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GABA Catabolism: Localization of Succinic Semialdehyde Dehydrogenase in Brain Motor and Sensory Nuclei

Abstract. *The localization of brain succinic semialdehyde dehydrogenase, a specific gamma-aminobutyric acid degradative enzyme, could potentially yield valuable information concerning the function of the enzyme. Application of a new histochemical technique for this enzyme has revealed characteristic patterns of neuronal staining that are consistent within embryologically and functionally similar nuclei of the brainstem of the rat.*

Cytochemical procedures for localizing neurotransmitters and their metabolic enzymes provide important morphological clues to the chemical circuitry of the central nervous system. At the light microscopic level, such work has been limited almost exclusively to the monoamines (*1*) and to acetylcholinesterase (*2*). In an attempt to develop methods that might be useful in locating synapses mediated by gamma-aminobutyric acid (GABA), we modified standard tetrazolium methods for oxidation reactions [see (*3*)] to localize succinic semialdehyde dehydrogenase (SSADH) in the brain of the rat (*4*). We now report that this staining method for SSADH produces a characteristic pattern of staining among cranial nerve nuclei; neurons of predominantly motor function can be clearly distinguished from neurons with predominantly sensory function. This result suggests that the cytochemical differences among these functionally distinguishable neurons may reflect similar characteristic patterns of their neurochemical innervation.

Sprague-Dawley rats (250 g) were decapitated, and the whole brain or the spinal cord was removed; the handling of the tissue and the histochemical procedure for SSADH were performed as previously described (*4*). The histochemical procedure utilizes direct reduction of nitroblue tetrazolium by reduced nicotinamide adenine dinucleotide at *p*H 9.0; deposition of formazan has been shown to be independent of tissue diaphorase activity (*4*). Coronal sections (14 μm) were taken serially at 56-μm intervals beginning at the midcervical spinal cord and extending through the telencephalon. Sagittal sections were similarly processed from midline to the lateral extent of the hemisected brain; the caudal limit of the sagittal sections was high thoracic spinal cord, but all rostral brain structures were included. Frequently, 14-μm sections were stained with toluidine blue to assess the population of neurons present in sections adjacent to those used for the SSADH staining reaction.

Comparison of sections reacted histochemically for SSADH with seriatim sections stained with toluidine blue reveals that only a small population of neurons present within the brainstem are stained for SSADH. Specific nuclear groups stained for SSADH uniformly exhibit either cell body staining, or neuropil staining, or both. Staining comparisons are based on comparative analysis of serial sections through the various nuclei; intranuclear morphological differences require that ranking of neuropil staining represents an overall as-

Table 1. Histochemical activity of SSADH in the brainstem of the rat. Staining intensity is based on a scale from 0 to 4+. Neuropil staining is compared in relation to other neuropil areas; that is, it is not judged relative to adjacent cellular staining.

PRIMARY MOTOR NUCLEI*

(A) *Somatic efferent column*			(B) *Special visceral efferent column*			(C) *General visceral efferent column*		
Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil
Oculomotor	++++	++	Trigeminal motor	++++	++	Edinger-Westphal	++++	+++
Trochlear	++++	++	Facial	++++	++			
Abducens	++++	++	Ambiguus	++++	++	Dorsal motor nucleus of vagus	++++	+++
Hypoglossal	++++	++	Spinal accessory	++++	++			

SENSORY NUCLEI†

(D) *General somatic afferent column*			(E) *Visceral afferent column*			(F) *Special somatic afferent column*‡		
Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil	Nucleus	Neuronal perikaryon	Neuropil
Mesencephalic root	++++	++	Tractus solitarius	0	++++	Ventral cochlear	++++	++
Principal trigeminal	0	++++				Dorsal cochlear	+	++++
Spinal tract trigeminal	0	++++	Parasolitarius	0	++++	Inferior vestibular	+	++++
Gracilis	0	++++				Medial vestibular	+	++++
Cuneatus	0	++++				Lateral vestibular	++++	+
Substantia gelatinosa	0	++++				Superior vestibular	+++	++

* Large (25 to 50 μm) multipolar, intensely stained neurons predominate as the cell type observed in columns A and B. Column C neurons are 15-by 40-μm fusiform or ovoid cells surrounded by homogeneous strong staining neuropil (see Fig. 1). † All sensory nuclei shown are secondary sensory nuclei with the notable exception of the mesencephalic root trigeminal nucleus. Cells of the mesencephalic root are primary sensory afferent cells analogous to the first order ganglion cells of the other sensory nuclei listed. ‡ The special afferent system also includes olfaction (visceral) and vision (somatic).

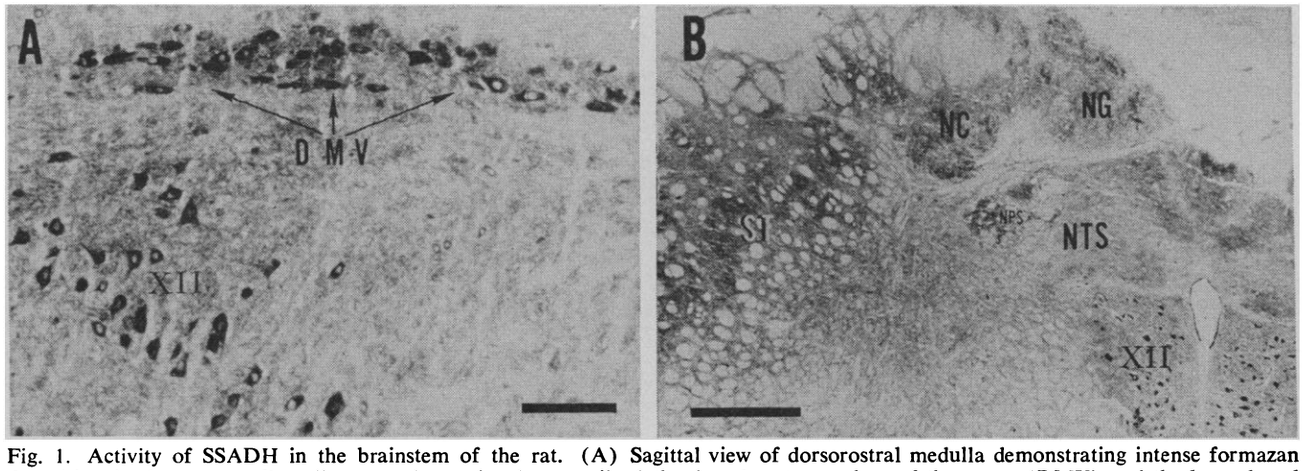

Fig. 1. Activity of SSADH in the brainstem of the rat. (A) Sagittal view of dorsorostral medulla demonstrating intense formazan deposition in the nerve cell perikarya and associated neuropil of the dorsal motor nucleus of the vagus (*DMV*) and the hypoglossal nucleus (*XII*). Scale line, 100 μm. (B) Coronal view through medulla. Nuclei are gracilis (*NG*), cuneatus (*NC*), tractus solitarius (*NTS*), parasolitarius (*NPS*), hypoglossal (*XII*), and spinal trigeminal (*ST*). Scale line, 500 μm.

sessment of the entire nucleus. Staining patterns are unrelated to neuronal size or shape.

The pattern of the histochemical activity of SSADH throughout the brainstem, the cranial nerve nuclei, and the analogous spinal cord structures corresponds closely to the functional and developmental organization of these nuclei into efferent and afferent systems (*5*). Primary efferent motor nuclei in both visceral and somatic systems exhibit the common characteristic of staining in nerve cell perikarya and surrounding neuropil (Table 1). For example, this pattern of staining is seen in general visceral efferent nuclei, such as the dorsal motor nucleus of the vagus (Fig. 1A), as well as in the somatic efferent nuclei (oculomotor, trochlear, abducens, and hypoglossal) (Fig. 1B) and their spinal cord homolog, the anterior horn cell. The other group of efferent cranial nuclei, the special visceral efferent column nuclei (spinal accessory, ambiguus, facial, and trigeminal motor) similarly show intense perikarya staining with moderate formazan deposition in the surrounding neuropil.

The pattern of staining in sensory nuclei after the histochemical reaction for SSADH is quite different than that observed for the efferent system (Table 1). The tractus solitarius nucleus (visceral afferent column) and the principal and spinal trigeminal sensory nuclei (general somatic afferent column) exhibit intense staining of neuropil with little or no staining of nerve cell perikarya (Fig. 1B). However, the neurons of the mesencephalic root of the trigeminal nucleus deviate markedly in SSADH staining from the pattern exhibited by other sensory trigeminal neurons. The characteristic spherical, 40-μm neurons of this nucleus are among the most intensely reactive cell bodies in the brain.

A more complex pattern of staining occurs in the special somatic column of hindbrain composed of the vestibular and cochlear nuclei (Table 1, column F). For example, the intense diffuse formazan deposition limited to neuropil seen in the general somatic and visceral afferent column nuclei is also observed in specific subgroups of the VIIIth nerve nuclei, such as the outer lamina of the dorsal cochlear nucleus and the medial vestibular nucleus. Those subdivisions that receive a substantial proportion of their afferent input from higher or lower modulatory centers, exemplified by the lateral vestibular (*6*) and ventral cochlear (*7*) nuclei, exhibit neuropil and perikaryonal staining similar to that observed in the efferent motor columns.

Thus, neurons of specific functional classifications exhibit similar characteristic patterns of cellular reactivity for an enzyme specifically related to the catabolism of a putative neurotransmitter, GABA. Further analysis of cellular staining patterns revealed by the histochemical method for SSADH may reveal common functional properties not readily achieved by other types of neurotransmitter histochemistry. The SSADH staining patterns of other neuronal systems (*8*) also appear to fall into functionally defined subgroups. While electron miscroscopic methods (*9*) and complementary electrophysiologic studies (*10*) will be required to elucidate the synaptic role played by GABA on each of the classes of neurons, the cytochemical similarities among functionally homologous neuronal subgroups suggest that common patterns of GABA-mediated synaptic innervation may exist. Although SSADH staining may only indirectly be related to GABA transmission points, the common cytochemical properties revealed by the reaction may provide useful indices for further physiologic exploration.

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