ACTA NEUROBIOL. EXP. 1990, 50: 505-513

Symposium "Recovery from brain damage: behavioral and neurochemical approaches"
4-7 July, 1989, Warsaw, Poland

IMPLICATIONS OF ALTERED BRAIN GANGLIOSIDE PROFILES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Maurice M. RAPPORT

Department of Neurology, Albert Einstein College of Medicine,
1300 Morris Park Avenue, Bronx, New York 10461, USA

Key words: receptors, growth factors, neurotrophic hormones, protein kinase, neuronal membranes, spinal cord

Abstract. Rapport et al. (11) reported that marked aberrations in brain ganglioside profiles were present in 17 of 21 patients with ALS. The aberrations were detected both in motor cortex and in unexpected regions such as frontal, temporal, and parahippocampal gyrus cortex. These results suggest that some underlying pathological process in ALS also occurs in some neurons that are less vulnerable than motor neurons to consequent deterioration. Since gangliosides are major membrane constituents whose carbohydrate residues establish structural configurations on the external face of the cell membrane, it is highly probable that aberrant ganglioside patterns reflect alterations in receptor structure and function. Receptors are inherently cell specific and the specificity would account for differences in response of sensory and motor neurons to the pathological process in ALS. An apparent absence of similar ganglioside aberrations in spinal cord suggests that the primary pathology is in the brain. Such aberrations are not seen in Alzheimer's disease. If receptor functions are altered in ALS, what ligands might be involved? A major consideration is neurotrophic hormones (2). Gangliosides are known to modulate the effect of nerve growth factor in some *in vitro* systems and very recent evidence implicates protein kinase activation as an important mechanism.

Amyotrophic lateral sclerosis is a progressive neuromuscular disease whose cause is unknown despite extensive searches for biochemical, endocrine, and metabolic abnormalities, exogenous toxins, nutritional deficiences, infectious agents, and immunological aberrations. The study I will discuss concerns abnormal ganglioside profiles in ALS brains obtained post mortem. It was published in 1985 and was based on examination of the gray matter from 4 cortical areas of 21 brains from ALS patients and 13 brains from non-ALS patients. All 4 areas, namely,

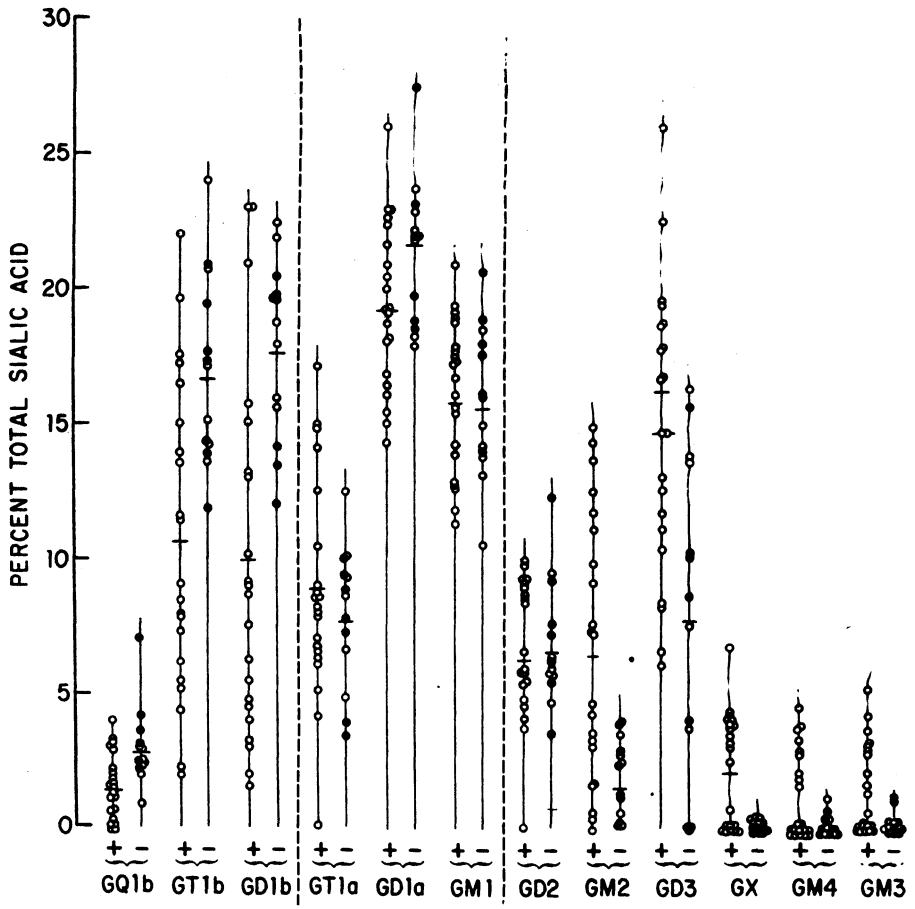

Fig. 1. Percentage distribution of 12 ganglioside species in the frontal cortex of 21 brains of patients with ALS and 13 non-ALS brains. Ordinate: "percent total sialic acid" represents sialic acid color obtained by scanning resorcinol sprayed bands. Each ganglioside band is the percentage of total color summed over all 12. bands, +, ALS brains; −, non-ALS brains; solid circles, neurologically involved cases; open circles, cases with no neurological involvement.

motor cortex, frontal cortex, temporal cortex, and parahippocampal gyrus cortex, showed abnormal ganglioside profiles. Two types of abnormal patterns were detected. One, present in 14 of the ALS brains, had reduced proportions of GQ_{1b}, GT_{1b}, and GD_{1b}, and elevated proportions of GM_2 and GD_3 (Fig. 1); proportions of GM_1, GD_{1a}, GD_2, and GT_{1a} were normal. The other abnormality, found in 13 of the ALS brains, was the occurrence of small amounts of G_x, a ganglioside whose structure is still to be established. Seventeen of the 21 ALS brains showed either one or the other type of abnormality and 10 showed both. When the studies were completed, the function of gangliosides was still too nebulous to project, with any conviction, a possible relation of alterations in ganglioside profiles to the disease process in ALS. However, these results showed that ALS pathology, as reflected in the abnormalities, is not restricted to motor cortex but is found also in other regions of the brain.

I would like to describe the manner in which these studies were begun and then extended since this may have important implications for further developments. This work was undertaken in 1975 principally for the reason that Dr. Donnenfeld (of the ALS Center at St. Vincent's Hospital) was able to secure ALS brain specimens within 3 to 5 hours of the patient's demise. In 1975, the separation of gangliosides on thin layer plates permitted only 4 major ganglioside species to be quantified conveniently. Our first two cases showed a deviation from the "normal values" presented in the literature. It was subsequently found that the literature values were not reliable, and therefore a baseline had to be obtained for non-Als brains. With the investigation at the stage where 4 ALS brains could be compared with 9 non-ALS brains, the abnormal signal was still firm (Fig. 2). However, the technology of ganglioside separation had by then advanced considerably (1), so that at least twelve ganglioside species were separable and quantifiable by densitometry. By this method, GD_{1b} was eventually found to have low values rather than high values; resolution by the earlier method had apparently been inadequate. The reason for mentioning this original incorrect observation is that ganglioside resolution has advanced once again, and the values obtained in the study we published in 1985 may still not correctly represent the aberration in ALS ganglioside profiles. Our study was completed in 1981, and the results based on 21 ALS brains and 13 non-Als controls, roughly age-matched, have, I believe, sufficient technical validity to sustain the conclusion that abnormal ganglioside patterns are present in several cortical areas of ALS brains. This conclusion is reinforced by the fact that 6 of the 13 non-ALS brains represented different types of neurological involvement: 2 brain infarcts, 2 multiple scle

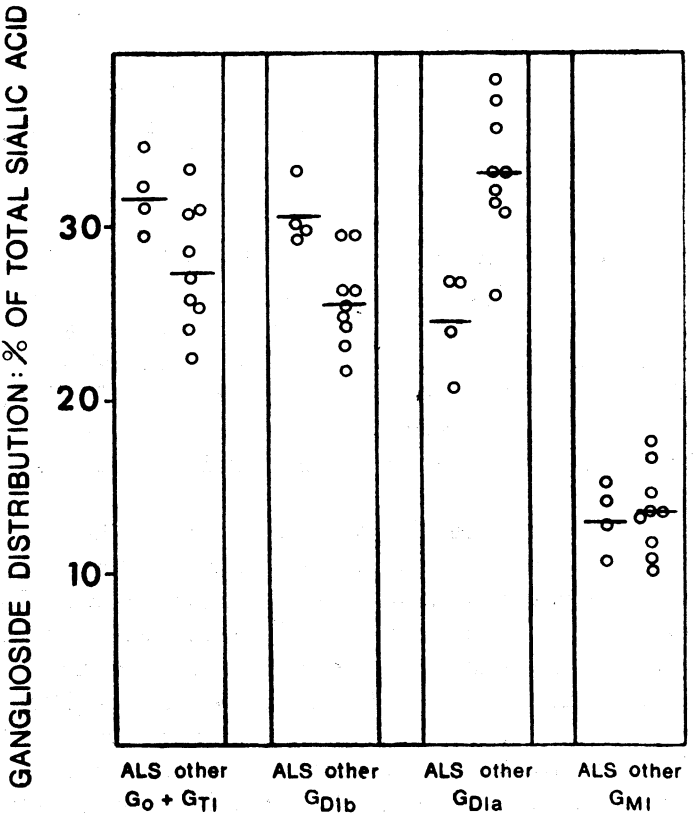

Fig. 2. Distribution of the 4 major ganglioside species in frontal cortex of 4 ALS and 9 non-ALS brains calculated as percentage of sialic acid recovered in these 4 species.

rosis, 1 Alzheimer's disease, and 1 progressive multifocal leucoencephalopathy.

The distribution of gangliosides in these ALS and non-ALS brains in frontal cortex is shown in Fig. 1. Loss of ganglioside sialic acid was detected only in motor cortex where the loss was about 10% (Table I). G_x, the structurally unidentified ganglioside, migrated between GD_{1b} and GD_2 (Fig. 3).

The pathological histology of the ALS brains (Table II) revealed nothing remarkable, and the clinical features (Table III) suggested nothing that might be relevant to the biochemical findings.

Two questions that were most frequently provoked by this study were first "Are there similar changes in other degenerative diseases of the nervous system?" and second, "Are similar changes seen in spinal cord?". With respect to the first question, since we were not able to obtain si-

TABLE I

Ganglioside sialic acid content of brain specimens (μg/g tissue)

	Frontal Cx	Temporal Cx	Motor Cx	Parahippocampal Gyrus Cx
ALS brains				
$n = 21$	716 (14)[a]	719 (18)	587 (22)	632 (26)
Non-ALS brains				
$n = 11$	718 (16)	767 (27)	658 (17) $p < 0.01$[b]	686 (26)

[a] Standard error of the mean; [b] two-tailed Student *t*-test.

TABLE II

ALS cases with neuropathological changes in motor cortex or other sites

Case	Motor cortex	Other sites
MSH	moderate neuronal loss and astrocytosis	
COP		anoxic cell change-Sommer's sector, astrocytosis and neuronal cell loss frontal cortex, marked. Posterior column degeneration, marked.
MRC	moderate neuronal loss and astrocytosis	
MSS		cystic infarct, small, right caudate
WCH	mild neuronal loss	
DSM	mild neuronal loss and astrocytosis	cystic infarction, moderate sized, left insular cortex
MRL	moderate neuronal loss	
SET	moderate astrocytosis of white matter	
SRO	mild astrocytosis	

milar areas of Alzheimer brain tissue, we examined cingulate gyrus cortex from 3 patients with Alzheimer's disease and 4 normal persons; no abnormality in ganglioside profiles was detected. As for spinal cords, questions were frequently raised regarding their profiles after the abnormalities in brain were found. However, I delayed moving in this direction since I believed that any differences between ALS and normal spinal cords could be attributed to the loss of neurons in ALS spinal cords. In the absence of information on the distribution of gangliosides in individual neurons, any conclusion based on a difference would have little significance. However, once the abnormalities in the ganglioside profiles had been established in areas of brain, it was certainly of interest to determine whether similar abnormalities were detectable in

TABLE III

Clinical characteristics of ALS patients

Patient	Age	Sex	Clinical status				PMT[a]
			Duration[b]	Progression	Reflexes[c]	Other	
DDE	58	F	120	Slow	Hyper		5
MRR	63	F	67	Slow	††		5.5
MSH	47	M	60	Slow	J++, Hyper, UE		7
CRT	47	M	60	Slow	J++, ††		7
ATS	56	F	53	Slow	J+++, S+, ††		8
RSE	70	M	50	Slow	J++, S++, Hyper	Dementia	3.5
STT	63	M	47	Slow	††		4.5
COP	59	M	43	Slow	Normal	Posterior column degeneration	4
MRC	78	M	42	Slow	J++, S++, Hyper		2.5
GEE	48	M	42	Slow	J+, Hyper, UE	Familial	3.5
MSS	75	F	36	Moderate	J++, S+, Absent		2
CMP	57	M	33	Moderate	††		6
SHR	63	M	33	Moderate	S+, Absent		2
WGU	58	F	30	Moderate	J+, S++, Hyper, ††		4
MMB	69	M	29	Moderate	Hyper, ††		6
WCH	60	F	27	Moderate	Absent, †	Mental deficiency	6
DSM	78	M	19	Fast	J++, S+++, ††	Dementia	3
NSH	64	M	16	Fast	Hyper, †		2.5
MRL	78	F	12	Fast	Hypo	Personality disorder	2.5
SET	67	M	10	Fast	J+++, S+++, Hyper, †		4
SRO	73	M	10	Fast	Not known		5

[a] Post mortem time (hours); [b] months; [c] reflex symbols: J = jaw (graded + to +++), S = snout (graded + to +++), Hyper = hyperflexia, † = unilateral Babinski sign, †† = bilateral Babinski sign, UE = upper extremity, Hypo = minimal reflex, Absent = no tendon reflex.

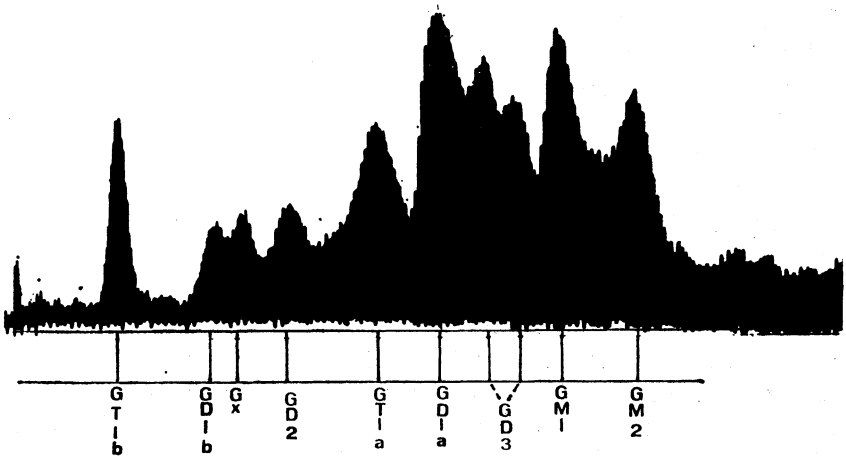

Fig. 3. Densitometer tracing of thin layer chromatogram of gangliosides from frontal cortex of ALS brain showing position of Gx.

spinal cord. In our design we compared dorsal gray matter with ventral gray matter, since the loss of motor neurons in the ventral gray of ALS patients is well recognized whereas a similar loss of sensory neurons in the dorsal roots is not seen. Nine spinal cords from ALS patients and 3 from non-ALS patients were studied. Gray matter specimens from cervical and lumbar regions of spinal cords that had been stored at −70°C were combined for analysis. Surprisingly, few differences were detected between the ganglioside profiles of dorsal and ventral roots, and, with a single exception, namely, the presence of G_x in one specimen of ventral gray matter, the profiles did not reflect the changes seen in brain. In general, the proportions of the different ganglioside species in both dorsal and ventral gray matter were in the order $GD_3 > > GD_{1b} > GT_{1b} > GM_1 > GD_{1a}$. The principal deviation was the reversal in this order of GM_1 and GT_{1b}. In one patient (ALS), GM_1 was the major ganglioside. We also noted that in ALS, the GD_{1a} content in ventral gray was considerably lower than in dorsal gray (6.7% vs. 11.3%). Also, the GD_3 in ventral gray was somewhat higher than in dorsal gray (29.3% vs. 23.9%). The ganglioside sialic acid content was similar for both. These results suggested that no information significant for ALS would be obtained by further studies of spinal cord.

What conclusions can we draw from the existence of aberrant ganglioside profiles in ALS brain? First, we must recognize that the observed abnormalities per se may not yet be informative since further advances in separation technology, for example, separating alkali stable from alkali unstable gangliosides, may alter the observed profile. Se-

cond, it seems reasonable to conclude that the disease process in ALS probably involves several regions of brain as well as the spinal cord. Third, and perhaps most significant, is the implication that abnormal ganglioside patterns are affecting receptors for growth factors or neurotrophic hormones that are essential for the maintenance of motor neurons. Within recent years, attention has focused on growth factors and their receptors in the plasma membranes of all cells including neurons and their roles in the maintenance of normal cellular physiology. As cited in the fourth edition of Basic Neurochemistry (p. 467), with a particular emphasis on target cells as a source of such factors, "Target cell derived signals are likely to be important regulators of neuronal cell body metabolism. The loss of target signals is a possible means by which the cell body learns that its axon has been damaged. Loss of such target-derived factors has been proposed as a general neurological defect". Several recent developments are consistent with this hypothesis. Roisen and cowokers (4, 5, 12) have observed that GM_1 gangliosides potentiate neuritogenic activity of conditioned media from several different sources, suggesting that at least a number of different growth factors may be involved. Cuello et al. (6) have found that gangliosides potentiate the effects of nerve growth factor on central cholinergic neurons.

At a more specific molecular level, Hilbush and Levine (8) have reported that GM_1 potentiates the stimulation by NGF of neurite outgrowth in PC12 cells, probably through stimulation of calcium-calmodulin protein kinase activity. Fukunaga and Soderling (7) observed that brain gangliosides stimulate calcium-calmodulin protein kinase in the absence of calcium-calmodulin with differences in effect among several gangliosides.

The involvement of gangliosides in related processes has also been seen. For example, Manev et al. (9) found that gangliosides protect NMDA-sensitive glutamate receptors, probably by limiting translocation of protein kinase C from cytoplasm to plasma membrane. The close connection of this observation with neuronal maintenance can be seen in the report of Morrison et al. (10) that suppression of protein kinase C activity potentiates the neurotrophic action of epidermal and basic fibroblast growth factors. Other mechanisms may also be involved, since Caceres et al. (3) report that the enhancement of neurite growth by gangliosides may result from the selective induction of high molecular weight MAP-2.

We have, therefore, an interesting group of observations that indicate that gangliosides are intimately involved in growth factor or neurotrophic hormone activities, and it would appear that an abnormality in

ganglioside patterns may well alter the responsiveness of particular neurons to such factors. This alteration in responsiveness may be responsible for the pathology in ALS (2).

REFERENCES

1. ANDO, S., CHANG, N. C., and YU, R. K. 1978. High performance thin-layer chromatography and densitometric determination of brain ganglioside compositions of several species. Anal. Biochem. 89: 437-450.
2. APPEL, S. H. 1981. A unifying hypothesis for the cause of amyotrophic lateral sclerosis, Parkinsonism, and Alzheimer disease. Ann. Neurol. 10: 499-505.
3. CACERES, A., FERREIRA, A., LANDA, C. and BUSCIGLIO, J. 1989. Ganglioside enhanced neurite growth: evidence for a selective induction of high molecular weight MAP-2. Soc. Neurosci. Abstr. 15: 1037.
4. CHEN, D. F., LESKAWA, K. C. and ROISEN, F. J. 1989. The role of ganglioside GM_1 in mediating neurotrophic interaction. Soc. Neurosci. Abstr. 15: 1363.
5. CHEN, D. F. and ROISEN, F. J. 1988. Action of ganglioside on responsiveness of sensory ganglia to trophic agents. Soc. Neurosci. Abstr. 14: 1247.
6. CUELLO, A. C., GAROFALO, L., KENIGSBERG, R. L. and MAYSINGER, D. 1989. Gangliosides potentiate *in vivo* and *in vitro* effects of nerve growth factor on central cholinergic neurons. Proc. Natl. Acad. Sci. USA 86: 2056-2060.
7. FUKUNAGA, K. and SODERLING, T. R. 1988. Stimulation of Ca^{2+}/calmodulin-dependent protein kinase II by brain ganglioside. Soc. Neurosci. Abstr. 14: 437.
8. HILBUSH, B. S. and LEVINE, J. M. 1988. Ganglioside GM_1 modulation of protein kinase activity in PC12 cells. Soc. Neurosci. Abstr. 14: 768.
9. MANEV, H., FAVARON, M., ALHO, H., BERTOLINO, M., GUIDOTTI, A. and COSTA, E. 1988. Gangliosides and NMDA-sensitive glutamate receptor antagonists prevent glutamate neurotoxicity via different mechanisms. Soc. Neurosci. Abstr. 14: 747.
10. MORRISON, R. S., GROSS, T. L. and MOSKAL, T. R. 1988. Suppression of protein kinase C activity potentials the neurotrophic action of epidermal and basic fibroblast growth factors. Soc. Neurosci. Abstr. 14: 363.
11. RAPPORT, M. M., DONNENFELD, H., BRUNNER, W., HUNGUND, B., and BARTFELD, H. 1985. Ganglioside patterns in amyotrophic lateral sclerosis brain regions. Ann. Neurol. 18: 60-67.
12. SPOERRI, P. E., DOZIER, A. K., CAPLE, C. G. and ROISEN, F. J. 1989. The effects of calcium on ganglioside-mediated neuritogenesis. Soc. Neurosci. Abstr. 15: 1260.