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Gangliosides stimulate dome formation in cultured canine kidney epithelial cell line (MDCK)

N. Rodrig*, T. Osanai*, M. Iwamori* and Y. Nagai*+

*Department of Biochemistry, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 and +Department of Neurobiology, Institute of Brain Research, Niigata University, Niigata, Japan

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The effect of exogenous gangliosides on the occurrence of domes in MDCK cell cultures was investigated in view of the involvement of both dome formation and gangliosides in cell growth, differentiation and transepithelial transport. Dome formation was increased by gangliosides in medium free of fetal calf serum. Among the gangliosides tested, GM3 and GD3 isolated from porcine kidney were most active, increasing the dome number 12–17-fold. Since gangliosides from kidney were more active than those from brain and erythrocytes, the hydrophobic moiety as well as sialic acid might be involved in this activity. These results indicate that tissue-specific molecules of gangliosides function as inducers or mediators of dome formation. The mechanism probably involves adenylate- cyclase or another transmembrane biosignal-transducing system.

Ganglioside; Dome formation; Epithelial transport; (MDCK cell)

1. INTRODUCTION

The cultured homogeneous epithelial cell line (Madin-Darby canine kidney or MDCK cells) derived from proximal or distal renal tubules of the kidney retains specific physiological and biochemical features of the in vivo intact tissue, thus providing an excellent model for the study of epithelial transport phenomena [1,2]. The cells form a continuous sheet or monolayer on culture dishes, exhibiting the asymmetric morphological polarity and occluding junctions that are characteristic of renal tubular epithelium. MDCK cells cultured in the presence of serum spontaneously form domes, which are fluid-filled, multicellular hemicysts, slightly raised from the surface of the monolayer of cultured cells [2]. The occurrence of domes has been reported in many cell cultures of transporting epithelia [3,4].

Correspondence address: Y. Nagai, Department of Biochemistry, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Different studies suggest that the formation of domes represents vectorial transport phenomena [4], and that it may be related to the induction of growth and differentiation in cell culture systems [5], or to secretory functions of the cell [3].

The important role of glycolipids, specifically of gangliosides, in reactions occurring at the cell surface has been widely recognized in control of cell growth and proliferation [6], induction of differentiation [6,7], modulation of transport in epithelial cells [8], recognition, adhesion and antigenic activity [9,10].

This situation prompted us to examine a possible correlation between ganglioside function and dome formation. Here, we have tested the effect of exogenous gangliosides applied to the apical surface of the monolayer on dome formation in MDCK cells. The results indicate an important role for specific gangliosides as inducers or mediators in the mechanism of dome formation, the molecular characteristics of which will be further investigated.

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2. MATERIALS AND METHODS

2.1. *Materials*

Chemicals were from Sigma (St. Louis, MO) unless otherwise indicated. Arginine-vasopressin (AVP) was from the Peptide Institute (Tokyo). Forskolin was from Calbiochem (La Jolla, CA). Anti-asialo GM1 and anti-fucosyl GM1 antisera for TLC immunostaining were prepared in our laboratory by immunizing rabbits with the purified antigens, and their specificity confirmed by ELISA [11].

2.2. *Isolation of gangliosides*

Extraction of total lipids followed by fractionation into neutral and acidic lipids by DEAE-Sephadex A-25 (acetate form) (Pharmacia, Sweden) column chromatography and purification by Iatrobeads (silica gel) (Iatron Lab, Tokyo) column chromatography were carried out according to [11].

GM3(NeuAc), GM3(NeuGc), GD3, fucosyl-GM1, GD1b, GT1b and $IV^3NeuAc_nLcOse_4Cer$ (SPG) were isolated from porcine kidney. GM3(NeuAc) was also isolated from canine erythrocytes and GM3(NeuGc) from horse erythrocytes. Lactosyl ceramide and other gangliosides were prepared in our laboratory by similar procedures.

Synthetic GM3 was kindly donated by Dr T. Ogawa (Institute of Physical and Chemical Research, Wako) and synthetic sialyl cholesterol by Dr H. Ogura (Kitasato University, Tokyo).

2.3. *Structural determination of porcine kidney gangliosides*

The structures of purified gangliosides were determined by thin-layer chromatography, treatment with sialidase (*Vibrio cholerae*; Calbiochem) and formic acid, TLC immunostaining [12], gas-liquid chromatography of partially methylated aldohexitol acetates [13] and densitometric scanning.

2.4. *Cell cultures*

MDCK cells were kindly donated by Dr T. Matsumura (Institute of Medical Science, University of Tokyo, Tokyo). Cells were cultured in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo), supplemented with $NaHCO_3$, L-glutamine (Wako, Tokyo), streptomycin and penicillin (Meiji, Tokyo) and 10% fetal calf serum (FCS) (Gibco) at 37°C, 5% CO_2.

2.5. *Dome induction experiments*

Cells were seeded at a density of $10^4/cm^2$ on 24-well culture dishes and grown to confluency (3–4 days). Medium was replaced by FCS-free medium for 2 days, then changed again to FCS-free medium including varying concentrations of gangliosides or other substances. After further incubation for 3–4 days cells were washed with PBS, fixed with methanol, stained with Giemsa (Merck), and examined for dome formation. All experiments were conducted in quadruplicate. Domes were counted in the entire area of each well, and results are expressed as the mean number of domes/cm^2.

3. RESULTS

3.1. *Gangliosides of porcine kidney*

As shown in table 1, about 70% of the total lipid-bound sialic acid in porcine kidney was distributed in lactose containing gangliosides GM3 and GD3.

3.2. *Spontaneous dome formation*

MDCK monolayers cultured in standard medium containing 10% FCS exhibited marked spontaneous incidence of domes (200/cm^2). Deletion of FCS or lowering its concentration in the medium caused a significant decrease in number of domes (table 2). Subconfluent cultures did not

Table 1

Composition of porcine kidney gangliosides

	% sialic acid
GM3(NeuAc)	12.9
GM3(NeuGc)	5.9
Fucosyl-GM1	5.6
GD3	56.0
GD1b	4.5
$IV^3NeuAcnLcOse_4Cer$ (SPG)	11.7
GT1b	2.1
X[a]	1.0

[a] Ganglioside X, insufficient amount for characterization

Table 2

Induction of domes in MDCK cultures

Addition	Source	Concentration	Domes/ cm^2	Induction/ control
None (control)			32	
FCS		5%	86	2.7
FCS		10%	200	6.2
Total gangliosides	PK	25 μM	126	3.9
Total gangliosides	BB	25 μM	96	3.0
Total gangliosides	MK	25 μM	147	4.6
GM3(NeuAc)	PK	25 μM	525	17.8
GM3(NeuAc)	CRBC	25 μM	74	2.3
GM3(NeuAc)	Syn	25 μM	58	1.8
GM3(NeuGc)	HRBC	25 μM	89	2.8
GM3(NeuGc)	Syn	25 μM	78	2.4
GM2	BB	25 μM	70	2.2
Fucosyl-GM1	PK	25 μM	107	3.3
GD3	PK	25 μM	399	12.5
GD1b	PK	25 μM	195	6.1
GT1b	PK	25 μM	109	3.4
GT1b	BB	25 μM	114	3.6
SPG	PK	25 μM	121	3.8
Lactosyl ceramide	PK	25 μM	67	2.1
Sialic acid		25 μM	61	1.9
Sialyl lactose		25 μM	52	1.6
Colominic acid		25 μM	67	2.1
Sialyl cholesterol	Syn	25 μM	70	2.2
Cholera toxin		10 μg/ml	128	4.0
AVP		10 mU/ml	212	6.6
IBMX		2 mM	149	4.6
8-Bromo cyclic AMP		0.5 mM	112	3.5
Dibutyryl cyclic AMP		0.5 mM	131	4.1
Forskolin		10^{-4} M	169	5.3

PK, porcine kidney; BB, bovine brain; MK, monkey kidney; CRBC, canine erythrocytes; HRBC, human erythrocytes; Syn, synthetic; FCS, fetal calf serum; IBMX, 3-isobutyl-1-methylxanthine; AVP, arginine-vasopressin; SPG, IV^3NeuAc $LcOse_4Cer$; synthetic GM3 – [$II^3\alpha$NeuLacCer (*N*-lignoceroyl sphingosine)]; synthetic sialyl cholesterol – 3-*O*-αNeuAc-cholesterol. Gangliosides are designated according to Svennerholm's system [17]

form domes, and domes occurred only after the cell density exceeded 1.5–2 × 10^5 cells/cm^2. Three to twenty cells were responsible for forming a dome spontaneously as well as that induced by various substances. The border of a dome is clearly defined by a unique alignment of cells surrounding the hemicyst when the cells were fixed and stained with Giemsa (fig.1).

3.3. *Induction of domes*

After depletion of FCS from the medium, gangliosides and other substances of interest were tested for their ability to induce dome formation which started 24–36 h after addition of inducer. Domes were counted 40–54 h after addition of inducer. The results are summarized in table 2. The addition of gangliosides significantly increased the

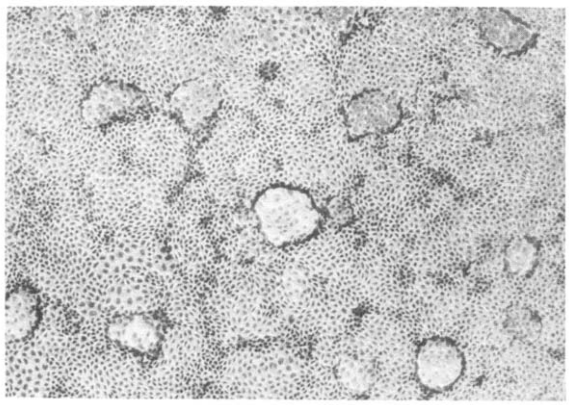

Fig.1. Dome formation in MDCK cells induced by GM3 of porcine kidney in FCS-free medium. Cells visualized by phase-contrast microscopy, after staining with Giemsa. Magnification 100 ×.

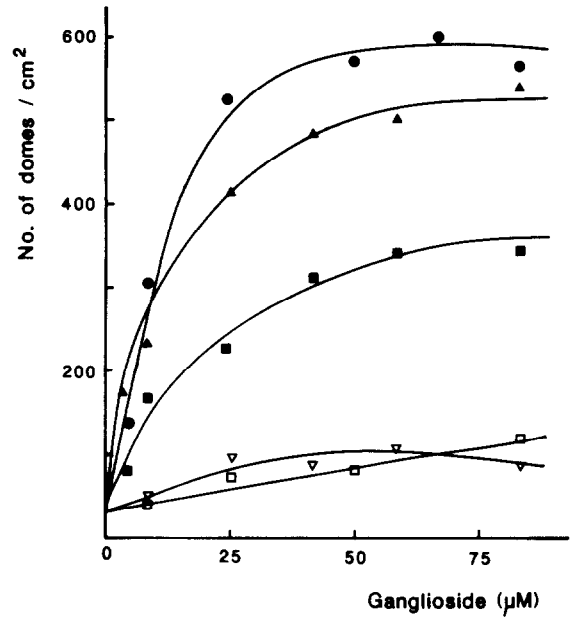

Fig.2. Effect of ganglioside concentration on dome formation. (●) GM3 (porcine kidney), (▲) GD3 (porcine kidney), (■) GD1b (porcine kidney), (□) total gangliosides (bovine brain), (▽) total gangliosides (porcine kidney).

number of domes. GM3, GD3 and GD1b from porcine kidney were most active, stimulating dome formation 17-, 12- and 6-fold, respectively. Porcine and monkey kidney gangliosides were more potent stimulants than those from bovine brain and erythrocytes, indicating some tissue specificity. Sialic acid and its derivatives caused slight increases in dome formation, whereas substances which increase cellular cyclic AMP levels stimulated dome formation 3–7-fold.

3.4. *Effect of ganglioside concentration on dome formation*

As shown in fig.2, the number of domes was dependent on ganglioside concentration and increased up to 50 μM GM3, GD3 and GD1b, giving the maximal level. Higher concentrations of gangliosides did not stimulate dome formation further.

4. DISCUSSION

In this study we have demonstrated the activity of gangliosides as inducers of dome formation in MDCK cells.

The glycosphingolipids of two strains of MDCK cells have been characterized [9,14,15]. These cells unequivocally contain acidic species, the major component being GM3, but levels of exogenous gangliosides inducing dome formation are 10–15-times higher than those of endogenous gangliosides [14,15].

The activity of compounds which elevate intracellular cyclic AMP levels as dome inducers, reported previously [5], was confirmed. However, the level of dome induction by these substances is much lower than that of the most active gangliosides. This either points to different mechanisms of dome formation by different groups of substances, or implies that gangliosides are indirect mediators in the process.

GM3, particularly of porcine kidney, was the most potent inducer of dome formation, but sialyl lactose or lactosyl ceramide was not active, indicating that the entire structure of GM3 (NeuAcα2→3Galβ1→4Glcβ1→1′ceramide) is required for this activity. In addition, GM3 from erythrocytes exhibited a relatively minor effect, suggesting that another prerequisite for activity is the ceramide portion having longer α-hydroxy fatty acids characteristic of kidney gangliosides.

Immunofluorescence studies showed a basolateral or intracellular localization of the

glycosphingolipids in MDCK cells [15], whereas in our experiments gangliosides were applied to the apical surface of the cells. This suggests that exogenous gangliosides are either metabolized or internalized by the cells although they do not redistribute to the basolateral membrane [16], or more likely act by affecting other components of the membrane.

Although the mechanism of ganglioside action is obscure at present, possible candidates for the action of gangliosides are the sodium channels which by changing the membrane permeability of Na^+ in association with particular gangliosides might induce dome formation. In this connection it is interesting to note a recent finding that gangliosides can modulate Na^+ transport in cultured kidney cells [8].

In summary our results show that gangliosides induce dome formation in epithelial cells. In future work, it is necessary to define some characteristic biochemical parameters rather than morphological features of the system. This approach will permit a more detailed study of the cellular functions involved in dome formation as demonstration of cell differentiation and transepithelial transport by gangliosides, as well as by other substances.

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