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Sympathetic neuroaxonal dystrophy in the aged rat pineal gland

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Abstract

Dysfunction of circadian melatonin production by the pineal gland in aged humans and rats is thought to reflect the functional loss of its sympathetic innervation. Our ultrastructural neuropathologic studies of the sympathetic innervation of the pineal gland of aged (24 months old) Fischer-344 and Sprague–Dawley rats showed loss of nerve terminals as well as the development of neuroaxonal dystrophy (NAD), an ultrastructurally distinctive distal axonopathy, far in excess of that in young control rats. Immunolocalization of tyrosine hydroxylase confirmed the age-related loss of normal noradrenergic innervation and development of NAD. NAD was more frequent in aged female rats compared to males and was particularly severe in aged female Sprague–Dawley rats compared to Fischer-344 rats. Pineal NGF content was significantly increased or unchanged in female and male aged Fischer-344 rats, respectively, compared to young controls. The rat pineal is a sensitive experimental model for the quantitative ultrastructural examination of age-related neuropathological changes in nerve terminals of postganglionic noradrenergic sympathetic axons, changes which may reflect similar changes in the diffusely distributed sympathetic innervation of other targeted endorgans.

Keywords: Sympathetic nervous system; Neuroaxonal dystrophy; Age; Pineal; Synapse; Distal axonopathy

1. Introduction

Dysfunction of the sympathetic nervous system is a recognized complication of aging in humans and experimental animals involving a variety of physiological processes (e.g., body temperature, blood pressure, alimentary tract motility, etc., see [50] for review) and affecting the response to a variety of stressors which reveal pathophysiology not present at an unstressed baseline [4,30]. The neuronal numbers in aged human [9,48,52] and rodent [15,43,44] sympathetic ganglia are preserved with aging. In contrast, structural alterations in intraganglionic terminal axons and synapses have been consistently identified in aged human [14,24,48,52] and rodent [51,57] sympathetic ganglia. The hallmark pathologic alteration in aged sympathetic ganglia is neuroaxonal dystrophy (NAD), a distinctive axonopathy characterized by marked enlargement of terminal axons and synapses by a variety of subcellular organelles [49], which is thought to reflect intraganglionic sprouting by sympathetic neurons. This pattern is likely to produce selective disconnection of ganglionic neurons and contribute to the loss of integrated reflexes.

Ultrastructural neuropathologic studies of the effect of age on the distal postganglionic sympathetic innervation of target organs are rare, due, in large degree, to the inherent difficulty of examining, identifying and quantitating the diffusely distributed sympathetic autonomic terminals in endorgans. In our previous experimental studies of acrylamide-induced sympathetic autonomic neuropathy [56], we established the rat superior cervical ganglia (SCG)/pineal gland system as a sensitive experimental model for the examination of neuropathological changes in the sympathetic innervation of a discrete target tissue. The postganglionic noradrenergic sympathetic innervation of the pineal arises from the SCG, ascends via the internal carotid arteries and forms the nervi conarii which ramify within the pineal capsule as minute 20–50 axon-containing nerve fascicles. Small branches arise from these capsular nerves and project along the vasculature

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culminating in delicate terminal axons which ramify adjacent to and are intimately apposed to pineocytes, although true synapses between sympathetic nerve terminals and pineocytes do not occur. Sympathetic axons arising in the SCG constitute the vast majority of a rich pineal innervation and are collected into an endorgan only 1–2 mm in diameter in the rat, allowing large numbers of the most distal elements of the sympathetic innervation to be easily sampled for ultrastructural analysis, simplifying the appreciation of a distally accentuated ("dying back") pattern of axonopathy and nerve terminal pathology.

Circadian changes in circulating melatonin levels reflect changes in sympathetic tone and its effect on the activity of the melatonin synthesizing enzyme arylalkylamine *N*-acetyltransferase [19]. Significantly, both aged rats (particularly females, [37,68]) and aged humans (especially patients with Alzheimer's disease, [27]) show attenuated diurnal variation of serum melatonin [31,34,38,39,41,61,63–65] and, in experimental animals, pineal synthetic enzyme activity [34,60], findings which may reflect deficient sympathetic nervous system innervation, activity or receptor content [20–22,42,62,67]. In support of an age-related change in sympathetic innervation of the pineal, it has been recently reported that the sympathetic innervation of the rat pineal loses functional plasticity [23] and terminal branching with age [21]. In order to more fully characterize the sympathetic innervation of the pineal gland in aged rats, we have examined its ultrastructural appearance. We report the loss of normal terminal axons and development of the distinctive pathology of NAD, determine its time course and establish an effect of strain and gender. In addition, we have investigated the effect of induced axonal sprouting in response to chronic exogenous treatment with NGF and NT-3.

2. Methods

2.1. Animals

Male and female 3-, 11–12- and 24-month-old Sprague–Dawley (Charles River Laboratories, Wilmington, Massachusetts) and 4–6, 12 and 20–24 months Fischer-344 rats (NIA supported colony at Charles River Laboratories, Wilmington Massachusetts) were used in this study. Rats were fed Purina rodent chow ad libitum and were housed individually or in small groups with a 0700–1900 light cycle.

2.2. Ultrastructural methods

Pineal glands were fixed by intracardiac perfusion with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) containing 0.45 mM Ca^{2+} preceded by saline containing heparin. After perfusion, pineal glands were dissected free of adjacent tissues and were placed in fixative overnight at 0–4 °C. A few pineal glands were fixed by immersion in the same fixative after rapid dissection, which did not affect the ultrastructural appearance of individual dystrophic axons or their frequency. Tissue samples for ultrastructural examination were postfixed in buffered OsO_4, dehydrated in graded alcohol solutions and propylene oxide and embedded in Epon. One micrometer thick sections were examined by light microscopy after staining with toluidine blue. Thin sections were cut onto formvar coated slot grids, which permitted the examination of nearly the entire pineal cross sectional area, stained with uranyl acetate and lead citrate and examined with a JEOL 1200 electron microscope.

The frequency of neuroaxonal dystrophy was determined by systematically scanning entire cross sections of individual pineal glands at 20,000× magnification counting the numbers of dystrophic axons and the numbers of nucleolated pineocytes. The frequency of dystrophic axonopathy is expressed as the ratio (as a percentage) of numbers of dystrophic axons divided by the numbers of nucleolated pineocytes, a method similar to that we have previously used to quantitate dystrophic elements in sympathetic ganglia [48].

2.3. Tyrosine hydroxylase immunohistochemistry

Animals were fixed by perfusion with freshly made 4% buffered paraformaldehyde at 0–4 °C and processed routinely for paraffin embedding. Paraffin embedded sections 5–8 μm thick were deparaffinized in xylene and rehydrated in a series of ethanol dilutions. Sections were preincubated for 20 min at room temperature in phosphate-buffered saline containing 2% BSA and 0.3% Triton X-100. Rabbit anti-tyrosine hydroxylase antibody (1:1000, Incstar, Stillwater, MN) was next added and the slides incubated overnight at 4 °C, washed and a secondary biotinylated goat anti-rabbit IgG (1:200) was applied, washed and followed by tyramide signal amplification using successive incubation with streptavidin HRP and cyanine-3 tyramide (Perkin-Elmer Life Science Products, Boston, MA).

2.4. Exogenous treatment of rats with NGF and NT-3

Three groups of rats are represented in this study:

(1) Untreated 12-month-old male Sprague–Dawley controls.
(2) Twelve-month-old rats treated with NT-3 (10 mg/kg, subcutaneously, 3× week, provided by Regeneron Pharmaceuticals, Tarrytown, NY) for the last 8 weeks of life ("control + NT-3").
(3) Twelve-month-old rats treated with rhNGF (1 mg/kg, subcutaneously, 3× week, provided by Genentech, Inc., San Francisco, CA) for the last 12 weeks of life ("control + NGF").

2.5. NGF assay

Fischer-344 male and female rats were euthanized at 4–5 and 24 months of age. The pineal was dissected, cleaned of adherent fat and frozen on dry ice. Pineals were maintained