# Suicide Transport: Destruction of Neurons by Retrograde Transport of Ricin, Abrin, and Modeccin

Abstract. *Certain toxic lectins, including ricin, are retrogradely transported along neuronal processes to the cell body where they inactivate ribosomes, resulting in neuronal death. This process of "suicide transport" suggests a powerful new experimental strategy for solving neurobiological problems.*

Many problems in neurobiology resist solution because of the limitations inherent in conventional lesioning techniques. The approach we describe was suggested by the observation that among the substances taken up and transported within axons by neurons are lectins (*1*), proteins which bind to surface glycoproteins of many cells. Some lectins are among the most potent cytotoxins known (*2, 3*). We examined the effects of ricin, and the closely related toxic lectins abrin and modeccin, on vagal and hypoglossal perikarya. We injected the lectins into the distal vagus or hypoglossal nerves to determine whether they could be used as "suicide transport" agents by examining whether they are retrogradely transported, whether they kill nerve cells, and whether their cytotoxicity is restricted to the neurons that transport the agent.

Ricin, a toxic lectin from castor beans *Ricinus communis*, abrin from *Abrus precatorius*, modeccin from *Adgia Adenia digitata*, and ricin conjugated to horseradish peroxidase (HRP) were dissolved in 0.9 percent NaCl at concentrations of 0.4 to 4.0 mg/ml (*4*). The vagus or hypoglossal nerve was exposed in the neck of 26 adult male Sprague-Dawley rats or four New Zealand White rabbits anesthetized with halothane. The lectins were applied (i) by dipping the central end of a transected nerve into lectin solution or (ii) by pressure microinjection (0.5 μl) with glass capillary micropipettes inserted into the intact nerve trunk. After surgery, the wounds were closed and anesthesia discontinued. After 1 to 52 days, the animals were killed. The nodose ganglia and medulla oblongata were processed routinely for histology. In experiments with ricin-HRP conjugate, Vibratome or cryostat sections were treated with tetramethylbenzidine and hydrogen peroxide and viewed with dark-field optics (*5*) to demonstrate the presence of transported ricin.

Ricin or ricin-HRP conjugate applied unilaterally to the cervical vagus nerve produced a characteristic sequence of morphological changes limited to neurons of the ipsilateral dorsal motor nucleus of the vagus and nodose ganglion. Virtually all neuronal Nissl substance had disappeared 24 to 48 hours after lectin application. The size, location, and appearance of nuclei and nucleoli were unchanged (Fig. 1, insets). After 4 to 7 days, the large motor neurons of the dorsal motor nucleus had disappeared and a glial reaction was evident (Fig. 1). At this time neurons of the nodose ganglion, while still present, appeared shrunken with irregular, pycnotic nuclei (Fig. 2). From 7 to 52 days the glial reaction in the dorsal motor nucleus partially subsided, and neurons of the nodose ganglion disappeared. Similar changes were observed in the ipsilateral hypoglossal nucleus after application of ricin to the distal hypoglossal nerve.

Abrin (0.25 μg) and modeccin (15-minute dip in 0.35 mg/ml), applied to the cervical vagus, produced similar changes that were also limited to the ipsilateral dorsal motor nucleus and nodose ganglion. In contrast, injection of large doses (15 to 20 μg) of wheat germ agglutinin–HRP conjugate into the nodose ganglion produced intense labeling of neurons in the dorsal motor nucleus of the vagus, but there was no evidence of cytotoxicity after 3 days, indicating that neuronal toxicity such as that observed with ricin, abrin, and modeccin is not seen with all lectins.

Because application of the toxic lectins to a nerve trunk produced local necrosis, it was possible that the cellular reaction observed represented the retrograde reaction, a nonspecific response to axonal damage. Therefore, as a control the hypoglossal and vagus nerves were surgically interrupted unilaterally by removal of a 1-cm segment proximal to the usual site of toxin application in 14 rats. This produced the classical morphological signs of the retrograde (axon) reaction in neurons of the ipsilateral dorsal motor nucleus, nodose ganglion, and hy-

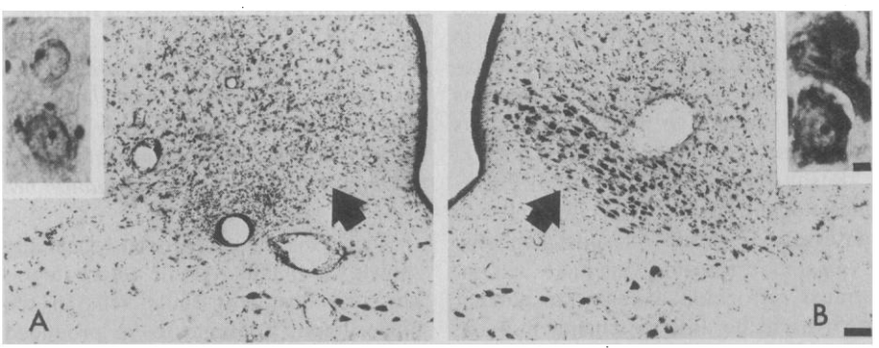


Fig. 1. Destruction of neurons in left dorsal motor nucleus of rabbit vagus 7 days after application of ricin to vagus nerve: (A) ipsilateral and (B) contralateral medulla. Cresyl violet stain was used. Arrows indicate dorsal motor nucleus of the vagus. Scale bar, 100 μm. Inset in (A), ipsilateral dorsal motor nucleus of vagus of a rat that survived for 2 days. Inset in (B), contralateral dorsal motor nucleus from same rat. Scale bar, 5 μm.

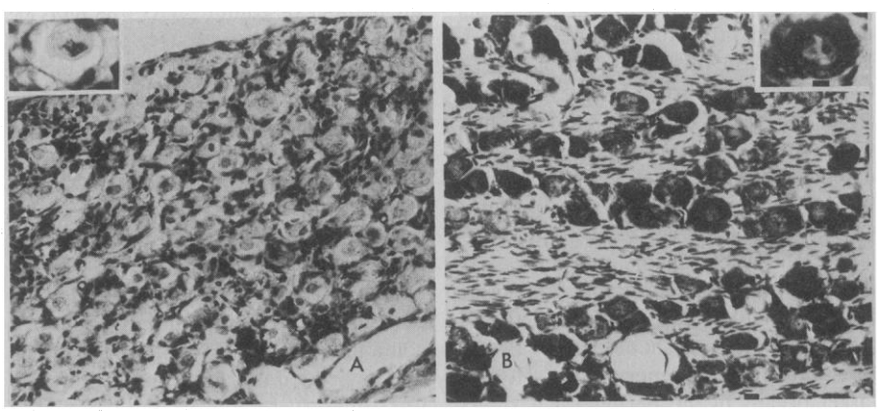


Fig. 2. Degenerating neurons in rat nodose ganglion ipsilateral to vagal ricin application after 6 days. (A) The ipsilateral nodose ganglion showing all neurons affected by ricin. (B) The contralateral normal nodose ganglion from the same animal. Scale bar, 20 μm. Insets are higher magnifications of a neuron from the same section. Scale bar, 10 μm.


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poglossal nucleus (*6*). There was variable subtotal Nissl dispersion beginning approximately 4 days after toxin application, with variable but incomplete cell loss, similar to that reported by others (*7*), beginning after 7 days. These changes after nerve transection were strikingly different in quality and timing from the changes observed after application of the toxic lectins.

To confirm that ricin was retrogradely transported, 0.5 μl of ricin-HRP conjugate (0.25 μg) was injected into the cervical vagus of three rats. After 24 to 48 hours, neuronal perikarya and processes were intensely labeled in the ipsilateral dorsal motor nucleus and nodose ganglion. Anterograde labeling of vagal sensory terminals in the nucleus of the tractus solitarius was not observed, suggesting that ricin killed nodose ganglion cells before the conjugate could be transported centrally. In five rats ricin-HRP conjugate (0.2 μg) was injected into the nucleus of the tractus solitarius. After 48 hours many cells were labeled in the nodose ganglia, but labeled neurons were rarely observed within the central nervous system. Although extensive necrosis occurred at the injection site and typical cytotoxic changes were seen in the nodose ganglia, no evidence of cytotoxicity was observed in central neurons known to project to the nucleus tractus solitarius.

Results obtained with ricin-HRP conjugate applied to the vagus are similar to observations made in postganglionic sympathetic neurons that have demonstrated retrograde axonal transport of ricin from the anterior chamber of the eye or submandibular salivary gland to the superior cervical ganglion (*1*, *8*). However, no permanent neuronal loss was reported (*1*, *8*).

Our study shows that ricin is retrogradely transported after direct application to a peripheral nerve. Neuronal death occurs after ricin reaches the perikaryon, and the resulting lesion is limited to neurons projecting to the injection site. The rapid (24 to 48 hours) and complete loss of Nissl substance after application of the toxic lectins is probably due to their action on polyribosomes (*2*, *9*).

The ability of sensory and motor axons to take up and retrogradely transport amounts of toxic lectin sufficient to kill the parent neuron should prove useful for permanently denervating selected target organs in the peripheral nervous system and for destroying the sensory and motor neurons whose axons project through a particular nerve. This process of suicide transport may also be adaptable to neurons with axons confined to the central nervous system. Local injection of a toxin into an appropriate terminal area of one set of collaterals may result in death of the parent cell body and of other collaterals. Neuronal death by suicide transport could be used to study adjustments made by axonal processes synapsing on the dying neurons. Moreover, by coupling the toxic moiety of ricin to a second molecule such as nerve growth factor, to another lectin, or to an antibody that is taken up only by certain neurons, it may be possible to selectively destroy specific classes of neurons.

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**References and Notes**

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# Neurons in the Rat Dentate Gyrus Granular Layer Substantially Increase During Juvenile and Adult Life

Abstract. *Volumetric estimates of the total number of granule cells in rats 30, 120, 200, and 365 days old increase linearly by approximately 35 to 43 percent between 1 month and 1 year. Total volume of the granular layer also grows linearly during that time. These results demonstrate a numerical increase in a neuronal population during adulthood in the mammalian brain.*

Since 1963 (*1*) it has been repeatedly shown with [$^3$H]thymidine autoradiography that dentate granule cells in the hippocampus continue to be produced during the adult period in rats (*2*, *3*). The question remained whether these neurons were adding to the population or were replacing those that may die during adult life. By 1975, Bayer and Altman (*4*) obtained circumstantial evidence that the number of granule cells was increasing until 120 days of age; the present study was designed to determine numerical age changes in this group of neurons.

We estimated the number of granule cells in the hippocampus of 17 male Purdue-Wistar rats, four animals each at 30, 120, and 200 days, and five at 365 days. After a transcardial perfusion with 10 percent neutral Formalin and Bouin's fixative, the brains were stored in 10 percent neutral Formalin until the block containing the entire right hippocampus was embedded in methacrylate. Serial 3-μm slices were cut in the horizontal plane with a JB-4 microtome (Sorvall) and stained with cresyl violet. A running count was made of all slices containing the granular layer; care was taken to assure that slices were of uniform thickness.

Two previous volumetric estimates of granule cell numbers in rats used modified Abercrombie correction factors to counts of neuronal nucleoli (*5*, *6*). This method was unworkable in our preparations since the Purdue-Wistar strain consistently shows double nucleolus-like bodies in many granule cell nuclei (arrows in Fig. 1). Consequently, we chose to quantify the nucleus. Our method was based on the equation:

$$N = V_t/V_a$$

where $N$ is the total number of cells, $V_t$ the total volume of all cell nuclei, and $V_a$ the average nuclear volume (*7*).

We experimentally determined $V_t$ from low-magnification photomicrographs of dorsal and ventral (*6*) granular layer slices at regularly spaced intervals. The granular layer was divided into 25-μm wide strips running perpendicular to the length; each strip was a possible sample. We randomly chose 200 strips from the total sample pool in all selected


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