Progressive loss of glutamic acid decarboxylase, parvalbumin, and calbindin D28K immunoreactive neurons in the cerebral cortex and hippocampus of adult rat with experimental hydrocephalus

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The authors investigated functional neuronal changes in experimental hydrocephalus using immunohistochemical techniques for glutamic acid decarboxylase (GAD) and two neuronal calcium-binding proteins: parvalbumin (PV) and calbindin D28K (CaBP).

Hydrocephalus was induced in 16 adult Wistar rats by intracisternal injection of a kaolin solution, which was confirmed microscopically via atlantooccipital dural puncture. Four control rats received the same volume of sterile saline. Immunohistochemical staining for GAD, PV, and CaBP and Nissl staining were performed at 1, 2, 3, and 4 weeks after the injection. Hydrocephalus occurred in 90% of kaolin-injected animals with various degrees of ventricular dilation. In the cerebral cortex, GAD-, PV-, and CaBP-immunoreactive (IR) interneurons initially lost their stained processes together with a concomitant loss of homogeneous neuropil staining, followed by the reduction of their total number. With progressive ventricular dilation, GAD- and PV-IR axon terminals on the cortical pyramidal cells disappeared, whereas the number of CaBP-IR pyramidal cells decreased, and ultimately in the most severe cases of hydrocephalus, GAD, PV, and CaBP immunoreactivity was almost entirely diminished. In the hippocampus, GAD-, PV-, and CaBP-IR interneurons demonstrated a reduction of their processes and terminals surrounding the pyramidal cells, with secondary reduction of CaBP-IR pyramidal and granular cells. On the other hand, Nissl staining revealed almost no morphological changes induced by ischemia or neuronal degeneration even in the most severe cases of hydrocephalus.

Hydrocephalus results in the progressive functional impairment of GAD-, PV-, and CaBP-IR neuronal systems in the cerebral cortex and hippocampus, often before there is evidence of morphological injury. The initial injury of cortical and hippocampal interneurons suggests that the functional deafferentation from intrinsic projection fibers may be the initial neuronal event in hydrocephalic brain injury. Although the mechanism of this impairment is still speculative, these findings emphasize the importance of investigating the neuronal pathophysiology in hydrocephalus.

Key Words * experimental hydrocephalus * immunohistochemical staining * glutamic acid decarboxylase * parvalbumin * calbindin D28K

Delayed diagnosis and treatment of progressive hydrocephalus can result in a variety of neurological deficits, including intellectual impairment, learning disabilities, epilepsy, and poor visual acuity.[41,52,59] These residual deficits suggest that irreversible neuronal injury occurs during the hydrocephalic process and that this occurs despite cerebrospinal fluid (CSF) shunting. Consequently a detailed pathological study of the neuronal changes in hydrocephalus is required to determine the critical time period in which a given surgical treatment would be effective. Although a number of previous experimental studies, and occasionally pathological studies, have focused on the cerebral white matter as the prime recipient of injury in hydrocephalus,[7,8,10,12,45,55] a few reports of concomitant neuronal injury in the cerebral cortex, hippocampus, and periventricular areas have been reported.[11,19,56,57]

Recent studies have identified significant reduction of various neurotransmitter levels in hydrocephalic brain tissue,[6,11,15,16,33,37] developmental impairment of cortical synaptogenesis in experimental or congenital hydrocephalic models,[38,51] and dendritic changes of pyramidal cells representative of a transneuronal effect.[32,36] To reveal possible functional changes of the central nervous system (CNS) in experimental hydrocephalus, we investigated immunohistochemical changes in gamma-aminobutyric acid (GABA)-positive neuronal systems, which consist of a vast subpopulation of cortical and hippocampal interneurons,[42] and in two neuronal populations with broad distributions containing calcium-binding proteins: parvalbumin (PV) and calbindin D28K (CaBP).[5]

Gamma-aminobutyric acid (GABA) has long been believed to be a major inhibitory neurotransmitter in the CNS, and its synthetic enzyme, glutamic acid decarboxylase (GAD), was thought to be a specific marker of those inhibitory neurons.[42] The enzyme is immunohistochemically recognized in the somata, dendrites, and axon terminals of a subclass of intrinsic interneurons in the cerebral cortex and the hippocampus. The axon terminals of these neurons form symmetric synapses predominantly with the somata and proximal dendrites of pyramidal cells. Therefore, these terminals are in a strategic position to mediate strong inhibition of pyramidal cells in the neocortex and hippocampus because of their concentration in those specific locations.[42]

Calcium ions (Ca^{++}) activate and regulate a number of key neuronal functions, including fast axonal transport of substances, synthesis and release of neurotransmitters, and membrane excitability.[20,21,25] In the CA1 hippocampal region, Ca^{++} might induce long-term potentiation and memory storage mechanisms.[14,34] With the tremendous interest and progress in Ca^{++} -related research, it has been demonstrated that both PV and CaBP strongly relate to Ca^{++} translocation and/or Ca^{++} buffering specifically to protect neurons against various insults.[3,24,31,35,39] Both PV and CaBP are valuable anatomical markers and have been shown to be present independently within the subpopulation of GABAergic interneurons in many brain areas; PV occurs in some chandelier and basket cells, whereas CaBP is found in some double-bouquet cells.[2,5,18,30,53]

In the cerebral cortex and hippocampus, the ischemic changes of GAD, PV, and CaBP immunoreactivity have been extensively studied[17,27,40] as have those changes observed in various neurodegenerative disorders, including Alzheimer's disease, dementia, Parkinson's disease, and epilepsy.[1,22,23,26,43,44,49,50,58] Thus, it is of great interest to determine if GAD-, PV-, and CaBP-IR neurons in these regions are indeed functionally impaired by the development of hydrocephalus compared with the morphological injury exhibited on Nissl staining.

MATERIALS AND METHODS

This research was approved by the Animal Care Committee of the University of Toronto.

Induction of Hydrocephalus

Twenty adult male Wistar rats, each weighing between 200 and 250 g, were anesthetized by intraperitoneal administration of chloral hydrate (28 mg/100 g body weight) and immobilized in a stereotaxic frame with neck flexed. A posterior midline incision was made, with the aid of an operating microscope, at the craniocervical junction to expose the atlantooccipital dura. Using a 27-gauge needle, a 0.05-ml volume of 25% kaolin solution (Sigma, St. Louis, MO) was manually injected into the cisterna magna via caudal puncture to prevent retrograde leakage of the solution. The same volume of sterile saline was injected into four control rats. Four kaolin-injected and one control animal were killed at 1, 2, 3, and 4 weeks after injection.

Immunohistochemical Staining

While the experimental and control animals were deeply anesthetized by intraperitoneal administration of chloral hydrate (42 mg/100 g body weight), they were transcardially perfused with saline (50 ml/100 g body weight), followed by 5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (100 ml/100 g body weight). Their brains were removed and immersed overnight in 0.1 M phosphate-buffered solution, pH 7.6, containing 20% sucrose. Serial sections were cut coronally at a thickness of 60 μ m on a freezing microtome. The sections were divided consecutively into four groups; each group consisted of every fourth section of a series of the serial sections. Three of the four groups were immunostained for GAD (polyclonal/rabbit AB108; Chemicon International Inc., Temecula, CA), PV (monoclonal/rabbit PV28; Swant, Bellinzona, Switzerland), and CaBP (monoclonal/mouse 300; Swant) using the avidin-biotin complex method; the floating sections were washed in several changes of 0.9% saline with 0.1 M phosphate buffer, pH 7.4, and exposed to antisera against GAD (diluted 1:5000), PV (diluted 1:5000) and CaBP (diluted 1:5000) for 24 hours. Biotinylated goat anti-rabbit immunoglobulin antibody (Vector, Burlingame, CA) was conjugated to the primary antibodies of GAD and PV, while biotinylated horse anti-mouse immunoglobulin antibody (Vector) was conjugated to that of CaBP for a period of over 3 hours each. Avidin-biotinylated-peroxidase complex (Vector) was then conjugated to the primary antibody over a 3-hour period. Immunolabeled peroxidase was visualized by incubation for 10 to 20 minutes with 0.02% diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in 50 mM Tris-HCl buffer, pH 7.6. The stained sections were serially mounted on gelatin-coated slides and examined by light microscopy.

Nissl Staining

Sections adjacent to the GAD, PV, and CaBP immunostained sections, were mounted on gelatin-coated slides and stained with cresyl violet.

Classification of Induced Hydrocephalus

The degree of hydrocephalus was classified according to the percent ratio of the maximum ventricular width divided by the maximum brain width on the section from the level of the anterior commissure. Classification was as follows: mild, less than 45%; moderate, from 45% to 55%; and severe, greater than 55%.

Quantitative Analysis of Hippocampal Interneurons

The number of hippocampal interneurons in each section was counted using the following method. The

number of GAD- and PV-IR interneurons in CA1 and CA2 of the stratum pyramidale of the unilateral dorsal hippocampus was counted with aid of a microscope and the numbers on the adjacent sections that contained the dorsal third ventricle were summed. The number of CaBP-IR interneurons in the whole unilateral dorsal hippocampus was counted in a similar fashion. The totals were related to the degree of hydrocephalus. Counts in the different regions were analyzed by analysis of variance with the Bonferroni correction for multiple comparisons.

RESULTS

Induction of Hydrocephalus

Following injection of kaolin, the animals transiently demonstrated reduced activity and loss of appetite and weight for several days. Two animals died at this stage due to acute hydrocephalus. Another two animals failed to develop hydrocephalus 2 and 4 weeks after injection and were immunohistochemically indistinguishable from controls. Experimental hydrocephalus was induced in 18 (90%) of 20 animals injected with kaolin which progressed to variable degrees of ventricular dilation that could be correlated to hydrocephalic duration. Ventricular ratios were 28.2% in control rats: 41.8% at 1 week, 47.5% at 2 weeks, 53% at 3 weeks, and 59.1% at 4 weeks. In the animals in which hydrocephalus was induced, there were three animals classified as mild and one moderate at 1 week; one mild, two moderate, and one severe at 2 weeks; one mild, one moderate, and two severe at 3 weeks; and two moderate and two severe at 4 weeks. The development of ventricular dilation was characterized by an initial rapid increase followed by a slower but steady progression, as shown in other models.[13]

Fig. 1. Photomicrographs showing changes in glutamic acid decarboxylase (GAD) (A-C) and parvalbumin (PV) (D-F) immunoreactivities in the cerebral cortex. A and D: Normal cortex revealing scattering of interneurons in all layers (except for Layer I in PV staining), homogeneous dense staining in upper layers, and circumferential perineuronal axon terminals in lower layers. B and E: Mild to moderate case of hydrocephalus showing the reduction of processes of GAD-immunoreactive interneurons, neuropil staining, and axon terminals. C and F: Severe case of hydrocephalus remarkably diminishes its cortical caliber, as well as both GAD and PV immunoreactivities and the number of interneurons. Bar = 200 μ m.

Immunohistochemical Staining of the Cerebral Cortex

In the normal cerebral cortex, many GAD-, PV-, and CaBP-IR neurons with various shapes, sizes, and immunoreactive intensities were scattered in all layers (except for the PV immunonegative Layer I). Homogeneous dense staining in Layers I to III (PV: Layers II - IV) prevented visualization of weakly stained cell bodies and tracing of fine neuronal processes. In deeper layers, the three kinds of immunoreactive neurons could be characterized as aspiny or sparsely spiny nonpyramidal interneurons (Figs. 1A and D, 2A).

Fig. 2. Photomicrographs showing changes in calbindin D28K (CaBP) immunoreactivity in the cerebral cortex.

A: Normal cortex showing strongly stained interneurons (arrowheads), weakly stained pyramidal cells (white arrow) and a homogeneously stained neuropil. B: Mild case of hydrocephalus revealing the somata of CaBP-immunoreactive interneurons losing their processes in deeper layers (arrowheads) and the slight decrease of immunoreactive fibers and staining in superficial layers. C: Moderate to severe case of hydrocephalus demonstrating loss of interneurons, with those remaining having a remnant somata (arrowheads) and further reduction of neuropil staining, with loss of even pyramidal cells in superficial layers. D: Most severe case of hydrocephalus demonstrating almost no CaBP immunoreactivity. I - V = cortical layers. Bar = 100 μ m.

In CaBP immunostaining, the majority of pyramidal cells in Layers II to V were also immunoreactive, albeit with considerably less intensity (Fig. 2A). Glutamic acid decarboxylase- and PV-IR punctate structures, which corresponded to axon terminals and varicosities, were distributed homogeneously within the neuropil, and some terminals were located on the surface of dendritic shaft, axon initial segment, and soma of pyramidal and nonpyramidal cells so as to form a continuous sheet around them (Fig. 3).

Fig. 3. Photomicrograph depicting normal cortex after parvalbumin (PV) immunostaining. Parvalbumin-immunoreactive punctate structures surround the somata of pyramidal cells so closely as to make their shapes distinct (arrows). Bar = 50 μ m.

In the GAD and PV immunostaining of mild and moderate cases of hydrocephalus, reduction of background neuropil immunoreactivity, beginning at the brain surface, highlighted the remaining interneurons. A few axon terminals still remained as punctate structures predominantly in Layer V (Fig. 1B and E). In the severe cases of hydrocephalus, all neuropil and axon terminals disappeared throughout the entire cerebral cortex; however, those interneurons still expressing GAD and PV immunoreactivity were observed in some patchy form more frequently in the deeper cortex than in the superficial cortex (Fig. 1C and F). In the CaBP immunostaining of mild cases of hydrocephalus, the interneurons in deeper layers lost their long processes, with only cell bodies and a few short dendrites being spared (Fig.

2B). The number of CaBP-IR interneurons in deeper layers was decreased with progressive ventricular dilation. There was reduced homogeneous staining of the neuropil in the upper layers, and the weakly

stained pyramidal cells in Layers II to V also disappeared (Fig. 2C). In the thin cerebral cortex of the most severe cases of hydrocephalus, all of the cortical CaBP immunoreactivity ultimately vanished, leaving vacuolations and weakly staining interneurons (Fig. 2D).

Fig. 4. Photomicrographs showing changes in glutamic acid decarboxylase (GAD) (A and B) and parvalbumin (PV) (C and D) immunoreactivities in the hippocampus. A and C: Normal hippocampus revealing a lot of interneurons with abundant processes in every subfield and axon terminals in the stratum pyramidale and stratum granulare. B and D: Severe case of hydrocephalus loses nearly all GAD and PV immunoreactivities, with remnant shrunken or pyknotic interneurons (arrowheads). Bar = 500 μm.

Immunohistochemical Staining of the Hippocampus

In the normal hippocampus, many GAD-, PV-, and CaBP-IR interneurons with abundant processes were scattered in the stratum oriens, stratum pyramidale, stratum radiatum, and stratum granulare of hippocampal subfields (Fig. 4A and C). In GAD and PV immunostaining, somata of the pyramidal cells were so closely surrounded by the axon terminals that the somatic shapes were distinct as an array of circles in the stratum pyramidale (Fig. 5A). In CaBP immunostaining, moderate immunoreactivity was expressed specifically in the majority of CA1 and CA2 pyramidal cells with their apical dendrites and in the granule cells with their dendrites and axons. Therefore the dentate gyrus and the CA3 and CA4 region, with no immunoreactive pyramidal cells, were recognized as CaBP-IR mossy fiber bundles (Fig. 6A).

Fig. 5. Photomicrographs revealing changes of glutamic acid decarboxylase (GAD) immunoreactivity in the CA2 and CA3 regions of the hippocampus. A: Normal hippocampus expressing numerous axon terminals as an array of circles in the stratum pyramidale. B: Moderate case of hydrocephalus demonstrating the reduction of neuronal processes and

axon terminals. C: Severe case of hydrocephalus revealing GAD-immunoreactive and shrunken interneurons (arrowheads), with almost no axon terminals. Bar = 100 μ m.

In the mild and moderate cases of hydrocephalus, many GAD-, PV-, and CaBP-IR interneurons demonstrated a reduction of processes in the hippocampal and dentate gyrus subfields, ultimately followed by an appearance of shrunken and dark "reactive" neurons with sparse short processes. In GAD and PV immunostaining, stratum pyramidale and stratum granulare gradually lost the immunoreactivity of axon terminals (Fig. 5B). In contrast to the interneurons, CaBP-IR pyramidal cells with prominent apical dendrites in the CA1 and CA2 regions were unaffected, even with some immunoreactive enhancement, by the progressive ventricular

dilation, and granule cells, together with their mossy fibers in the dentate gyrus and the CA3 and CA4 regions, also remained intact in the moderate cases of hydrocephalus (Fig. 7). In the severe cases of hydrocephalus, the reduction of all immunoreactivity was remarkable, with only some patchy stained areas remaining (Fig. 4B and D, Fig. 6B). The somata of remaining interneurons were shrunken and wrinkled, with short or absent dendrites (Fig. 5C).

Fig. 6. Photomicrographs showing changes of calbindin D28K (CaBP) immunoreactivity in the hippocampal formation. A: Normal hippocampus revealing many intensely stained interneurons (arrowheads), weakly stained pyramidal cells (small arrows), and moderately stained granule cells (white arrowheads). The dentate gyrus and the CA3 region are moderately stained with mossy fibers from the granule cells. B: Severe case of hydrocephalus revealing the remarkable reduction of CaBP immunoreactivity, with a small number of residual pyramidal and granule cells (arrows), and the appearance of various-sized vacuolations (arrowheads). Bar = 250 μ m.

Nissl Staining

The moderate and severe hydrocephalic cortex maintained an arrangement of cortical layers containing no degenerative neurons with chromatolysis or pyknosis in any layers, in contrast with the remarkable immunohistochemical changes (Fig. 8A and B). In the most severely hydrocephalic animals, the ventricular ependyma was disrupted, especially in the anterosuperior and occipitotemporal portion of lateral ventricles, followed by destruction of areas of the corpus callosum, periventricular white matter, and, ultimately, the cerebral cortex with dilated extracellular spaces.

Fig. 7. Photomicrograph demonstrating calbindin D28K (CaBP) immunoreactivity in the CA2 region of the strata radiatum and pyramidale of the hippocampus. A moderate case of hydrocephalus exhibiting the preservation of CaBP-immunoreactive pyramidal and granule cells with some immunoreactive enhancement, in contrast with pyknotic interneurons with no dendrites (arrowheads). Bar = 200 μ m.

In the hippocampus, only the most severe cases of hydrocephalus revealed a minimally disordered cellular arrangement, with scattered and variable-sized vacuolations mainly in the stratum pyramidale and the stratum radiatum. There was, however, a pronounced difference between the absence of any CaBP immunoreactivity in pyramidal and granule cells and the well-ordered lines of cells that stained normally by Nissl staining (Fig. 8C and D).

Fig. 8. Photomicrographs illustrating a comparison between calbindin D28K (CaBP) immunostaining and Nissl staining in the cerebral cortex and hippocampus. A: Moderate case of hydrocephalus revealing the loss of CaBP immunoreactivity in deeper cortical layers. B: Adjacent section of same specimen as in A that has been stained by cresyl violet conversely demonstrating preserved cellular arrangement with minimal degenerative changes even in deeper layers. Arrowheads in A and B indicate profiles of blood vessels as landmarks. C: Severe case of hydrocephalus manifesting faint intensity of CaBP immunoreactivity in the pyramidal (arrows) and granule cell layers (arrowheads). D: Adjacent section of same specimen as in C that has been Nissl stained strongly exhibiting stained intact pyramidal (arrows) and granule cells (arrowheads). A and B, bar = 100 μ m; C and D, bar = 250 μ m.

Quantitative Analysis of Hippocampal Interneurons

The numbers of GAD- and CaBP-IR interneurons in the prescribed subfields gradually decreased with progression of the ventricular dilation, whereas the PV-IR neuronal number was unchanged (Table 1). The mean CaBP-IR neuronal numbers, at each level of hydrocephalus, were significantly different from the control numbers, as well as all of the numbers at other levels (p less than 0.05). The mean GAD-IR neuronal numbers were significantly reduced from those of the control in the moderate and severe hydrocephalic groups (p less than 0.05).

TABLE 1 NUMBERS OF GLUTAMIC ACID DECARBOXYLASE-, PARVALBUMIN-, AND CALBINDIN D28K-IMMUNOREACTIVE INTERNEURONS IN THE UNILATERAL DORSAL HIPPOCAMPUS*				
Immunostain	Control (4 rats)	Degree of Hydrocephalus		
		Mild (5 rats)	Moderate (6 rats)	Severe (5 rats)
GAD	99.9 ± 7.5	87.4 ± 8.1	80.7 ± 7.3†	49.5 ± 11.8†
PV	65.3 ± 8.3	60.4 ± 10.3	59.3 ± 5.2	53.3 ± 7.3
CaBP	45.4 ± 6.5	34.3 ± 4.1†	26.1 ± 2.3†	13.8 ± 3.0†

* Data are presented as the mean ± the standard deviation. Abbreviations: CaBP = calbindin D28K; GAD = glutamic acid decarboxylase; PV = parvalbumin.
† Statistically different from control group using analysis of variance with the Bonferroni correction for multiple comparisons ($p < 0.05$).

DISCUSSION

The salient finding in this study is that there was progressive and characteristic loss of GAD-, PV-, and CaBP-IR neurons in the cerebral cortex and hippocampus during the hydrocephalic process. Nissl counterstaining revealed minimal pathological changes in these areas, even in the most severe cases of hydrocephalus. These findings indicate a functional impairment of GAD-, PV-, and CaBP-IR neurons prior to the appearance of any morphological change and underline the importance of neuronal systems in the pathophysiology of hydrocephalus.

Animal Model

An injection of kaolin resulted in progressive hydrocephalus in 90% (18 of 20) of the adult rats in this series. The higher rate of hydrocephalus induction in this report[54] may be related to our use of microscopic guidance. Cisternal injection of kaolin in rats causes an inflammatory obstructive hydrocephalus and results in progressive and variable ventricular dilation in common with a number of other models.[8,10,11,13,47] The kaolin had minimal, if any, direct affect on the pathological changes observed because the animals that failed to develop hydrocephalus were immunohistochemically indistinguishable from controls.

Neuronal Vulnerability to Hydrocephalus

The calcium-binding proteins are thought to play a neuroprotective role by acting as an intracellular buffer, although both resistance and susceptibility to various neurodegenerative disorders has been reported.[1,3,17,24,26,27,35,39,46,49,50] In general there should be a simultaneous reduction of immunoreactivity among all neuronal groups studied. However, our quantitative analysis in the hippocampus, where the PV-IR neuronal numbers were unchanged, suggests that PV might have a certain neuroprotective effect. This has been reported in kindling epileptogenesis in the hippocampus, in which the coexistence of PV in GABAergic neurons exerts a protective effect.[28] It is also possible that the enhancement of CaBP immunoreactivity in the pyramidal and granule cells of moderate hydrocephalic hippocampus represents an increase in a calcium-loaded form of CaBP, with strong immunoreactivity and/or an increased content of CaBP acting as a neuroprotective buffer.[28] With respect to affected neuronal type, our results show that the nonpyramidal intrinsic interneurons are most vulnerable, and consequently the most impaired neuronal function in hydrocephalus may be inhibitory innervation from the GABAergic interneurons to the long projection neurons.

Mechanism of Neuronal Injury in Hydrocephalus

The likely mechanism of neuronal injury in hydrocephalus includes direct compression, chronic ischemia, and metabolic derangement with anaerobic glycolysis.[10] The fine aspiny or sparsely spiny dendrites of intrinsic interneurons that project in a diffuse manner may be most sensitive to mechanical distortion. The axonal transport of thin and mostly unmyelinated axons may be easily affected.[42] Other neurons, including pyramidal cells, may well be functionally damaged with overexcitation resulting from the deafferentation of their inhibitory input from the injured intrinsic interneurons.[32,46] We were unable to confirm the morphological and, possibly, transneuronal changes of pyramidal cells reported by McAllister, et al.,[36] because of weak CaBP immunoreactivity in cortical pyramidal cells.

Ischemia has been shown to cause a selective reduction of GAD-IR axon terminals in the cerebral cortex and hippocampus akin to that observed in this study. However, the distribution of the loss of PV and CaBP immunoreactivity is quite unlike that reported in acute four-vessel occlusion,[17,27] in which PV-IR interneurons disappear transiently, with no reduction of stained axon terminals, and CaBP-IR pyramidal cells are more vulnerable than CaBP-IR nonpyramidal neurons. In addition, levels of cerebral blood flow in gray matter have often been normal or only moderately reduced so that ischemia may not have a prominent role in neuronal injury of hydrocephalic brain.[9]

The GABAergic axon terminals have been shown to possess a higher reliance on aerobic metabolism than the other types of axon terminals.[42] In particular, PV-IR interneurons are recognized to function as "fast-firing" neurons with a high metabolic rate and a high cytochrome oxidase activity.[43] Evidence of aerobic metabolism has been demonstrated in the white matter in hydrocephalus,[7] although the effects of possible impairment of aerobic metabolism in gray matter is still uncertain.

Functional Injury by Hydrocephalus

There is progressive loss of GAD-, PV-, and CaBP-IR neurons in the cortex corresponding to the degree of ventricular enlargement and duration of the hydrocephalus with total sparing of neuronal architecture, as shown by Nissl staining, although GAD-IR interneurons occupy approximately 48% of the cortical neurons.[48] Also in the hippocampus, it is quite obvious that the CaBP-IR pyramidal and granule cells have lost their immunoreactivity, while these cells have maintained their morphological architecture. These findings indicate that the progressive, selective, functional injury of the specific or general protein synthesis occurs in these neuronal groups, and exhaustion of intracellular proteins is expressed as progressive loss of GAD-, PV-, and CaBP immunoreactivity prior to the appearance of morphological changes. Recovery of immunoreactivity after CSF shunting might confirm a functional cessation of the protein synthesis. A burst of high GABA concentration in the CSF with hydrocephalus has been reported in two clinical studies, with the CSF-GABA level of the shunt-effective group returning to normal levels after shunt insertion.[4,29] A critical period for functional recovery may depend on the degree of neuronal degenerative damage, such as GABAergic interneurons and axon terminals.

Clinical Correlates

Decreases of GAD-, PV-, and CaBP-IR interneurons and axon terminals in the cerebral cortex have been reported in dementia and Alzheimer's disease in humans[1,22,23,26] and cortical focal epilepsy in monkeys induced by alumina gel.[43,44] Also loss of GAD-IR axon terminals in the hippocampus, as shown in the present study, was found in kindling seizure models.[28,50] These may have some relationship to the intellectual impairment, learning disabilities, and epilepsy seen in

hydrocephalus,[41,52,59] which may be more easily attributable to neuronal systems rather than to white matter pathology. The maintenance of neuronal function requires physiologically significant synaptic arrangements with anterograde and/or retrograde feedback connections extant as intrinsic short circuits.[32,46] The initial injury of interneurons and axon terminals presented in this study, suggests that the functional deafferentation from intrinsic projection fibers might be the inceptive neuronal event leading to permanent deficits in hydrocephalus.

CONCLUSIONS

The progressive loss of GAD-, PV-, and CaBP-IR neurons in the cerebral cortex and hippocampus, with the absence of overt changes in neuronal architecture, indicates a functional impairment of the neuronal systems in the hydrocephalic process prior to the appearance of morphological injury. Although the mechanism of these impairments is still speculative, these data emphasize the importance of the investigation of neuronal pathophysiology in hydrocephalus.

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