Retrograde Transport of the Lectin *Phaseolus vulgaris* Leucoagglutinin in Frog Central Nervous System

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The transport properties of the lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) were tested in the frog central nervous system. After delivery of the lectin to the lower brainstem by iontophoresis, stained axons and axon terminals, as well as neurons with richly arborizing dendrites, were observed in different regions of the brain and spinal cord even far away from the site of application. The large number and the Golgi-like appearance of labeled neurons situated retro- and caudal to the site of PHA-L deposit indicate that PHA-L is transported equally in both the anterograde and the retrograde direction in the central nervous system of the frog. This is in contrast with the mammalian nervous system, in which PHA-L is transported predominantly in the anterograde direction and the retrograde transport is poor. (J Histochem Cytochem 38:1913-1917, 1990)

**Key Words:** PHA-L; Neuronal tracing; Immunostaining; Frog

**Introduction**

The kidney bean lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) introduced by Gerfen and Sawchenko (4) is used as a highly sensitive anterograde tracing substance in the mammalian central nervous system. PHA-L is transported equally in both the anterograde and the retrograde direction (3,19), but fibers of passage are also reported to take up predominant transport by axons originating in the site of injection (3,19), but fibers of passage are also reported to take up and transport the lectin anterogradely (2). However, Kita and Kitai (9) observed a faint granular labeling in neurons of the globus pallidus after injection of PHA-L into the subthalamic nucleus of the rat. After delivery of the lectin into skeletal muscles of the rat, a similar faint granular labeling has been detected in spinal motoneurons (10). Although the efficiency of retrograde labeling has almost always been found to be poor, these findings suggest that, in contrast to the general assumption, PHA-L may not be exclusively an anterograde neural tracer. To investigate this possibility, we tested the transport properties of PHA-L in the central nervous system of the frog. After deposit of the tracer in the lower brainstem, the extensive afferent and efferent fiber connections of this region provide an excellent opportunity for the study of anterograde and retrograde transport in the same specimen.

**Materials and Methods**

The experiments were carried out on frogs, *Rana esculenta*. The animals were deeply anesthetized with tricaine methanesulfonate (MS 222; Sigma, St Louis, MO) and the caudal portion of the occipital bone was excised with a malleoscope. Glass micropipettes with a tip diameter of 10–20 μm were filled with 2.5% solution of PHA-L (Vector Labs; Burlingame, CA) dissolved in 0.05 M Tris-buffered saline (TBS; pH 7.4). The tracer was delivered into the lower brainstem at the level of exit of either the eighth or the ninth cranial nerve by iontophoresis, using positive direct current of 5 mA with a pulse duration of 7 sec followed by 3-sec intervals for a period of 15–20 min. At each level, the tracer was injected into two sites: 200 μm and 600 μm from the midline and 210 μm deep from the dorsal surface. After a survival period of 7 days, the animals were given an overdose of anesthetic and perfused transcardially first with Tyrode’s solution (oxygenated with a mixture of 95% O2 and 5% CO2) for 1–2 min, followed by a fixative containing 2.5% glutaraldehyde, 0.5% paraformaldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB; pH 7.4). The brain was removed and fixed by immersion in the same fixative overnight. Diencephalon, brainstem, and spinal cord were dissected and washed in 10% solution of TS containing 1% normal goat serum and 0.5% Triton X-100. The immunoreaction was completed with a nickel-enhanced DAB chromogen react-

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BRIEF REPORT
Results

Injection Sites

Camera lucida drawings show typical sites of injection of PHA-L into the medulla (Figure 1). Although the lectin was delivered to two different locations in both cases, the injections were placed so close to each other that the infiltrated sites fused and covered relatively large areas with a diameter of 600–700 μm. At the level of the eighth cranial nerve, the medial, lateral, and descending vestibular nuclei, the cochlear and saccular auditory nuclei, the spinal nucleus of the trigeminus, the superior olive, and the middle reticular nucleus were located within the region of PHA-L application (Figure 1A). At the level of the ninth cranial nerve, the medial and lateral nuclei were involved within the region of PHA-L application (Figure 1A). Neurons with ventro-laterally oriented dendritic arbors were found in the ventral and dorsal horns contralateral to the site of injection (Figures 2E and 2F). Neurons with ventro-laterally oriented dendritic arbors were found in the ventral part of the diencephalon (Figure 3C), and small stellate-like neurons were stained in the mesencephalic tegmentum presented a large number of stained neurons in both sides. The shape and size of these neurons varied over a wide range (Figures 3K and 3L). In addition, Purkinje cells in the cerebellum (Figure 3J) and large ganglionic cells in the optic tectum (Figures 3F and 3G) were also encountered when the PHA-L was delivered at the level of the eighth cranial nerve. Large ganglionic cells in the tectum were found both ipsi- and contralateral to the injection site and were located exclusively in Layer 7 (Figure 3F) or in the superficial part of Layer 6 (Figure 3G).

Retrograde Labeling of Neurons

Stained perikarya with richly arborizing dendritic trees were revealed in all regions where labeled axons and axon terminals were observed. The morphology of the dendritic arbors, even far away from the application sites, resembled the best Golgi-impregnated neurons (Figures 2E, 2F, 3C, 3E–G, 3J–L). Fine details of dendrites, including small appendages (Figure 3F) or beads (Figure 3C), were also stained. In the spinal cord, labeled neurons were located both in the ventral and dorsal horns contralateral to the site of injection (Figures 2E and 2F). Neurons with ventro-laterally oriented dendritic arbors were found in the ventral part of the diencephalon (Figure 3C), and small stellate-like neurons were stained in the pretectal region (Figure 3E) ipsilateral to the application. The mesencephalic tegmentum presented a large number of stained neurons in both sides. The shape and size of these neurons varied over a wide range (Figures 3K and 3L). In addition, Purkinje cells in the cerebellum (Figure 3J) and large ganglionic cells in the optic tectum (Figures 3F and 3G) were also encountered when the PHA-L was delivered at the level of the eighth cranial nerve. Large ganglionic cells in the tectum were found both ipsi- and contralateral to the injection site and were located exclusively in Layer 7 (Figure 3F) or in the superficial part of Layer 6 (Figure 3G).

Discussion

Phaseolus vulgaris leucoagglutinin is regarded as a highly sensitive anterograde tracing substance in the mammalian central nervous system (3,4,19). Although it has been reported that neurons at the site of application, or in the close vicinity, are intensely labeled with PHA-L, long-distance retrograde transport has only occasionally been observed in the mammalian central nervous system. Moreover, the retrograde labeling has always been found to be conspicuously poor, resulting only in granular reaction product in the cytoplasm (9). In contrast to this, we describe here a very good degree of PHA-L transport in both the anterograde and the retrograde direction in the frog brain. The anterograde transport is as...
effective as in mammals, and Golgi-like labeling of neurons indicates extensive retrograde transport far away from the site of injection. The distribution and number of retrogradely labeled neurons are very similar to the results obtained from experiments in which HRP, the well-established retrograde tracer, was applied at a similar location in the frog brainstem (11). In addition, most of the PHA-L-stained neurons disclosed more extensive dendritic arbors than those labeled with HRP.

The difference in the mechanism of transport of PHA-L in mammals and frogs remains to be explored. The distribution of specific glycoconjugates that are responsible for uptake of the lectin into the cell (13,15) may be different in mammals and frogs. They may be equally present in dendrites, perikarya, and axon terminals in the frog, and may occur predominantly in dendrites and perikarya in the mammalian brain. Another possibility is that the difference between mammals and frogs may be in the intracellular mechanism involved in the transport of endocytosed lectin–glycoprotein complexes. In a recent study, Trudrung and Schumacher (16) found that the glycoprotein binding pattern of the lectin wheat germ agglutinin, which is equally transported in the anterograde and the retrograde direction, very much resembles the binding pattern of PHA-L in the rat brain. This suggests that the final sorting process resulting in different transport directions of lectins may be an intracellular phenomenon independent of transmembrane glycoproteins.

The dual transport directions of the PHA-L, the Golgi-like labeling of nerve cells, and the fact that PHA-L does not cause any significant degeneration in labeled structures (3,19) have a number of advantages in the study of neuronal connectivities in the frog's brain. (a) Dendritic morphology of efferent neurons can be described, and electron microscopic studies can then be made on the
Figure 3. Photomicrographs showing PHA-L labeling rostral to the sites of application. (A,B) Axons and axon terminals in the diencephalon. (C) Axon terminals and retrogradely labeled neurons in the ventral part of the diencephalon. Arrows point to a beaded dendrite. (D) Fine terminal arborization of stained axons in the pretectal region. (E) A stellate-like neuron in the pretectal region. (F,G) Large ganglionic neurons in Layer 7 (F) and in the superficial part of Layer 6 (G) of the optic tectum. Arrows in F point to dendritic appendages. (H) Mossy fibers in the cerebellum. (I) A climbing fiber in the molecular layer of the cerebellum. (J) Retrogradely labeled Purkinje cell in the cerebellum. (K,L) Retrogradely labeled neurons in ventral (K) and dorsal (L) part of the mesencephalic tegmentum. Bars: A–H,K,L = 50 \mu m; I,J = 25 \mu m.
synaptology of identified neurons. (b) Feedback loops between brain regions can be identified by injecting PHA-L into a nucleus that receives afferents from another brain region and sends efferents to the same area. Synaptic contacts between labeled neurons and axon terminals can be mapped in a consecutive electron microscopic analysis.

(c) It has been demonstrated that PHA-L conjugated with small molecules, biotin or dinitrophenol, has transport properties similar to those of the unconjugated lectin (1). Unconjugated and conjugated PHA-L can be simultaneously visualized in contrasting colors by double immunohistochemical procedures, permitting the detection of structures of different origin labeled in the same target area (1). The dual-color labeling and the anterograde and retrograde transport of PHA-L render the investigation of afferent input to efferent neurons possible. Neurons in a certain nucleus can be retrogradely labeled from their target area, and their contacts with presynaptic terminals labeled anterogradely from a third brain region can be investigated.

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Literature Cited


5. Grove EA, Domesick VB, Nauta WJH: Light microscopic evidence of striatal input to intrapallidal neurons of cholinergic cell group Ch4 in the rat: a study employing the anterograde tracer Phaseolus vulgaris leucagglutinin (PHA-L). Brain Res 367:379, 1986


17. Wouterlood FG: Anterograde neuroanatomical tracing with Phaseolus vulgaris leucagglutinin combined with immunocytochemistry of gamma-aminobutyric acid, choline acetyltransferase or serotonin. Histochemistry 89:421, 1989
