Neuritogenic Activity of Gangliosides from Echinoderms and Their Structure–Activity Relationship

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The effects of the gangliosides isolated from echinoderms on the neuritogenesis of a rat pheochromocytoma cell line (PC-12 cells) in the presence of nerve growth factor were investigated. The results show that they displayed neuritogenic activity. Based on the observed results, a structure–activity relationship has been established.

Key words ganglioside; echinoderm; neuritogenic activity; structure–activity relationship

Gangliosides, sialic acid containing glycosphingolipids, have received much attention owing to their biological functions.[1)] Meanwhile, it is known that the gangliosides present in echinoderms possess unique structures,[2—4)] and therefore they can be expected to represent components of pharmacological interest. A series of studies on the isolation and structural elucidation of gangliosides from echinoderms have been performed in our laboratory, and a ganglioside has been found to support the survival of cultured neuronal cells[5)] and an another ganglioside showed neuritogenic and growth-inhibitory activities toward the mouse neuroblastoma cell line (Neuro 2a).[6)] In this paper, we report a biological profile on the neuritogenic activity toward the rat pheochromocytoma cell line PC-12 in the presence of nerve growth factor of the so far isolated thirteen gangliosides from echinoderms and their structure–activity relationships.

Experimental

Gangliosides Gangliosides used in this study were isolated from starfish and sea cucumber species, and their structures are shown in Fig. 1 according to the number of sialic acid. Although the ceramide moieties of the gangliosides of echinoderms are various and the effects of them to the biological activities can not be ignored, they are expressed only as ceramide to simplify this study. The structures of the ceramide moieties of each ganglioside are shown in Fig. 2. Gangliosides and their origin are as follows. SJG-2 and SJG-1[7,8)]: sea cucumber *Stichopus japonicus*, LLG-5 and LLG-3[9,10)]: starfish *Linckia laevigata*, GAA-7[6)]: starfish *Asterias amurensis versicolor*, HLG-2, HLG-3, and HLG-1[11)]: sea cucumber *Holothuria leucospilota*, LMG-4 and LMG-2[12,13)]: starfish *Luidia maculata*, GP-3[14)]: starfish *Asterina pectinifera*, AG-2 and AG-3[15,16)]: starfish *Acanthaster planci*.

Generally speaking, gangliosides from starfish possess trisacharide moiety **NeuAc(Gc)**α2→3Galβ1→4Glc as basic structure of sugar part, meanwhile gangliosides from sea cucumber have disaccharide moiety **NeuAc(Gc)**α2→6Glc. On the other hand, both SJG-2 and GAA-7 have unique tetrasaccharide **NeuAc(Gc)**α2→3GalNAcβ1→3Galβ1→4Glc as basic sugar moiety.

Biological Assay The neuritogenic activity of gangliosides on PC-12 cells was observed according to a method previously reported.[7)]

Results and Discussion

The effects of the above mentioned gangliosides on the neuritogenesis of PC-12 cells were investigated. The results show that they displayed neuritogenic activity in the presence of nerve growth factor (NGF). The proportion of cells with neurites longer than the diameter of the cell body of the gangliosides at a concentration of 10 μM was shown in Table 1 when compared with the control (NGF 5 ng/ml: 20.6±2.2%). The evaluation of the activity led to the following correlation between structure and activity.

(1) The presence of sialic acid (NeuAc or NeuGc) is essential since CDH (ceramide lactoside)[17)] has no activity.

(2) The presence of two terminal sialic acids plays cru-

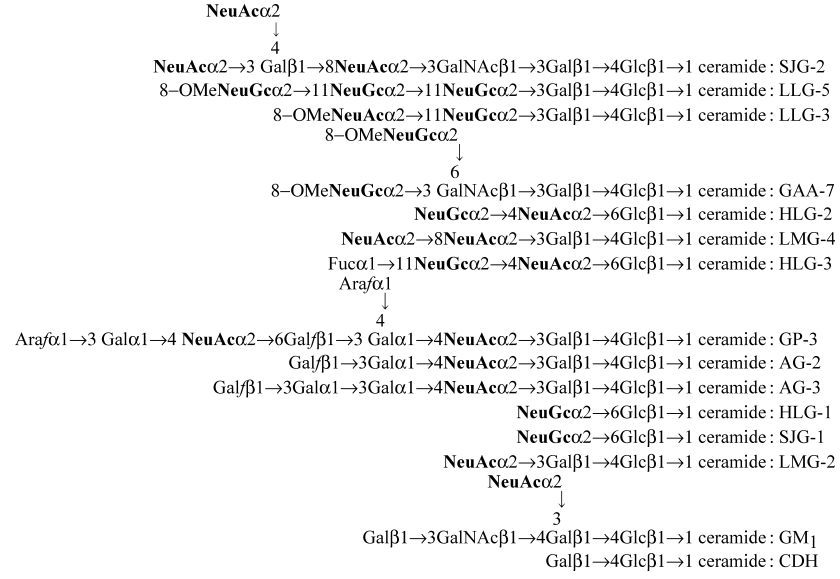

Fig. 1. Gangliosides from Echinoderms

Bold: sialic acid, GM_1: mammalian ganglioside, CDH: ceramide lactoside.

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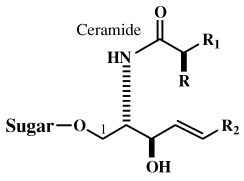
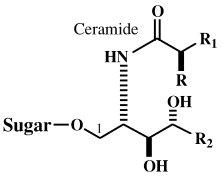


HLG-2: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_9CH(CH_3)_2$
GM_1 : **R** = **H**, R_1 = -$(CH_2)_{15}CH_3$, R_2 = -$(CH_2)_{12}CH_3$



SJG-2: **R** = **H**, R_1 = -$(CH_2)_{15}CH_3$, R_2 = -$(CH_2)_{10}CH(CH_3)_2$
SJG-1: **R** = **H**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_{10}CH(CH_3)_2$
LLG-5: **R** = **OH**, R_1 = -$(CH_2)_{19}CH_3$, R_2 = -$(CH_2)_{13}CH_3$
LLG-3: **R** = **OH**, R_1 = -$(CH_2)_{20}CH_3$, R_2 = -$(CH_2)_{12}CH_3$
GAA-7: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_x$-CH=CH-$(CH_2)_y CH_3$ (x + y = 15)
HLG-3: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_{10}CH(CH_3)_2$
HLG-1: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_{10}CH(CH_3)_2$
LMG-4: **R** = **OH**, R_1 = -$(CH_2)_{19}CH_3$, R_2 = -$(CH_2)_{12}CH_3$
LMG-2: **R** = **OH**, R_1 = -$(CH_2)_{19}CH_3$, R_2 = -$(CH_2)_{14}CH_3$
GP-3: **R** = **OH**, R_1 = -$(CH_2)_{19}CH_3$, R_2 = -$(CH_2)_{10}CH(CH_3)_2$
AG-2: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_{11}CH_3$
AG-3: **R** = **OH**, R_1 = -$(CH_2)_{21}CH_3$, R_2 = -$(CH_2)_{11}CH_3$
CDH: **R** = **OH**, R_1 = -$(CH_2)_{19}CH_3$, R_2 = -$(CH_2)_{12}CH_3$

Fig. 2. Structures of the Major Ceramide Moiety of Gangliosides

Table 1. Neuritogenic Activity of Gangliosides from Echinoderms toward PC-12 Cells

Compounds	Neurite bearing cells (%)
Trisialo-gangliosides	
SJG-2	64.8±7.6
LLG-5	59.3±6.1
Disialo-gangliosides	
LLG-3	63.1±6.3
GAA-7	61.2±2.3
HLG-2	48.4±3.0
LMG-4	47.7±2.3
HLG-3	45.2±4.3
GP-3	38.2±2.5
Monosialo-gangliosides	
AG-2	50.2±4.3
AG-3	45.7±3.1
HLG-1	44.7±3.5
SJG-1	35.4±4.0
LMG-2	32.7±5.0
GM_1	47.0±2.5
CDH	18.7±4.1

The concentration of the gangliosides was 10 μM with NGF (5 ng/ml). Each value represents the mean±S.E. (n=5).

cial role for strong activity as shown in SJG-2 and GAA-7.

(3) When compared the gangliosides possessing tandem disialoyl moiety (LLG-5, LLG-3, HLG-2, LMG-4, and HLG-3), gangliosides having 8-*O*-Me silalic acid (LLG-5 and LLG-3) showed stronger activity than the other gangliosides.

(4) Comparison of the monosialo-gangliosides indicates that gangliosides possessing sialic acid inside of the oligosaccharide moiety (AG-2 and AG-3) shows more strong activity than those having terminal sialic acid.

(5) The difference of the activity between HLG-1 and SJG-1, despite they have the same sugar moiety, must be occurred from the difference of the structure of their ceramide moieties.

(6) The effect of SJG-2, LLG-5, LLG-3, and GAA-7 are more considerable than that of mammalian ganglioside GM_1, which has been known to show positive effects in neurological diseases.[18)]

On the other hand, these gangliosides showed no activity without NGF. Therefore, it is suggested that these gangliosides are potentiated neuritogenesis activity of NGF.

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