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Research paper

Wisteria floribunda lectin is associated with specific cell types in the ventral cochlear nucleus of the gerbil, *Meriones unguiculatus*

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Abstract

The cochlear nucleus is made up of a number of diverse cell types with different anatomical and physiological properties. A plant lectin, *Wisteria floribunda* agglutinin, that recognizes specific carbohydrate residues in the extracellular matrix binds to some cell types in the ventral cochlear nucleus but not to cells in the dorsal cochlear nucleus. In the ventral cochlear nucleus, the most intensely labeled cells are octopus cells, a subset of multipolar cells and cochlear root neurons. The multipolar cells that are labeled may correspond to the population that projects to the inferior colliculus.

Keywords: Auditory system; Octopus cells; Multipolar cells; Perineuronal nets

1. Introduction

The mammalian cochlear nucleus is populated by a variety of neuronal types that can be distinguished by differences in morphology, physiological properties, neurotransmitter chemistry, and connectivity (reviewed in Romand and Avan, 1997; Cant and Benson, 2003; Young and Oertel, 2004). The cell types first defined in cats (Osen, 1969; Brawer et al., 1974) and rats (Harrison and Irving, 1965, 1966) are also recognized in other species, including humans (reviewed by Cant, 1992; human: Moore and Osen, 1979; Richter, 1983; Adams, 1986, 1997). Many of the distinct cell populations in the cochlear nucleus, as well as the tracts formed by their axons, are segregated from one another, making it possible to target different cell groups in physiological or anatomical studies. Because of this, the cochlear nucleus is one of the most well-understood parts of the brain with respect to its component neuronal types and their physiology and connections. However, segregation of the different cell populations is not complete. Some of them are intermingled, and the identification of specific markers for each type can aid in distinguishing them in neuroanatomical, neurophysiological and other types of studies.

Lectins, proteins that bind to sugar residues in carbohydrate-containing macromolecules, have been shown to bind specifically to some cell groups in the central nervous system and not to others (reviewed by Spicer and Schulte, 1992). Because they can be conjugated to markers such as fluorescent tags or biotin that can be visualized histochemically, lectins can be used to detect the presence of specific carbohydrate-rich components of the extracellular matrix (ECM) in tissues throughout the body, including nervous tissue (see Section 4). In this paper, we describe the binding patterns of one such lectin, *Wisteria floribunda* agglutinin (WFA), which has been shown to bind to the ECM around some but not all neurons in the central nervous system (e.g., Celio et al., 1998). We suggest, based on the staining patterns in the ventral cochlear nucleus,

Abbreviations: AVCN, anteroventral cochlear nucleus; BDA, biotinylated dextran amine; cbm, cerebellum; cnr, cochlear nerve root; DCN, dorsal cochlear nucleus; ECM, extracellular matrix; gcl, granule cell layer; icp, inferior cerebellar peduncle; OCA, octopus cell area; PVCN, posteroventral cochlear nucleus; SCA, spherical cell area; scp, superior cerebellar peduncle; tb, trapezoid body; V, descending branch, trigeminal nerve root; VCN, ventral cochlear nucleus; vnr, vestibular nerve root; WFA, *Wisteria floribunda* agglutinin

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that WFA can be used in the gerbil to mark the locations of some specific cell types.

2. Materials and methods

Young female gerbils were obtained from Charles River Laboratories and housed in Duke University animal quarters until use. The animals used in this study were between 9 and 17 weeks old at the time of sacrifice. All procedures involving animals were approved by the Duke University Institutional Animal Care and Use Committee and were in accord with guidelines established by the NIH.

2.1. Lectin histochemistry

Animals were given an overdose of pentobarbital (Nembutal; >70 mg/kg). When withdrawal reflexes were absent and breathing ceased, the chest was quickly opened and the animal was perfused through the heart with a brief rinse of phosphate buffer (0.1 M, pH 7.6) followed by approximately 200 ml of buffered 4% paraformaldehyde. The brain was removed and frozen sections 40 μm thick were cut in one of the three standard planes. The sections were collected in serial order in phosphate buffer. Before they were processed for lectin binding, they were placed in a solution of 0.6% hydrogen peroxide for 30 min. They were then rinsed thoroughly in buffer and incubated in the following solution for 15–20 h at 4 °C: 0.5–1.0 μg/ml biotinylated *Wisteria floribunda* agglutinin (WFA, Sigma #L-1766 or Vector Laboratories #B-1355), 2% bovine serum albumin, phosphate buffered normal saline (PBS). At the end of the incubation period, the sections were rinsed in PBS, and placed in buffered Vector Elite ABC solution (Vector Laboratories; 1:100) for 1 h at room temperature. After rinses in PBS, the lectin was visualized by placing the sections in 0.05% diaminobenzidine in phosphate buffer with heavy metal intensification (Adams, 1981). The sections were mounted on glass slides, allowed to dry, dehydrated in a series of alcohols and sealed under coverslips with Permount.

2.2. Retrograde tracing study

The spatial distribution of lectin binding was compared to the distribution of cells that project from the ventral cochlear nucleus to the inferior colliculus, as determined in an earlier study (Cant and Benson, 2006). Details of the experiments are provided in the description of that study. Briefly, biotinylated dextran amine (BDA) was injected iontophoretically into the central nucleus of the inferior colliculus. After a survival period of 5–11 days, the animals were perfused as above and sections were processed for the presence of BDA. Maps were made of the positions of labeled cells on alternate horizontal sections through the contralateral cochlear nucleus of 25 animals. Each section was then matched to a section at the comparable dorsal-to-ventral level in one horizontal series chosen as a reference; all of the labeled cells from every case were re-plotted onto these reference sections. Because the BDA injections included the entire topographic (tonotopic) axis of the inferior colliculus, this procedure provided an estimate of the spatial distribution of all cells in the cochlear nucleus that project to it.

3. Results

In the gerbil, the lectin *Wisteria floribunda* agglutinin (WFA) binds selectively to the ECM around some but not all of the cell populations in the cochlear nucleus (Figs. 1–6). The dorsal cochlear nucleus (DCN) and the granule cell layer covering the surface of much of the ventral cochlear nucleus (VCN) are almost unstained after incubation with WFA, although occasional cell bodies in the DCN may sometimes be lightly outlined (e.g., Fig. 1F, arrowhead). In the VCN, octopus cells, some but not all multipolar cells, and cochlear root neurons are intensely stained compared to other cell groups.

The octopus cells in the PVCN are the most intensely stained cells in the cochlear nucleus. The lectin binds to the ECM around both the cell body and also the large primary and secondary dendrites (Fig. 1F and G, large black arrows; Fig. 2A–C, OCA; Fig. 3A and B, small black arrow). Staining around the cell body has the characteristic reticular appearance often seen with lectin binding (Fig. 3A, inset). The differential staining in the caudal PVCN allows visualization of the "octopus cell area" (Osen, 1969), made up exclusively of this cell type. However, some of the labeled octopus cells on the boundaries of this region appear to intermingle with unstained cells (Figs. 1F and 2C, lower boxed-in area; Fig. 3B). The octopus cells in the gerbil extend dorsally and medially along the path of the intermediate acoustic stria (Fig. 1G, arrow; Fig. 2, panel A, OCA).

Most of the other neurons in the VCN labeled by the WFA are multipolar cells located in both the PVCN and AVCN (Figs. 1–6). These cells are smaller than the octopus cells, and the lectin staining is intense only around the cell bodies; dendrites are rarely outlined (Fig. 3B and C, black arrows; Figs. 5 and 6). The lectin-labeled multipolar cells are not distributed homogeneously throughout the VCN. A few are found scattered among large numbers of unlabeled spherical bushy cells in the rostral AVCN (Fig. 1A and B, small arrows; Fig. 5, SCA, arrows). Most of the labeled multipolar cells lie in the middle of the VCN, straddling the border between the AVCN and PVCN (Figs. 4 and 5). In the middle of the PVCN, between the labeled multipolar cells and the octopus cell area, lies a small area that is almost devoid of WFA-binding (Fig. 1F, white arrow; Fig. 2C, lower boxed-in area; Fig. 3B, white arrows). Most of the unlabeled cells in this area are probably multipolar cells, as the globular bushy cells located in the PVCN are situated more ventrally (unpublished observations).

We could not distinguish the globular bushy cells from multipolar cells based on lectin-staining patterns. In the