Calcineurin and synaptophysin in the human spinal cord of normal individuals and patients with familial dysautonomia

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Summary. This report concerns the immunohistochemical demonstration of two neuronal Ca²⁺-binding proteins, calcineurin and synaptophysin, in the spinal cord of normal controls and from patients with familial dysautonomia. In controls, calcineurin immunoreactivity was highly concentrated in small nerve cells and fibers of the substantia gelatinosa. Synaptophysin immunoreactivity was normally distributed throughout the spinal cord gray matter, being highly concentrated in the substantia gelatinosa, the dorsal nucleus of Clarke and the anterior horn. In patients with familial dysautonomia, no apparent changes in calcineurin immunoreactivity were found in the substantia gelatinosa. By contrast, there was a significant depletion of synaptophysin-positive axon terminals in the substantia gelatinosa and in the dorsal nucleus of Clarke of patients with familial dysautonomia.

Key words: Calcineurin — Synaptophysin — Familial dysautonomia — Spinal cord — Substantia gelatinosa

Familial dysautonomia (Riley-Day syndrome) is an autosomal recessive disorder observed in Ashkenazi Jews, which is characterized clinically by sensory deficits and autonomic abnormalities [17, 22, 23]. Pathologically there is severe impairment of autonomic and spinal root ganglia neurons, with evidence of developmental and degenerative changes [1, 5, 16—18, 21]. Marked depletion of synaptophysin (SP)-containing axons in the substantia gelatinosa is found as a reflection of diminution of the primary sensory afferents [19]. Although abnormal function of nerve growth factor has been postulated to be related to developmental failure in sensory and sympathetic ganglia in familial dysautonomia [21], the pathogenesis of this disease remains unclear.

We have performed an immunohistochemical assay to determine the distribution patterns of two neuronal Ca²⁺-binding proteins, calcineurin [6—8, 13] and SP [14, 20, 24], in the human spinal cord and to elucidate whether or how they are changed in familial dysautonomia. On the basis of the findings obtained, we discuss the anatomical abnormalities occurring in the spinal cord gray matter of familial dysautonomia.

Materials and methods

Spinal cord was obtained at autopsy from four patients with familial dysautonomia, aged 9 months 3, 15 and 18 years, and from 10 controls, aged 6 months to 45 years, who had no neuropathology. Spinal cord tissues were formalin fixed and paraffin embedded. Neuropathological examination utilized hematoxylin-eosin and luxol fast blue/periodic acid-Schiff (LFB-PAS) stainings.

An affinity-purified rabbit antibody to calcineurin, previously characterized and employed in other immunocytochemical studies, was used [6—10]. Mouse monoclonal antibody to SP (Boehringer-Mannheim) and rabbit polyclonal antibody to SP (Cambridge Research Biochemicals) were used at dilutions of 1:100 and 1:1000, respectively, with overnight incubation at 4°C. Bound antibodies were visualized by employing biotinylated secondary antibodies bridged to biotinylated horseradish peroxidase with avidin using the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame) following manufacturer's instructions. 3,3'-Diaminobenzidine was used as chromogen. Staining specificity was assessed by the methods described previously [6—10].

Results

In control individuals, calcineurin immunoreactivity was seen only in the gray matter of the spinal cord. The highest concentration of calcineurin immunoreactivity was seen in the substantia gelatinosa of the dorsal horn (Fig. 1 A), where a number of calcineurin-posi-
Fig. 1 A–F. Immunohistochemical distributions of calcineurin and synaptophysin (SP) in the spinal cord. 

A Calcineurin-staining of the lumbar cord from a control subject. Strong calcineurin immunoreactivity in the substantia gelatinosa can be seen. ×10.

B Enlargement of square in A. Calcineurin-positive small cells and fibers can be seen. ×50. 

C SP-staining of the lumbar cord from a control subject. Dense fiber networks positive for SP are present in the substantia gelatinosa. ×10. 

D SP-staining of the thoracic cord from a 14-year-old patient with familial dysautonomia. SP-like immunoreactivity is faint in the substantia gelatinosa (arrow) ×16. 

E Calcineurin-staining of the thoracic cord from a control subject. ×8. 

F Calcineurin-staining of the thoracic cord from a 3-year-old patient with familial dysautonomia. No abnormalities in calcineurin immunoreactivity are found in the substantia gelatinosa. The dorsal column appears to be of small size. ×10.