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The carbohydrate deposits detected by histochemical methods in the molecular layer of the dentate gyrus in the hippocampal formation of patients with schizophrenia, Down's syndrome and dementia, and aged person

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Post-mortem brain tissue was obtained from 28 patients with brain disorders, of which 15 had clinically diagnosed schizophrenia, 6 Alzheimer type dementia, 5 dementia with tangles and 2 cases of Down's syndrome. The controls were 22 cases from autopsies without brain disorders or with no known episodes of brain disorder. The tissues were stained for the detection of carbohydrate deposits in the hippocampal formation, using lectin, immunohistochemical and conventional staining methods. The staining revealed the existence of spherical deposits in the inner and middle molecular layers of the dentate gyrus in the hippocampal formation which contained fucose, galactose, N-acetyl galactosamine, N-acetyl glucosamine, sialic acid, mannose and chondroitin sulfate. The number of the deposits was higher in patients with brain disorder such as schizophrenia, Alzheimer type dementia, dementia with tangles or Down's syndrome, and in some aged individuals, in comparison to those in younger individuals. No deposits were detected in a few younger or aged individuals. Spherical deposits 3–10 μm in diameter may be an immature form of the corpora amylacea, since they were similar in the histochemical characteristics with lectin, immunohistochemical and conventional staining methods. However, differing staining ability by hematoxylin, periodic acid Schiff's reagent and antibodies against the intracellular degraded proteins such as ubiquitin and tau-protein was observed. The antibodies against ubiquitin and tau-protein showed clear reactivity with the corpora amylacea and no reactivity with spherical deposits, indicating that the corpora amylacea has an intracellular origin and spherical deposits an extracellular matrix origin. The results obtained in this study indicate that not only neuronal degeneration but also unusual glycometabolism in neurons may disturb the neuronal function and cause brain disorders, and that spherical deposits may cause dysfunction of the neuronal network in the dentate gyrus of the hippocampus which is closely linked with recognition and memory functions.

Keywords: **glycoconjugates, degenerative changes, brain tissue, Alzheimer type dementia, Down's syndrome, schizophrenia, lectin-histochemical staining**

Introduction

The development during embryogenesis is a most complex morphogenetic process in cell-cell recognition in which glycoconjugates have been implicated to play a major role [1]. However, the number of diseases known to be caused by abnormalities in sugar chains has expanded tremendously in recent years. A distinguishing trait of these newly described

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Table 1. Summary of clinical and/or pathological diagnosis of individuals and results obtained by histochemical staining in the hippocampal formation

Case no.	*Autopsy no.*	*Age at death, Y*	*Sex*	*Cause of death*	*Postmortem interval, h*	*SP*[6]*	*NFT*[6]*	*Carbohydrate desposits*			
								Vas[3]	*Str[3]*	*CA*	*SPD*
Schizophrenia, with clinical diagnosis											
1	N-116	21	M	Malignant syndrome	10	−	−	−	−	1+	3+
2	N-221	25	M	Asphyxia, accident	21	−	−	−	−	−	2+
3	98-44	30	M	Asphyxia, homicide	11	−	−	−	−	2+	4+
4	44154	32	F	Poisoning, suicide	9	−	−	−	−	2+	3+
5	N-80	32	F	Burned in a fire, suicide	9	−	−	−	−	2+	3+
6	N-147	32	M	Abdominal wound, suicide	20	−	−	−	−	2+	3+
7	N-97	32	M	Poisoning, suicide	15	−	−	−	−	3+	3+
8	N-90	33	M	Wound in the neck, suicide	12	−	−	−	−	−	2+
9	N-163	37	M	Bronchopneumonia	9	−	−	−	−	−	4+
10	99-21	44	M	Asphyxia, homicide	9	−	−	−	1+	3+	4+
11	N-130	44	F	Cardiac death	32	−	−	−	−	2+	3+
12	N-34	45	M	Cardiac stab wound, suicide	13	−	−	−	−	−	3+
13	N-214	47	F	Asphyxia, accident	36	−	−	−	−	4+	4+
14	N-212	48	M	Bronchopneumonia	17	−	−	−	−	3+	4+
15	44153	55	M	Pneumonia	11	−	−	1+	−	2+	3+
Pathologically diagnosed Alzheimer type dementia [4]											
16	42208	63	F	Cardiac death	16	2+	1+	−	−	2+	−
17	N-157	74	M	Pneumonia	16	4+	4+	1+	2+	4+	4+
18	N-0	76	F	Cardiac death	11	4+	4+	3+	1+	4+	3+
19	N-29	80	F	Cardiac death	6	4+	4+	−	1+	2+	2+
20	HS	84	F	Cardiac death	20	4+	4+	−	−	−	4+
21	98-17	91	F	Cerebral contusion, homicide	24	3+	3+	−	2+	2+	1+
Pathologically diagnosed dementia with neurofibrillary tangles [4]											
22	43220	62	M	Cerebral hemorrhage	10	−	4+	3+	3+	4+	−
23	43051	69	M	Cardiac death	4	−	3+	−	−	3+	4+
24	N-86	82	F	Cardiac death	6	−	4+	−	3+	4+	3+
25	97-47	83	M	Hypothermia, accident	72	2+	4+	−	−	4+	2+
26	N-53	88	F	Cardiac death	10	−	3+	−	1+	2+	4+
Down's syndrome											
27	41520	32	M	Cardiac death	12	4+	−	3+	3+	3+	3+
28	99-76	44	F	Cardiac death	8	4+	−	2+	3+	2+	1+
Adult individual, with unknown episode											
29	99-29	22	F	Brainstem laceration, accident	9	−	−	−	−	−	−
30	99-27	24	M	Drowning, accident	96	−	−	−	−	−	−
31	97-43	31	M	Cardiac death	7	−	−	−	−	2+	−
32	97-42	33	F	Pulmonary embolism	11	−	−	−	−	−	1+
33	42206	35	M	Pneumonia	38	−	−	−	−	−	1+
34	N-13	35	M	Cardiac death	5	−	−	−	1+	2+	1+
35	41700	36	M	Cerebral hemorrhage	33	−	−	−	−	2+	−
36	43052	38	M	Cardiac death	4	−	−	1+	−	3+	−
37	N-153	43	M	Subarachnoidal hemorrhage	13	−	−	−	−	2+	−
38	42014	45	M	Cardiac death	12	−	−	−	−	2+	1+
39	97-12	46	M	Aortic laceration, accident	8	−	−	2+	−	2+	−
40	N-129	47	M	Cardiac death	8	−	−	−	1+	3+	−
41	99-04	48	M	Burned in a fire, accident	22	−	−	−	2+	−	−
42	98-02	55	F	Drowning, suicide	40	−	−	−	−	1+	−
43	42171	62	M	Cardiac death	15	−	−	−	−	−	−
44	97-33	64	F	Cerebral contusion, accident	12	−	−	−	−	1+	−

Table 1 (*Continued*)

Case no.	*Autopsy no.*	*Age at death, Y*	*Sex*	*Cause of death*	*Postmortem interval, h*	*SP*[6]*	*NFT*[6]*	*Carbohydrate desposits*			
								Vas[3]	*Str[3]*	*CA*	*SPD*
Aged individual, with unknown episode											
45	43502	73	M	Pneumonia	5	2+	−	−	2+	−	−
46	N-164	73	M	Subarachnoidal hemorrhage	10	3+	−	1+	1+	3+	2+
47	42313	73	M	Cardiac death	17	−	−	−	−	2+	−
48	42349	79	M	Cardiac death	36	−	−	−	−	3+	−
49	N-68	80	M	Drowning, accident	14	−	1+	−	1+	3+	−
50	N-33	83	M	Hepatic cancer	5	−	−	−	1+	2+	−

M: male, F: female, SP: Senile plaques, NFT: Neurofibrillary tangles, *: detected by Gallyus-Braak silver stain, Vas: Vascular type of carbohydrate deposits, Str: Stratiform type of carbohydrate deposits, CA: corpora amylacea, SPD: Spherical shape of carbohydrate deposit. Staining intensity of SP, NFT, Vas and Str was the results from previous studies [3,6]. Number of carbohydrate depositions from CA was expressed in figures, i.e. 4+: over 100 deposits, 3+: over 50 deposits, 2+: over 10 deposits, 1+: not greater than 10 deposits and − : no deposit in the whole area of hippocampal formation and parahippocampal gyrus. Number of carbohydrate depositions from SPD was expressed in figures, i.e. 4+: over 100 deposits, 3+: over 50 deposits, 2+: over 10 deposits, 1+: not greater than 10 deposits and − : no deposit in the whole area of the molecular layer of the dentate gyrus.

diseases is that they are related to abnormalities in the biosynthesis of the sugar chains [2]. Abnormal accumulation or deposition of the sugar chains in brains of patients with neuro-degenerative diseases was also reported [2]. In our previous study we showed that amorphous depositions, which are composed of vascular and stratiform type, associated with sugar chains were observed in the white matter of brains of patients with Alzheimer type dementia and Down's syndrome, in addition to the existence of sugar chains detected in senile plaques, neurofibrillary tangles and corpora amylacea in the brains of patients with the diseases and aged persons [3]. In this study we show that spherical deposits associated with sugar chains in the molecular layer of the hippocampal formation, may play a key part in the perturbation of the memory of the patients with schizophrenia, Alzheimer type dementia, Down's syndrome, and/or related diseases.

Materials and methods

Brain tissue sections from the hippocampus were routinely obtained at autopsy to prepare tissue sections for the pathology diagnosis in our departments. In this study we examined the hippocampal formation from 50 individuals. The cause of death, postmortem interval, symptoms and episode of the individuals are given in Table 1 of which 15 (cases 1 to 15) had been clinically diagnosed and under medical treatment for schizophrenia. Two individuals were clinically diagnosed as having Down's syndrome (cases 27 and 28) and the rest were patients with dementia, (cases 16 to 26), and individuals with other diseases or unknown episodes (cases 29 to 50). In these cases, we found no genetic disorder, e.g., Krabbe's disease, Gaucher's disease, CDGS (Carbohydrate Deficient Glycoprotein Syndrome) etc. in which a metabolic enzyme deficiency or an altered glycoprotein has been characterized as the cause.

The tissue samples were fixed in 10% formalin, dehydrated in graded ethanol series, and embedded in paraffin. For the histochemical study, serial paraffin sections were cut at 6 μ, mounted, deparaffinized, hydrated and stained by means of the following conventional, lectin and immunohistochemical methods. Staining methods by Periodic acid Schiff's reagent (PAS), Alcian blue (AB) at pH 1.0 and at pH 2.5 were performed in order to detect neutral, acidic or sulfate glycoconjugates in the depositions, respectively. The reactivity with 10 lectins (Table 2) conjugated by peroxidase or biotin was also examined to determine the nature of saccharide residues in spherical depositions and corpora amylacea, and the distribution pattern of these depositions. Control for lectin staining and negative controls for immunostaining were carried out as described previously [4,5]. Antibodies (Table 2) against chondroitin sulfate, heparan sulfate, CD 15 (Lewis x), β amyloid, tau protein, ubiquitin, amyloid component P, glial fibrillary acidic protein (GFAP), human IgG and alanyl aminopeptidase [6] were also employed to detect the correspondent epitope in spherical deposits and corpora amylacea. Binding with lectins and antibodies was performed as reported previously [3–9] and peroxidase activity was visualized with 3-3-diaminobenzidine-hydrogen peroxidase medium. Vascular and stratiform-type carbohydrate deposits have been previously reported [3]. Senile plaques and neurofibrillary tangles in the hippocampal formation from all individuals utilized in this study were detected and divided into five groups, from − (negative) to 4+ (strongly positive), by means of our method using two kinds of triple staining methods. In these methods, two serial sections were stained firstly with Galleys–Braak silver stain, secondly by anti-β amyloid antibody and by antibodies against tau protein or GFAP as in final steps, respectively [7]. According to the results from the two triple stainings, individuals who were suspected to be patients with dementia were pathologically diagnosed as Alzheimer type dementia in which both senile plaques and neurofibrillary tangles were obviously detected or non-Alzheimer type dementia with neurofibrillary tangles, in

Table 2. Carbohydrate binding specificity of lectins used in this study

Lectin and antibodies	*Abbreviation*	*Carbohydrate binding specificity and epitope of antibodies*	*Company*	*Concentration*
Archis hypogaea agglutinin	PNA	Gal(β1,3)GalNAc	Sigma	0.5 mg/ml
Canavalia ensiformis agglutinin	Con A	Branched α-Man	E.Y.	0.5 mg/ml
Datura stramonium	DSA	GlcNAc	Sigma	0.5 mg/ml
Dolichos biflorus agglutinin	DBA	α-GalNAc	E.Y.	0.5 mg/ml
Erythrina cristagalli	ECA	Gal(β1,3)GlcNAc	E.Y.	0.5 mg/ml
Glycine max agglutinin	SBA	GalNAc(α1,3)Gal	E.Y.	0.5 mg/ml
Griffonia simplicifolia iso agglutinin I-B4	GSA-I-B4	α-Gal	E.Y.	0.5 mg/ml
Pisum sativum agglutinin	PSA	Branched α-Man with α-Fuc as determinent	E.Y.	0.5mg/ml
Triticum vulgare	WGA	Manβ(1,4)GlcNAc(1,4)GlcNAc	E.Y.	0.5 mg//ml
Ulex europaeus agglutinin	UEA-I	α-Fuc	E.Y.	0.5 mg/ml
Anti-chondroitin sulfate		[GlcAβ (1,3)GalNAc]n	Seikagaku Kogyo	0.05 mg/ml
Anti-heparan sulfate		[GlcAβ (1,4)GlcNAc]n	Seikagaku Kogyo	0.05 mg/ml
Anti-Lewis x (CD15)		Galβ (1,4)(Fuc 1,3)GlcNAc	Dako	1:200
Anti-lactoferin		Human lactoferin	Dako	1:400
Anti-component P		Component P of human amyloid	Dako	1:400
Anti-tau		Tau	Dako	1:800
Anti-ubiquitin		Ubiqichin	Dako	1:800
Anti-human IgG		Human IgG	Dako	1:400
Anti-alanyl aminopeptidase		Human alanyl aminopeptidase	Yamamoto, Y *et al.* [6]	1:400

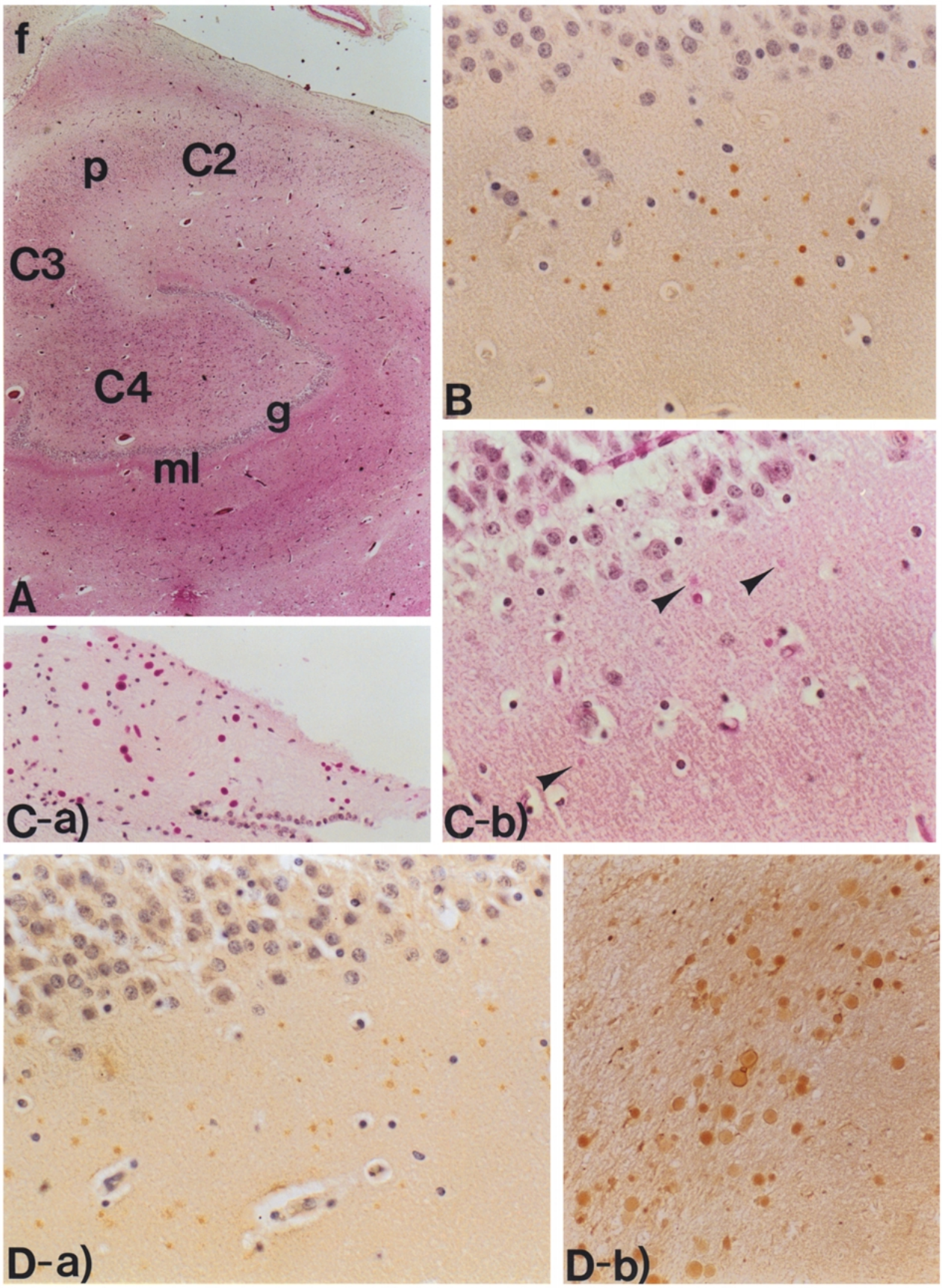
Man: Mannose, Gal: galactose, Fuc: Fucose, GalNAc: N-acetylgalactosamine, GlcNAc: N-acetylglucosamine.

which neurofibrillary tangles with or without a few senile plaques gave an intensive reaction. The existence of senile plaques and neurofibrillary tangles in the hippocampal formation was additionally detected by lectin stains [3]. The frequency of the presence in the hippocampal formation, the cerebellum and the white matter of the frontal lobe from each individual studied was divided into 5 groups and expressed in figures. The frequency of spherical deposits and corpora amylacea detected by lectin stains was also divided into 5 groups according to the presence in the molecular layer of the hippocampal formation for spherical deposits, and in the total area of the hippocampal formation for corpora amylacea on each of three slides stained by DBA, GSAIB4 and UEAI lectins, respectively, since these lectins intensively stained blood vessels of donors corresponding to their ABO blood group specificity, so failing to distinguish spherical deposits and corpora amylacea from small blood vessels. We counted the number of deposits in a serial section from individuals, stained by 7 lectins (PNA, Con A, DBA, ECA, SBA, GSI-B4 and UEA-I). We divided them into 5 groups, according to the mean number of the deposits, that is, 4+; over 100 deposits, 3+; over 50 deposits, 2+; over 10 deposits, 1+; under 10 deposits and −; no deposits were observed in the whole of the dentate gyrus.

Results

Spherical deposits with 3 to 10 μ in diameter detected by lectin stains were observed mainly within the inner and middle molecular layer of the dentate gyrus of the hippocampal formation. Corpora amylacea 10 to 100 μ in diameter detected by lectins were found mainly in the sub-pial and the fimbria of the hippocampus. Although the number of deposits composed of spherical deposits and/or corpora amylacea varied among the patients or individuals, the appearance was distinctive in the respective regions from all patients with Schizophrenia,

Figure 1. (A) The hippocampal formation stained by Hematoxylin Eosin. The granular (g), pyramidal (p) cell and molecular (ml) layers and the fimbria (f) are shown, and C2, C3 and C4 indicate anatomical regions of the hippocampal formation (×20). (B) Spherical deposits detected by lectin stains. The large number of spherical deposits observed in the molecular layer from case 3, by staining with GSAI–B4 without counterstaining by hematoxylin (×400). (C) Spherical deposits and corpora amylacea stained by PAS which showed intensive reactivity with a) corpora amylacea (×200) and weak reactivity with b) spherical deposits (arrowheads) (×400) on the same section obtained from case 3. (D) Spherical deposits and corpora amylacea stained by antibodies. a) Spherical deposits showed good reactivity with anti chondroitin sulfate (case 3, ×400) and b) corpora amylacea showed good reactivity with anti tau protein antibody (case 18, ×400).


f
p
C2
C3
C4
g
ml
A
B
C-a)
C-b)
D-a)
D-b)

Alzheimer type dementia, dementia with neurofibrillary tangles or Down's syndrome and some aged individuals. The presence of spherical deposits with a few corpora amylacea was mainly observed in young patients with schizophrenia, and the co-localization of spherical deposits and corpora amylacea was recognized in middle-aged patients with schizophrenia and patients with Alzheimer type dementia, dementia with neurofibrillary tangles and Down's syndrome. The vascular and/or stratiform type deposits were mainly observed in patients with Alzheimer type dementia, dementia with neurofibrillary tangles and Down's syndrome. Although the co-localization of spherical deposits and corpora amylacea was also recognized in one aged individual, case 46, and two adult individuals, cases 34 and 38, with unknown episodes, spherical deposits were generally observed thinly or rarely in the regions from younger individuals with unknown episode. In the molecular layer of the dentate gyrus from one patient with schizophrenia (30-year-old male, case no. 3), numerous spherical deposits in the molecular layer and other part of the hippocampal formation were detected, and small amounts of corpora amylacea in the sub-pia of the hippocampus were also detected, as shown in Figure 1. On the other hand, in the hippocampus from an eldest person with pathologically diagnosed Alzheimer type dementia (91-year-old female, case no. 21), a few spherical deposits in the molecular layer was detected, although numerous corpora amylacea were detected in the brain. In the hippocampal formation from patients with schizophrenia, no senile plaques and neurofibrillary tangles were detected and vascular and stratiform type carbohydrate deposits were detected only in one patient. The presence of carbohydrate deposits, i.e. vascular, stratiform, spherical and corpora amylacea, and degenerative neuronal changes, i.e. senile plaques and neurofibrillary tangles, in each individual tested in this study are summarized in Table 1.

AB stains at pH 1.0 and 2.5 clearly revealed both spherical deposits and/or corpora amylacea. Spherical deposits exhibited weak PAS and no hematoxylin reaction, whereas corpora amylacea showed strong or moderate reactivity with these staining methods, respectively. Both spherical deposits and corpora amylacea exhibited intensive affinity towards GSAIB4, UEA-I and DBA lectins, which stained endothelial cells of blood vessel from blood group B, O or A donors, respectively. SBA, PNA and ECA lectins reacted clearly with spherical deposits and weakly with corpora amylacea. No binding of spherical deposits and corpora amylacea was seen with PSA lectin, but Con A lectin showed weak reactivity with corpora amylacea and good with spherical deposits. With hematoxylin staining prior to lectin staining, spherical deposits could be clearly distinguished from corpora amylacea. Spherical deposits which showed reactivity with lectin and none with hematoxylin were stained brown by diaminobenzidine, and corpora amylacea which reacted both with hematoxylin and lectin were colored blue-brown.

Although anti Le x (CD 15) antibody intensively stained astrocytes and microglia in the hippocampal formation and clearly delineated the surface of the unstained granular cells which were strongly stained by PAS and Con A lectins, both spherical deposits and corpora amylacea showed no reactivity. Although antibodies against chondroitin sulfate and heparan sulfate stained glial and granular cells in the hippocampal formation, spherical deposits and corpora amylacea showed reactivity only with anti chondroitin sulfate antibody. Antibodies against tau protein, ubiquitin and alanyl aminopeptidase, barring lactoferrin, IgG, amyloid component P and β amyloid could stain corpora amylacea with various reactivity but spherical deposits showed no reactivity with these antibodies. The staining results obtained by lectins and antibodies with spherical deposits and corpora amylacea are summarized in Table 3.

Table 3. Stainability of spherical deposits and corpora amylacea with various histochemical methods

	Spherical deposits	*Corpora amylacea*
Conventional staining		
Hematoxylin	−	+
AB pH 1.0	weak	+
pH 2.5	weak	+
PAS	weak	+
Lectin stain		
PNA	+	weak
Con A	+	weak
DSA	−	+
DBA	+	+
ECA	+	weak
SBA	+	weak
GSA-I-B4	+	+
PSA	−	weak
WGA	weak	weak
UEA-I	+	+
Immunochemical stain		
Chondroitin sulfate	+	+
Heparan sulfate	−	−
Lewis x (CD15)	−	−
Beta amyloid (8–17)	−	−
Beta amyloid (1–40)	−	−
Beta amyloid (1–42)	−	−
Beta amyloid (1–43)	−	−
GFAP	−	−
Lactoferin	−	−
Component P	−	weak
Tau	−	+
Ubiquitin	−	+
Human IgG	−	−
Alanyl aminopeptidase	−	+

−: negative, weak: weakly positive, +: positive.

Discussion

The mass-presence of spherical deposits in the molecular layer of the dentate gyrus of the hippocampal formation is a new finding. The first step to understanding the properties and the origin of spherical deposits is the characterization of its component, and the localization, distribution and extent of them is the second step to understanding the pathological significance in the brain of patients with brain disorders. The staining ability of AB at pH 2.5 and 1.0 and PAS suggests the presence of carboxylated, sulfated and neutral carbohydrate in both spherical deposits and corpora amylacea. Spherical deposits 3 to 10 μm in diameter may be immature forms of corpora amylacea, since they have similar characteristics by AB, PAS and lectin staining, and show only small differences in the stainability with SBA, PNA and ECA lectins. However, hematoxylin staining could clearly distinguish spherical deposits from corpora amylacea. The hematoxylin staining followed by lectin staining was an effective means to demonstrate the distribution and number of spherical deposits in the molecular layer of the dentate gyrus, since spherical deposits showed good reactivity with lectin and none with hematoxylin, and stained pure brown by DAB.

Corpora amylacea which are composed of cell-cytosol or cyto-skeleton, showed good reactivity with antibodies against tau protein, ubiquitin and alanyl aminopeptidase but were not or feebly stained by lactoferrin, component P and IgG which are mainly found in body fluids. These results confirm the hypothesis [10] that corpora amylacea have an intracellular origin, because the antigens detected are associated with cytoskeletal and intracellularly degraded proteins during prolonged neuronal damage. However, the absence of these antigens may indicate that spherical deposits were not of both intracellular and body fluid origin and were not associated with the neuronal cell degradation.

The expression and distribution of the Le x antigen is related to phosphacan [11] in the brain changes during the stages of gestation and postnatal development [3]. In the granular cell layer of the dentate gyrus, the Le x antigen was expressed on the outer surface of the unstained granular cells which were stained by PSA lectin in a basket-like form. The carbohydrate epitopes bound by anti-Le x and anti-chondroitin sulfate were Gal-(Fuc)-GlcNAc and (GlcA-GalNAc) respectively [12,13], and N-CAM which is closely associated with neuronal cell migration in the hippocampus even in adults, contains GlcNAc, Fuc, Man, and sialic acids in the carbohydrate portion of the extracellular segment of the transmembrane protein [14]. Many kinds of sugar chains are involved as a tag in the synthesis and transport of proteins within the cell [15] and the development and migration of neurons is also controlled by glycoconjugates such as the Le x antigen, phosphacan and N-CAM. These mechanisms are based on a subtle balance of a combination of glycosyltransferases, glycosidases [16] and sugar transport. The carbohydrate residues detected by lectins and anti-chondroitin sulfate in the molecular layer may result from the degraded carbohydrate chains of the Le x antigen, phosphacan and/or N-CAM on the surface of granular cells and glial cells. The carbohydrate structures are cleaved and released from the extracellular domain into the extracellular space, causing an unbalance of the mechanism during the neuropeptide metabolism, neuronal development and/or migration [17].

The molecular layer of the dentate gyrus in the hippocampal formation contains dendrite fibers of granular cells in the dentate gyrus, which is considered to be the first step in the intrinsic hippocampal circuit, perforant pathway from entorhinal cortex and glias [18]. The perforant pathway synapses on the outer portion of the dentate that arises from the dentate gyrus granule cells. This subtends approximately two-thirds of the granule cell dendrite and the perforant pathway contributes 80–85% of the synaptic terminals that end in this zone. By contrast, the inner one-third of the molecular layer receives afferent nerves from the CA4 zone and from the septum [18]. Eriksson *et al.* [19] reported that new neurons were generated from dividing progenitor cells in the dentate gyrus of adult human, and indicated that the human hippocampus retained its ability to generate neurons throughout life. The physiological function of the hippocampus appears to be particularly concerned with memory and long-term potentiation [20]. Although long-term potentiation is likely to serve as a mechanism for the storage of recent memory by the hippocampus, the formation of permanent memory traces is likely to involve the synthesis of new proteins and the formation of new synapses with the assistance of sugar chains. Since many lectin-positive spherical deposits were mainly observed in the inner one-third portion of the molecular layer of the dentate gyrus, it is suggested that there may exist a disadvantageous interaction between these deposits and dendrites of neurons of the C4 zone. The presence of spherical deposits was obvious in patients with schizophrenia, Alzheimer type dementia and Down's syndrome. These results indicate that spherical deposits may play a key role in formation of the neuronal network in the molecular layer of the hippocampal formation. Although the postmortem assignment of psychiatric diagnoses presents major practical difficulties [21], the presence of spherical deposits could be an indicator for psychiatric disorders of patients, since Grace [22] proposed that schizophrenia is a developmentally related disorder, in which disruption of the hippocampal influence over the limbic system during ontogeny results in a pathological alteration of cortico-accumbens interaction in the adult organism.

There are many studies concerning the relationship between glycoconjugates and brain disorder, such as, the glycated tau protein in Alzheimer type dementia [23], heparan sulfate proteoglycan [24] or saccharide [25] in senile plaques and neurofibrillary tangles in Alzheimer type dementia [26]. Since the biosynthesis of sugar chains is not controlled by the interpolation of a template, the structures of sugar chains are much less rigidly pre-programmed than are those of proteins and nucleic acids. Accordingly, age-related alterations in the

sugar chains of various glycoproteins is an important target to solve various pathological problems found in aged individuals [2]. Although at present we have no clear explanation for the pathogenesis and origin of the spherical deposits in the molecular layer of the hippocampal formation, the results obtained in this and previous studies [3] indicate that not only neuronal degeneration but also unusual glyco-metabolism in neurons may disturb the neuronal network and cause the brain disorder.

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