Some Neurons of the Rat Central Nervous System Contain Aromatic-L-Amino-Acid Decarboxylase but Not Monoamines

Abstract. *Neurons containing the enzyme aromatic-L-amino-acid decarboxylase (AADC) but lacking either tyrosine hydroxylase or serotonin were found in the spinal cord of neonatal and adult rats by light and electron microscopic immunocytochemistry. The majority of these neurons localized to area X of Rexed contact ependyma. Thus, spinal AADC neurons have the enzymatic capacity to catalyze directly the conversion of the amino acids tyrosine, tryptophan, or phenylalanine to their respective amines tyramine, tryptamine, or phenylethylamine. These amines normally present in the central nervous system may be of potential clinical significance as endogenous psychotomimetics.*

The enzyme aromatic-L-amino-acid decarboxylase (AADC) (E.C. 4.1.1.28) catalyzes the decarboxylation of L-dihydroxyphenylalanine (L-dopa), 5-hydroxytryptophan, and aromatic L-amino acids including L-tryptophan, L-tyrosine, and L-phenylalanine (*1*). Although the enzyme is widely distributed in the mammalian body, it is generally assumed that in neurons of the central nervous system (CNS) AADC is restricted to those cells that synthesize, store, and release monoamine neurotransmitters, specifically the catecholamines (dopamine, norepinephrine, or epinephrine) or serotonin (*2*). In these neurons AADC is expressed along with other specific enzymes required for monoamine biosynthesis, so that in monoamine neurons the substrate for AADC is synthesized in situ from amino acid precursors. Thus, in catecholamine neurons L-dopa is synthesized by tyrosine hydroxylase from tyrosine and in serotonin neurons 5-hydroxytryptophan is synthesized by tryptophan hydroxylase from tryptophan.

In the periphery, AADC is not restricted to tissues synthesizing catecholamines or serotonin. The enzyme is found in islet cells of the pancreas and in the kidney and liver (*3*). The presence of AADC in nonmonoaminergic cells raises the question of whether AADC also is expressed in cells of the CNS that synthesize neither catecholamines nor serotonin.

We have recently observed that in the rat a group of cells containing AADC appears within the neural tube during embryogenesis (*4*). Since monoamine neurons are not found in adult spinal cord (*5*), the observation raises the questions: (i) Are these AADC cells in the developing spinal cord neurons that, like their peripheral counterpart, persist throughout adult life? and (ii) Do they contain monoamines?

Eighteen Sprague-Dawley rats of various ages (4, 15, 30, 90, or 180 days) were anesthetized with pentobarbital (60 mg per kilogram of body weight, intraperitoneal) and perfused through the heart with 4 percent paraformaldehyde in phosphate buffer. The tissues were prepared for routine light and electron microscopic immunocytochemistry by methods described in (*6*). Highly specific antibodies were prepared in rabbits to AADC, tyrosine hydroxylase (TH), and phenylethanolamine *N*-methyltransferase (PNMT) (*7*). The criteria establishing the purity of the antigens and the specificity of antibodies for immunocytochemistry (*6*) also included a different pattern of staining on adjacent sections incubated with different antisera.

Cells containing AADC were localized throughout the spinal cord in animals of all ages. The densest distribution of these AADC-positive cells was in the cervical cord (Fig. 1). Thoracic and lumbar segments of the cord had fewer AADC cells; in the thoracic cord, intermittent clusters of AADC cells occurred (Fig. 1). In the cervical segments, three to eight spinal AADC cells were seen in the average section. The majority of the AADC cells were localized to area X of Rexed (*8*) surrounding the central canal.

Most AADC cells were juxtaposed to the ependymal cells of the central canal (Fig. 2, a and b). They had small (8 to 11 μm in diameter), round-to-oval cell bodies and several short processes (Fig. 2, a and b). With few exceptions, AADC-containing cells of the spinal cord extended at least one of their processes into the lumen of the central canal (Fig. 2, a and e). Fine tortuous processes of the AADC cells extended laterally from the soma (Fig. 2a). Lateral processes projected rostrally and caudally for short segments parallel to the lumen of the central canal. Some spinal AADC cells lacked a central process.

Serial sections were prepared, and adjacent sections were stained with antibodies to monoamine synthetic enzymes TH and PNMT and to serotonin (purchased from Immunonuclear Corporation, Stillwaters, Minnesota) No perikarya in the spinal cord stained with antibodies to TH or PNMT or even serotonin (Fig. 2c), although each antibody stained processes in patterns characteristic for each antigen (*5*). Even though the failure to stain with antibodies to PNMT or TH or serotonin does not rule out the possibility that immunocytochemically undetectable amounts of enzymes sufficient for in situ biosynthesis of monoamines could be present in AADC cells, in the light of the earlier findings (*5*), this seems highly unlikely.

To establish whether the AADC cells had morphological characteristics of neurons, we examined them by electron microscopic immunocytochemistry. Profiles through the soma of the AADC cells often had triangular or oval shapes (Fig. 2d). They had small rounded nuclei with patches of excentric chromatin. Mitochondria formed clusters near prominent cisterns of Golgi apparatuses and rough endoplasmic reticulum. Occasionally, multivesicular bodies were seen in the cytoplasm. Axon terminals containing pleomorphic vesicles abutted the surfaces of the AADC cells at different positions. Primary processes of AADC cells were contacted by axon terminals similar to those seen on perikarya. Typical asymmetric synapses were found between small axonal butons and distal

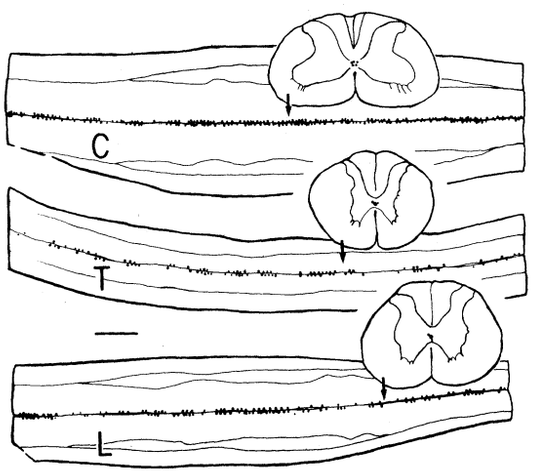

Fig. 1. The distribution of AADC cells in the spinal cord area X of Rexed. Cells (dots) were mapped from three adjacent longitudinal sections (25 μm thick) of the cervical (*C*), midthoracic (*T*), and lumbar (*L*) segments of a 30-day-old rat (case 24E). Arrows indicate the approximate level of representative cross sections (case 22D). Rostral is on the left. There is a clustering of AADC cells in the thoracic region. Space bar = 1 mm.

processes of the AADC cells (Fig. 2f). A central process of AADC neurons projected into the lumen of the central canal where it formed a bulbous enlargement (Fig. 2e). Central as well as lateral processes of AADC cells contained vesicles of various sizes and granularity. Thus, on the basis of the usual criteria of their fine structure, the spinal AADC cells were neurons (*9*).

This study demonstrates an anatomically defined group of cells in the adult CNS that contains AADC. On the basis of their morphological features and ultrastructure, AADC cells are neurons.

The spinal AADC neurons appear to have some characteristics similar to a group of neurons called "CSF [cerebrospinal fluid] contacting neurons" (*10*) found in various vertebrates and defined by the fact that one of their processes extends into the lumen of the central canal. These CSF contacting neurons may possess neurosecretory as well as neuroreceptor function (*10*) and have been considered as members of the large group of paraneurons (*11*).

Our observations also raise questions with respect to the identity of the neurotransmitter that the spinal AADC cells synthesize. Since AADC will decarboxylate a number of amino acids (*1*), it is possible that AADC cells may produce substances that are not conventionally considered as vertebrate neurotransmitters as, for example, tyramine from tyrosine, tryptamine from tryptophan, or phenylethylamine from phenylalanine. These "trace amines" (*12*) are contained in the mammalian brain and spinal cord, they have potent pharmacological and psychotropic action (*13*), and their participation in certain mental diseases has been postulated (*14*). The fact that the AADC neurons that we found presumably do not contain the enzymes required for the hydroxylation of either tyrosine, tryptophan, or phenylalanine establishes a biochemical basis whereby their direct decarboxylation could occur in situ in mammalian neurons.

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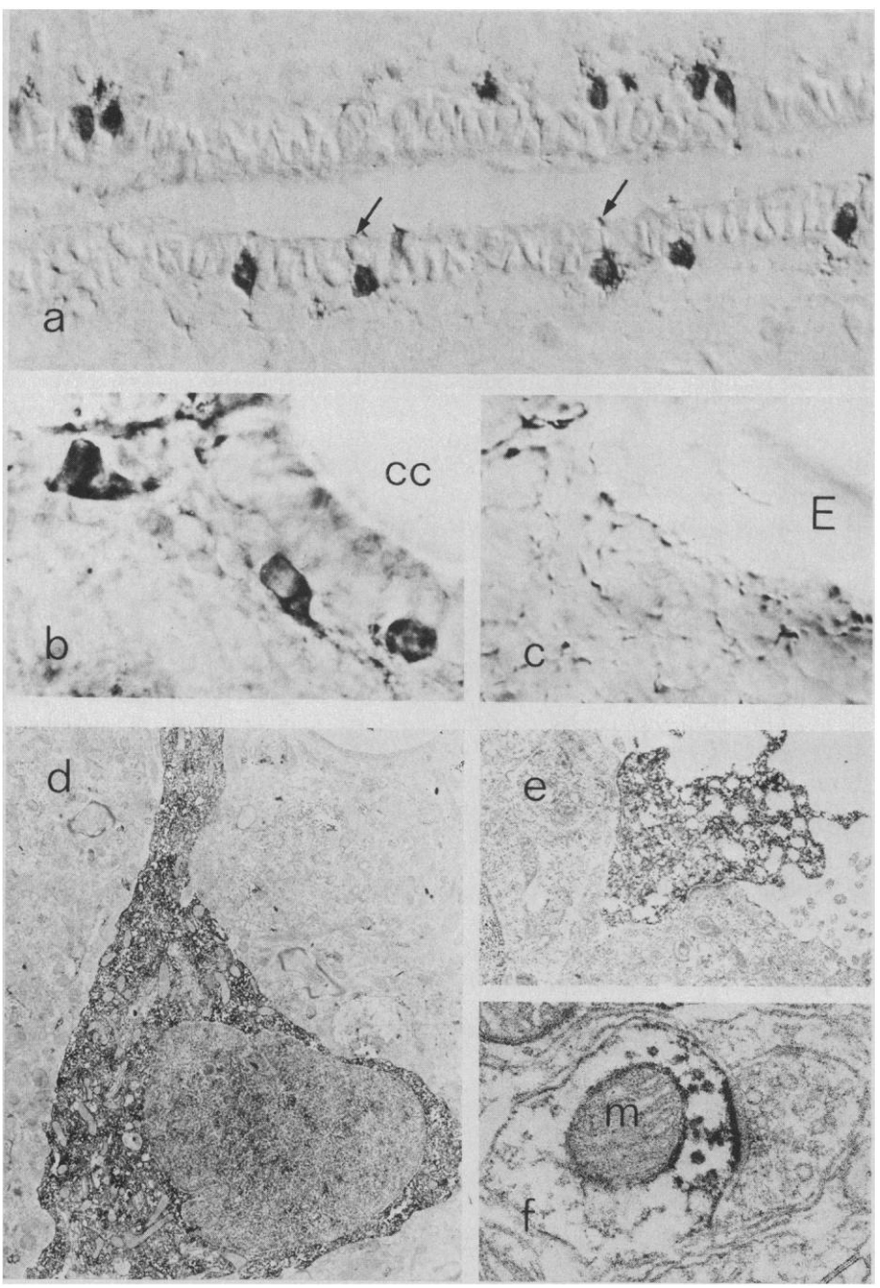

Fig. 2. (a) Localization of AADC in the perikarya of spinal subependymal neurons. Longitudinal plane through a cervical enlargement of a 4-day-old rat. Arrows point to the central process contacting the central canal. Antisera dilution 1:500 (×450). (b) Localization of AADC cells in the spinal cord (cut transversely) of an adult rat (90 days); *cc*, central canal. Phase-contrast optics (×950). (c) Localization of serotonin in varicose processes surrounding ependyma (*E*). Note the absence of cellular staining (×950). (d) AADC-containing perikarya (×5100). (e) A central process of the AADC cells protruding into the central canal. Note the terminal bars between the AADC cell and the neighboring ependyma (×11,200). (f) Asymmetric synapse between an AADC-positive dendrite and a small axon terminal; *m*, mitochondria (×43,500).

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lose, Sephadex G-200, and hydroxyapatite. The final product was then subjected to polyacrylamide gel electrophoresis, and the enzymatically active protein band was identified by the assay of gel slices. Gel slices that contained enzymatically active protein were collected, homogenized with 0.9 percent saline, mixed with Freund's complete adjuvant, and injected subcutaneously into rabbits. Four injections with a 2-week interval were required to produce high-titer antisera.
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Neuromuscular Patterns and the Origin of Trophic Specialization in Fishes

Abstract. *The pattern of muscle electrical activity in the pharyngeal muscles of the mollusc-eating sunfish* Lepomis microlophus *is highly specialized in comparison with the pattern displayed by most other members of the sunfish family and does not change when different prey are eaten. The closest genealogical relative of this species has the specialized muscle activity pattern for crushing prey when it feeds on snails but uses the primitive sequence of muscle activity during swallowing of other prey. The ability of species that crush snails to use molluscan prey effectively is due primarily to the evolutionary transformation of the neuromuscular program controlling the trophic apparatus.*

The process by which populations of organisms evolve to specialize on a particular resource in the environment is of considerable interest to evolutionary biologists. The phenomena of niche partitioning, character displacement, competitive exclusion of species, and evolutionary diversification within clades have all been linked to the ways in which organisms use environmental resources to obtain energy for growth and reproduction (*1*). Most analyses of trophic specialization have focused on morphological features of organisms as a reflection of their ability to collect and process food (*2*). I present experimental data from fishes on muscle activity patterns involved in the use of a specialized food resource. The results indicate that an evolutionary transformation in neuromuscular pattern resulted in a specialized method of acquiring energy.

The North American sunfish family Centrarchidae contains 32 species that display a wide range of food and habitat preferences (*3*). One species in the largest genus, *Lepomis microlophus* (redear sunfish), feeds primarily on freshwater snails (*4*). This food choice is specialized in that only one other species in the family, *Lepomis gibbosus*, feeds on snails to any significant degree; phylogenetically primitive species such as the bass *Micropterus* and the rock bass *Ambloplites*, as well as the other *Lepomis* species, are insectivorous and piscivorous; and, snails are not common elements of the diet in other morphologically generalized perciform fishes, which are predominately insectivores and piscivores (*5*).

Most teleost fishes capture prey by rapidly expanding the mouth and drawing water and the prey into the buccal cavity in a process known as suction feeding. Very little mastication occurs in the mouth, and prey are usually swallowed whole by movements of modified gill arch elements—the pharyngeal jaws (*6*). The pattern of pharyngeal muscle activity during intraoral prey manipulation and transport into the esophagus was studied by electromyographic recordings in six insectivorous and piscivorous sunfishes and in the perch *Perca* (*7*). All species exhibited a similar highly stereotyped pattern, with minor variations dependent on prey type and size (Fig. 1A). The key feature of the sequence of pharyngeal muscle electrical activity is the regular rhythmic pattern of activity (Fig. 1A) that may last a minute during prolonged swallowing sequences. For example, the muscle protracting the lower pharyngeal jaws alternates, occasionally in a double-burst pattern as in Fig. 1, with activity in the muscle retracting the lower pharyngeal jaws [pharyngohyoideus (PH) and pharyngocleithralis internus (PC_i), respectively, in Fig. 1A]. These features are consistently present in perch, as well as in all insectivorous and piscivorous sunfishes examined.

In the redear sunfish a radically different pattern of muscle activity occurs (Fig. 1B). All pharyngeal muscles are active together in a closely synchronized pattern to appose the pharyngeal jaws and crush the prey. When snails are being eaten, pieces of shell fall out of the mouth cavity as the shell is cracked, and sound recordings of snail crushing show that shell fracture occurs at the end of muscle bursts (Fig. 1B). Repeated crushing sequences occur until the snail shell has been completely fragmented and then the body and adhering shell pieces are swallowed. This same pattern of muscle activity occurs when the redear sunfish feeds on worms and fish. This species possesses only a single very stereotyped "crushing pattern" used without regard to prey consistency or size.

The closest genealogical relative (sister species) of *L. microlophus* (*8*) possesses a versatile activity pattern that can be modulated for different prey types. *Lepomis gibbosus* uses the primitive muscle activity pattern when eating fish and worms (Fig. 1A) but employs a stereotyped crushing pattern when eating snails (Fig. 1C). Pharyngeal muscle activity during snail crushing is similar in all respects to that of *L. microlophus* except that the duration of the burst in all muscles is significantly shorter [$t(70) = 5.46$, $P < .001$].

These results allow reconstruction of the sequence of evolutionary modification in muscle activity patterns involved in utilizing a specialized food type. A rhythmic pattern of coordinated pharyngeal muscle activity (Fig. 1A) transported prey into the esophagus in early sunfishes. Use of a new prey type, snails, in addition to fishes and invertebrates, was associated with the addition of a distinctive crushing pattern of muscle activity in which all the pharyngeal muscles are nearly synchronously active (Fig. 1C). The primitive pattern of rhythmic activity is retained as a component of the behavioral repertoire and is elicited by other types of prey. Finally, the snail-crushing pattern became the only component of the neuromuscular output involved in feeding to the extent that all prey are treated as though they were snails.

Morphological modifications associated with snail crushing are found in *L. microlophus* and include an increase in the proportion of molariform teeth on the pharyngeal jaws and an increase in physiological cross section of several of the pharyngeal muscles (*9*). However, no major structural reorganization has oc-