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14. This research was supported in part by Public Health Service grants NS 14319 and NS 14325 and by the Jack Wechter Memorial Fund. We thank M. Ganapathi for technical assistance.

13 April 1979; revised 5 July 1979

Tyrosine Hydroxylase Immunoreactivity in Familial Dysautonomia

Abstract. *Tyrosine hydroxylase antigen was localized immunohistochemically in sympathetic neurons from human autopsy tissue. The reaction persists in paraffin-embedded tissue, and the method is applicable to archival specimens. Increased amounts in this antigen per cell may partially compensate for decreased numbers of sympathetic neurons in familial dysautonomia.*

Tyrosine hydroxylase (TH) antigen was detected immunocytochemically in neurons of human sympathetic ganglia and found to be increased in the disease familial dysautonomia (FD). The antigen was detectable unchanged in control tissue embedded for 11 years in paraffin after fixation in formalin. Thus, the technique could be used in retrospective studies. The TH-immunoreactivity (TH-IR) as measured by the peroxidase-anti-peroxidase (PAP) technique (*1*) does not indicate enzymatic activity but, when all staining factors are kept constant and with substrates in excess, the quantity of reaction product is determined by the amount of TH protein present. Studies of rat PC12 pheochromocytoma in which TH enzymatic activity was manipulated over long periods indicated corresponding changes in TH-IR.

Familial dysautonomia is an autosomal recessive disease which appears in infancy and is characterized by motor, sensory, and autonomic abnormalities (*2*). Clinical, biochemical, pharmacologic, and anatomic data indicate diminution in the numbers of sympathetic neurons (*3*). Superior cervical sympathetic ganglia (SCSG) from nine controls and nine FD patients (*3*) were stored in paraffin after formalin fixation (Table 1). Sections (10 μm) of this tissue were subjected to simultaneous processing in the same reagents to ensure that the only variable was the amount of TH antigen present; the sections were stained as described (*4*). Rabbit antibodies to TH antigen purified from human pheochromocytoma (*5*) have been used to demonstrate catecholaminergic neurons in human brain, adrenal medullary cells, and sympathetic axons (*4*).

Control sympathetic neurons exhibited varied staining intensity (Fig. 1A), with a few showing virtually no reaction product. Variation in catecholamine content of sympathetic neurons has been demonstrated by induced histofluorescence (*6*). In some species, adrenergic (A) and dopaminergic (DA) neurons are present in addition to the predominating noradrenergic (NA) neurons (*7*). If human ganglia are similar, variability in TH-IR might be anticipated since amounts of TH appear to be higher in A and DA neurons than in NA neurons (*8*). Neurons without TH-IR possibly contain noncatecholamine transmitters, as has been suggested for other species (*9*).

Within FD neurons, skeins of fine fibrils stained more intensely than in controls. Fibrils extended into the axons where they formed spirals around one another. Axons in FD were wider than in controls, and the number of fibrils was greater. In every FD patient, almost all SCSG neurons stained with greater intensity than controls (Fig. 1B). Mean densitometer readings of TH/PAP end product in 50 neurons of each of the patients and controls appear in Fig. 1C. Mean absorbancy of the controls was 0.39 ± 0.05. In FD it was 0.80 ± 0.15 ($P < .01$). Significant difference persisted if the two older controls were omitted. Individual patients differed sig-

Table 1. Ages and causes of death of FD patients and controls. All FD patients had complex clinical histories including episodes of hypoxia and hypotension. Pharmacology: during vomiting crises six patients were treated for brief periods (usually a few days and not just prior to death) with chlorpromazine (a) and one had received this drug over a long period (b). One patient was occasionally treated with methacholine (c). Two patients received no neuropharmacologic agents. No relation was found between the form of therapy and TH-IR in neurons. Except as indicated by diagnosis, controls were apparently in good health until their deaths.

Code number	Age (years)	Period of block storage (years)	Pharmacology	Cause of death
		Patients		
54436	1	11	0	Respiratory failure
UA 49 73	4	6	0	Aspiration pneumonia
N 71 73	9	6	a, c	Aspiration of vomitus
F 2909	15	1	a	Respiratory failure
UA 178 72	16	7	a	Aspiration pneumonia
RI 74 106	17	5	a	Aspiration pneumonia
UA 113 77	18	2	a	Cardiac arrest
F 2560	28	3	b	Aspiration of vomitus
UA 17 76	33	3	a	Renal failure
		Controls		
54632	1	11	0	Werdnig-Hoffman disease
F 2596	1	3	0	Drowned
57839	3	3	0	Fanconi syndrome
F 2615	14	3	0	Barbiturate poisoning
F 2920	25	1	0	Gunshot
F 2585	28	3	0	Car crash
B 76-1656	36	3	0	Gunshot
B 2919	50	1	0	Gunshot
58379	57	1	0	Myocardial infarct

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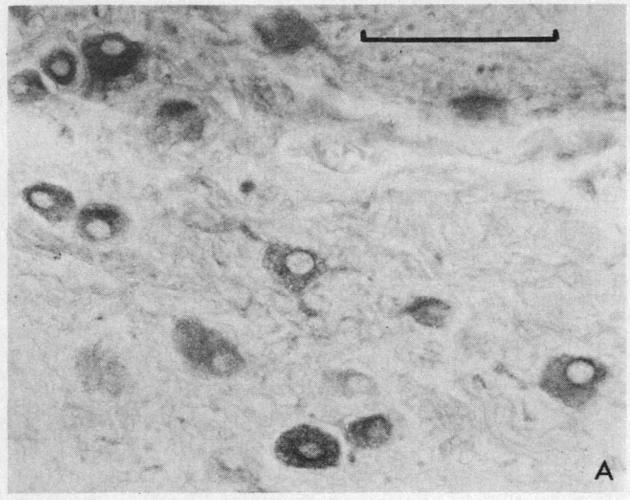

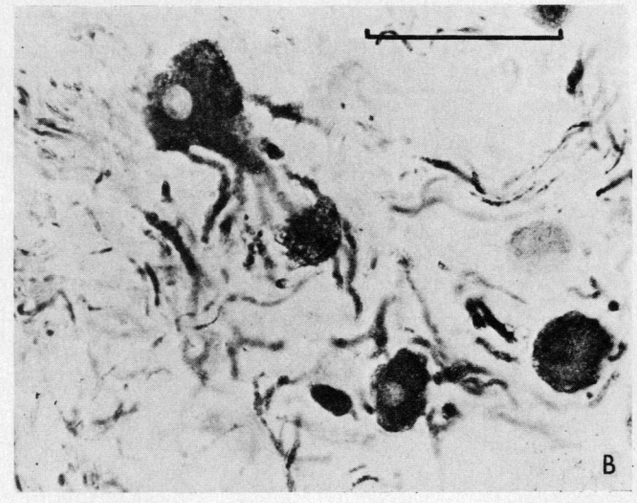

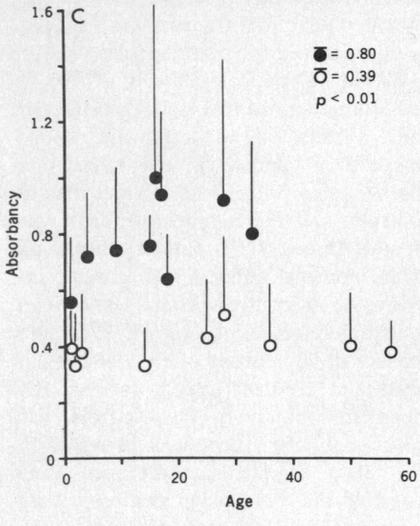

Fig. 1. (A) Superior cervical sympathetic ganglion TH/PAP immunoreactivity from a control subject, aged 14 years. (B) Specimen from a 15-year-old patient; the specimen received the same treatment in the same reagents at the same time. Staining in perikarya and axons is more intense. Although the neuron diameter is characteristically greater in the patient, this cannot account for the difference in staining intensity since the section thickness is 10 μm for all tests and controls, thus ensuring light paths of equal length through neuronal cytoplasm (bar, 100 μm; photographic conditions automated and constant). (C) The means of microdensitometer (Zeiss PMI) readings at a wavelength of 490 nm of TH/PAP immunoreaction product in 50 sympathetic neurons. Only neurons cut through the planes of their nuclei were measured. Random sampling was assured by measuring neurons sequentially as they "passed" a point on a filar micrometer while the stage was moved in one axis. (●) FD patients; (○) controls; bars, 1 S.D.

nificantly from controls of similar age ($P < .01$; Student's t-test).

These results were not an artifact attributable to systematic differences in postmortem intervals since the range was similar in patients and controls. Ganglia from a patient where the postmortem interval was 57 hours between death and autopsy, the longest in this study, showed the same intensity of TH reaction product as other FD cases. Time of storage appears not to be a factor. No change in staining intensity occurred in control tissue stored between 1 and 11 years. FD ganglia stored for 11 years showed the same increased staining as did ganglia stored for 1 year. Separate studies indicated that the antigen was stable in formalin for at least 2 weeks; ganglia were processed after no more than 2 days in fixative.

In all FD patients, SCSG were hypoplastic (*3*). Neuron populations were low (mean for FD was 120,000, with a range of 107,000 to 184,000; mean for the control group was 1,060,000, with a range of 894,000 to 1,120,000) (*3*). In SCSG from two patients Black (*10*) found TH activity per ganglion to be within normal range. In opposite SCSG from these two cases, we counted totals of 126,000 and 136,000 neurons (F2909 and UA11377). Since ganglia with less than 14 percent of the normal populations of neurons had normal TH activity, there must have been an increase in TH activity per neuron. Immunohistochemical studies indicated increased TH antigen per neuron. Both biochemical and immunohistochemical studies are consistent with the hypothesis of a compensatory increase of TH in the surviving neurons. The slight increase in neuronal size in FD (*3*) does not appear sufficient to account for these observations. Selective survival of neurons normally rich in TH would not in itself be a sufficient explanation because even the most intensely stained control neurons took less stain than the majority of those in the patients. With diminution of peripheral sympathetic axons in FD (*3*), there might be some "damming back" of TH in perikarya. Destruction by 6-OH-dopamine of the peripheral terminals of neurons stimulated by NGF results in increased TH and accumulation of neurofibrils (*11*).

The SCSG neurons tend to diminish in number in older patients (*3*). In Fig. 1C, the trend toward increased TH-IR per neuron with increasing age is apparent. This may represent adaptive biochemical modification in those that are survivors.

Useful information regarding neurotransmitter synthetic enzymes can be derived from archival human autopsy tissue. The detail demonstrated at the cellular level is unattainable at present by biochemical techniques. Biochemical hypertrophy may be a means, other than axonal sprouting and denervation supersensitivity, by which neurons, although incapable of cellular regeneration, retain some degree of plasticity in pathologic conditions.

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12. Supported by the Dysautonomia Foundation and NIH grant R01 HD12260-02.

27 February 1979; revised 8 June 1979