interest to know the ganglioside composition of the plasma membrane of the ROS and the actual photoreceptor disks.

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