Calcineurin immunoreactivity in striatonigral degeneration

S. Goto¹, A Hirano¹, and R. R. Rojas-Corona²

Divisions of ¹ Neuropathology and ² Immunopathology, Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, 111 East 210th Street, Bronx, NY 10467, USA

Summary. The basal ganglia (including substantia nigra) of two patients with striatonigral degeneration, who had clinical histories of Parkinson's disease, were studied immunohistochemically using a purified antibody to calcineurin (CaN). Marked loss of CaN-immunoreactive neurons in the putamen and neuromelanin-pigmented neurons in the zona compacta of the substantia nigra was seen in both cases. A small number of CaN-immunoreactive neurons remained dispersed in “clusters” or “islands” in the medial portion of the putamen. In one case there was loss of CaN-immunoreactive neurons in the caudate nucleus to a lesser degree than that in the putamen. In addition, both cases showed marked depletion of CaN-immunoreactive afferent nerve fibers in the external and internal segments of the globus pallidus and the zona reticulata of the substantia nigra. This report emphasizes the usefulness of the CaN-immunostaining technique for assessing the striatal efferents in human basal ganglia, and shows the neuropathological changes in the basal ganglia with striatonigral degeneration which were not possible to ascertain with previous techniques.

Key words: Striatonigral degeneration – Basal ganglia – Calcineurin – Immunohistochemistry

Striatonigral degeneration (SND) was first described as a distinct clinico-pathological entity by Adams, van Bogaert and van der Eecken [1]. Its clinical symptoms were those of Parkinson's disease (PD) in which rigidity and slowness of movement of extremities were prominent [2]. Also, neuropathological features were reported which showed neuronal degeneration in the corpus striatum and substantia nigra; however, there are some variations from case to case [1 – 3, 16, 18, 27]. The classification of SND is controversial [2, 21] because it is occasionally associated with olivo-pontocerebellar atrophy (OPCA) and/or Shy-Drager Syndrome [23]. Takei and Mirra [27] proposed that SND is a form of the multiple system atrophy described by Graham and Oppenheimer [14]. In this report, we use “striatonigral degeneration” in a purely neuropathological sense.

Calcineurin (CaN), one of the Ca²⁺/calmodulin-binding proteins, is highly concentrated in mammalian brains [17], especially in the basal ganglia [8, 9, 28, 29]. It is presently thought to be a multifunctional enzyme because of its ability to dephosphorylate many phosphoproteins [17], such as microtubule proteins [7]. While the exact physiological function of CaN is unclear, its unique calmodulin-regulated phosphatase function may play an important role in extrapyramidal function. We have previously demonstrated that CaN was present only in striatal neurons and their projections to the globus pallidus and the substantia nigra of the rat basal ganglia [9]. This was confirmed by the immunohistochemical observations (marked depletion of CaN immunoreactivity in the globus pallidus and substantia nigra according to the number of CaN-immunoreactive neurons in the striatum) in autopsy materials from cases of Huntington's chorea [10].

Also, several morphological investigations have indicated that most CaN-immunoreactive neurons in the rat striatum could be classified as medium-size densely spiny neurons acting as major projection neurons and sending their axons to the globus pallidus and the substantia nigra, or both [11].

In the work reported in this paper, we show the usefulness of the CaN-immunostaining technique for visualizing the striatal efferents in the human basal ganglia, and demonstrate neuropathological changes of the basal ganglia in SND.
Materials and methods

Materials

We examined two autopsied patients with SND: case 1 was a 72-year-old female with a history of PD. Case 2 was a 56-year-old female with a history of PD and Shy-Drager syndrome. Autopsy material from a 62-year-old male, who had died of esophageal cancer and had no neuropathology, was used as a control. Postmortem brain tissue from these patients was fixed with 10% neutral formalin for 3 weeks, then serial coronal sections of the cerebrum and cross sections of the brain stem were made. Neuropathological examinations were performed utilizing conventional techniques such as hematoxylin and eosin, Nissl, Holzer, iron, luxol fast blue and Bielschowsky stains. For immunohistochemical study, we prepared 5-µm sections from paraffin-embedded tissues.

Methods

The antiserum to CaN was produced in a rabbit as described previously [8]. In this experiment only the purified antibody prepared by the CaN-affinity column was used. The CaN antiserum was tested for mono-specificity [8, 12, 13]. Immunostaining procedures were carried out according to the instructions of the Vectastain ABC kit (Vector Labs, Burlingame, Calif) [15], using 3,3'-diaminobenzidine as a chromogen. The staining specificity was carefully assessed as previously described by Goto et al. [11, 12].

Results

Case 1

Neuropathological findings. The brain, weighing 1000 g in the unfixed condition, showed moderate atrophy, and a definitive shrinkage of the putamen on both sides with moderate brownish discoloration. The globus pallidus on both sides was also small, while the caudate nucleus was of normal size on both sides and no ventricular dilatation was observed. Marked depigmentation of the substantia nigra and the locus ceruleus were seen. Cerebellum and spinal cord were unremarkable. No apparent neuronal loss was found in the cerebral cortex on light microscopic examination. There was marked loss of large and small neurons of the putamen with prominent astrocytosis and parenchymal rarefaction. Marked enlargement of perivascular spaces in the putamen was seen. At the anterior level, the ventromedial portion of the putamen was less affected than the lateral portion. The caudate nucleus and the gray matter bridging the internal capsule between the putamen and the caudate nucleus showed no definite neuropathological change. The globus pallidus revealed loose parenchyma with moderate astrocytosis but no apparent decrease and/or degeneration of its neurons was observed. A number of macrophages laden with brown pigment was seen in the putamen and globus pallidus. The external capsule had moderate gliosis and rarefaction but the claustrum was unremarkable. The substantia nigra and locus ceruleus showed a marked depletion of the pigmented neurons with astrocytosis. Inclusion bodies were not seen in these areas nor in the substantia innominata. Other regions such as the cerebellum, the vagal dorsal nucleus and the Ammon's horn appeared normal. Neuropathological diagnosis was SND.

Immunohistochemical findings. Initially we demonstrated the CaN-immunostained basal ganglia (Fig. 1a) and substantia nigra (Fig. 1b) in the control case. Strong CaN immunoreactivity was seen in the putamen, the globus pallidus externus, the globus pallidus internus and the substantia nigra. As reported previously, CaN immunoreactivity of the globus pallidus and substantia nigra was present mostly in the projection neurons originating from the striatum. The pallidal and nigral neurons did not contain CaN immunoreactivity.

The macroscopic immunohistochemical findings in the basal ganglia of case 1 are shown in Fig. 2a. The putamen showed marked loss of CaN immunoreactivity in contrast to the strong reactivity of the head of the caudate nucleus and the ponticuli substantiae griseae (the gray matter bridging the internal capsule between the putamen and the caudate nucleus). However, the remaining CaN immunoreactivity was observed randomly distributed in the medial and ventral portions of the putamen adjacent to the external medullary lamina. In addition, the ventral portion of the globus pallidus externus showed marked depletion of CaN immunoreactivity, while strong reactivity was seen in the dorsal area and ventromedial area adjacent to the internal medullary lamina as in the putamen. In the globus pallidus internus, an almost complete loss of CaN immunoreactivity in its ventrolateral area was observed.

Under the light microscope, the caudate nucleus (Fig. 2b) and ponticuli substantiae griseae (Fig. 2c) were seen to contain many CaN-immunoreactive neuronal bodies and neurites. In the putamen, some clusters of the strongly reactive neurons were observed in the medial portion (Fig. 3a), but the lateral portion showed an almost complete loss of CaN-immunoreactive cell bodies and their processes (Fig. 3b). The ventrolateral portions of the globus pallidus internus (Fig. 4b) and externus (Fig. 4d) revealed marked depletion of CaN-immunoreactive nerve fibers, which can be contrasted to the findings of their dorsal portions (Fig. 4a, c). In the substantia nigra, CaN immunoreactivity was originally present only in the zona reticulata which is known to have morphological structures similar to the globus pallidus. As shown in Fig. 5a, CaN immunoreactivity in the substantia nigra zona reticulata appeared thin, and weak in its lateral