Experimental sensory ganglionectomy by way of suicide axoplasmic transport

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In attempts to destroy selectively the sensory ganglion cells via retrograde axoplasmic transport, either one or the other of the Ricinus communis agglutinins (RCA 60 and RCA 120), highly toxic lectins from castor beans, was topically applied to the proximal stump of the rat trigeminal branches (the mental and supraorbital nerves) or to the sciatic nerve. Within several days, the sensory ganglion cells associated with the nerve to which RCA was applied developed diffuse chromatolysis and subsequent dissolution of neuronal cell bodies. The resultant Wallerian degeneration of their primary afferent fibers could be traced within the brain stem and, in cases with RCA application to the sciatic nerve, within the spinal cord. This observation implies that the central counterpart of the peripheral nerve may be effectively destroyed by way of retrograde axoplasmic transport without direct attack on the target structure, and thus this method may be utilized in the future as a means for controlling various pain problems.

KEY WORDS • axoplasmic transport • dorsal rhizotomy • lectin • pain • retrogasserian rhizotomy • sensory ganglionectomy • trigeminal nerve

CONTROL of intractable pain is one of the major tasks imposed on neurosurgeons. Surgical destruction of pain-related structures, in particular peripheral neurectomy and dorsal rhizotomy, has been widely employed in the management of intractable pain problems such as neuralgias and cancer pain. There are, however, several drawbacks inherent in these surgical procedures, including difficulty in selectively destroying the pain-related nerve structures, recurrence of pain, anesthesia dolorosa, and the inevitable risks of surgery.

Recently, Wiley, et al., reported that the injection of several toxic lectins (plant proteins with hemagglutinating activity) into the rat vagal nerve trunk had resulted in degeneration and ultimate loss of neurons in the nodose ganglion by way of "suicide transport" of the lectins.

The present investigation was undertaken to elucidate the effects of these toxic lectins on the neurons involved in pain conduction, namely, those in the trigeminal and dorsal root ganglia, and their primary afferent fibers. We demonstrate that intraneural injection of Ricinus communis agglutinins (RCA's) in rats causes severe and selective degeneration of sensory ganglion cells associated with the injected peripheral nerves, and consequent Wallerian degeneration of the primary afferents in the brain stem or in the spinal cord. The results warrant further investigations of this noninvasive technique of ganglionectomy as a unique and novel adjunct to pain-controlling procedures.

Materials and Methods

Approximately 50 Wistar rats of either sex, each weighing 150 to 200 gm, were used for this study. All surgical procedures were carried out under intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight).

One of two small branches of the trigeminal nerve, either the supraorbital or the mental nerve, was exposed under an operating microscope. The supraorbital nerve was identified at the superior orbital ridge and a subcutaneous portion of the nerve, 5 to 7 mm in length, was freed from adjacent tissue. The distal end of the exposed nerve was sharply transected and the proximal stump was dipped for 30 to 60 minutes in a small rubber bag containing a solution of 0.1% RCA (RCA 60 or RCA 120, of 60,000 or 120,000 molecular weight, respectively)* in 0.01 M phosphate buffer. The mental branch was exposed distal to the mental foramen, 0.1 to 1.0 μl of 0.1% RCA was intraneurally injected with a microsyringe, and then the injection site was crushed with a forceps.

* Ricinus communis agglutinins (RCA) and anti-RCA rabbit IgG obtained from E. Y. Laboratories, San Mateo, California.
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In another series of experiments, the sciatic nerve was exposed posterior to the hip joint. Intraneural injection of 0.3 to 2.0 μl of 0.1% RCA was followed by crushing the injection site with a forceps. As a control, the contralateral homologous nerve was also transected in several animals of this group in order to compare the effect of simple transection of the nerve with that of RCA treatment. The wound was irrigated with normal saline and the skin was approximated with silk.

Two to 14 days after the surgery, 10% neutral buffered formalin was perfused into the heart with the rats under deep chloroform anesthesia. Three animals with a RCA-treated sciatic nerve were perfused with a 4% paraformaldehyde and 0.5% glutaraldehyde mixture in Millonig’s phosphate buffer for electron microscopic studies of the dorsal root ganglia and their primary afferents. The tissues were postfixed with 1% OsO₄ in the same buffer solution, dehydrated in graded ethanol, and embedded in epoxy resin.

The trigeminal and dorsal root ganglia, with their peripheral nerves and dorsal roots attached, were embedded in paraffin and sections were stained with hematoxylin and eosin, Luxol fast blue-cresyl violet, and by the Bodian method. The brain stem and spinal cord were cut into sections 35 μm thick on a cryomicrotome. These sections were then impregnated according to the Fink-Heimer method for degenerating axons and terminals.

In order to confirm the retrograde axoplasmic transport of RCA into the ganglion cell bodies, RCA 120-horseradish peroxidase (HRP) conjugate (in a 1% concentration) or a larger dose of RCA 120 (0.5%, 3 to 5 μl) was injected similarly into the sciatic nerve. In this series of experiments, the rats were perfused with 4% paraformaldehyde in phosphate buffer 19 to 36 hours later. After fixation, bilateral dorsal root ganglia were removed and stored overnight in Millonig’s wash buffer. Frozen sections, 30 to 60 μm thick, were cut and mounted on gelatinized slides. Slides made from animals injected with RCA-HRP were treated with the tetramethylbenzidine method of de Olmos, et al., for the detection of peroxidase activity. Slides from RCA 120-injected animals were used for the unlabeled antibody method of Sternberger, et al., (peroxidase-antiperoxidase method; PAP). The slides were covered with anti-RCA rabbit immunoglobulin G (IgG) solution (diluted 400 to 800 times with 0.01 M phosphate-buffered saline with 0.2% Triton X-100; PBS-TX) and incubated for 48 hours at 4°C in a moist chamber. Thereafter, they were thoroughly rinsed with PBS, and a second antibody, an anti-rabbit IgG goat IgG fraction (diluted 1:100 with PBS-TX), was applied for 1 hour, after which a rabbit peroxidase-antiperoxidase complex (diluted 1:50 with PBS-TX) was applied for 40 minutes. After washing with several changes of PBS, the slides were incubated with diaminobenzidine solution (100 mg/200 ml of Millonig’s phosphate buffer solution at pH 7.3, supplemented with 5 ml of 1% CoC₁₂ and 4 ml of 1% nickel ammonium sulfate for 15 minutes, after the further addition of 0.6 ml of 3% H₂O₂ for 7 to 15 minutes). The slides were then rinsed with PBS, dehydrated with a graded series of ethanol, and cleared in xylene.

Results

Histological Findings

A diffuse chromatolytic change in sensory ganglion cells in the trigeminal and spinal sensory ganglia was first detected as early as 24 hours after the application of RCA to the corresponding peripheral nerves. Dissolution of perikarya and nuclei developed within 2 days (Fig. 1A). Empty baskets and residual nodules of Nageotte due to loss of ganglion cells and proliferation of capsule cells were often seen in animals sacrificed 1 week after RCA injection. In animals injected with RCA into the mental nerve, the empty baskets were confined to the posterolateral portion of the trigeminal ganglion (posterolateral protuberance at which point the mandibular nerve fused to the maxillo-ophthalmic division) (Fig. 1B). On the other hand, in animals with RCA application to the supraorbital nerve, the empty baskets were scattered less conspicuously within the medial aspects of the maxillo-ophthalmic division (Fig. 1C). A majority of neurons adjacent to the empty baskets appeared to be normal, indicating that the RCA was confined to the injected nerves and their parent ganglion cells, and that it had not been diffused out within a ganglion. The mental nerve proximal to the injection site also showed disintegration of the myelin sheath and a marked increase in the number of Schwann cells when examined 5 days after the RCA injection. Intraneural injections into the sciatic nerve led to a similar neuronal loss in the ipsilateral dorsal root ganglia, most severely at the L4-5 vertebral levels and less conspicuously at L-6. The motoneurons located in the ventrolateral quadrant of the ipsilateral ventral horn also showed diffuse chromatolysis between the levels of L-4 and L-6 in animals surviving for more than 3 days, indicating that the RCA exerted a toxic effect even on somatic motoneurons.

After simple transection of the sciatic nerve, it took more than 1 week for a mild central chromatolysis to develop in the corresponding ganglia, in contrast to a 48-hour interval between the development of severe neuronal degeneration and the peripheral nerve application of RCA’s.

Electron Microscopic Findings

The earliest changes in dorsal root ganglion cells, manifested within 24 hours, were disintegration of the endoplasmic reticulum and dispersal of free ribosomes. The endoplasmic reticula lost the polyribosomes at-

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† Anti-rabbit IgG goat IgG fraction obtained from Cappel Laboratories, West Chester, Pennsylvania. Peroxidase-antiperoxidase obtained from Dakopatts, Denmark.
FIG. 1. Photomicrographs of sensory ganglia. A: Section of an L-5 dorsal root ganglion of an animal surviving for 2 days after intraneural injection of RCA into the sciatic nerve. Many ganglion cells show dissolution of perikarya and nuclei with an increase in capsule cells. Only a few neurons appear preserved. H & E, × 240. B: Section from a rat with 7 days survival after RCA injection into the mental nerve. The mandibular division of the trigeminal ganglion shows severe loss of ganglion cells in its medial portion with capsular cell proliferation. Small ganglion cells along the medial border (upper left) have survived. H & E, × 75. C: The maxillo-ophthalmic division of the trigeminal ganglion, following dipping of the supraorbital nerve into RCA and 5 days survival. Notice several empty baskets intermixed with normal-looking ganglion cells. The arrow indicates dissolution of a ganglion cell while its nucleus remains identifiable. Luxol fast blue-cresyl violet, × 150.

attached to them, and their usual laminar pattern of tubular arrangements was markedly disturbed. The majority of free ribosomes were no longer in clusters or in rosettes but were randomly dispersed as grains in the cytoplasm. Mitochondria increased in number and some of them were quite elongated (Fig. 2A). A rat sacrificed 3 days after RCA injection showed ballooning of mitochondria with loss of their cristae, a decrease in the number of ribosomes, and disruption of the nuclear membrane, as well as further disintegration of the endoplasmic reticulum. At this stage, these degenerating neurons were often encircled by a number of capsule cells, possibly engaged in phagocytotic activity (Fig. 2B and C).

The Fink-Heimer Study

In rats with trigeminal nerve RCA injection, degenerating axons in passage were clearly demonstrated within the brain stem of those sacrificed between Days 5 and 12. When the RCA was applied to the mental nerve, the degenerating axons were found in the dorsal aspect of the spinal tract of the fifth cranial nerve in a section through the root entry of the nerve to the pons. Many degenerating axons penetrated the tract into the dorsal aspect of the principal nucleus, where dense plexus of terminal degeneration was formed. Caudally, the degenerating fibers occupied the dorsal aspect of the spinal tract throughout its course and formed terminal degeneration fields in the oral, interpolar, and caudal portions of the spinal tract nucleus of the fifth cranial nerve (Fig. 3A and B). Particularly in the pars caudalis, terminal degeneration was observed as a dense belt in the substantia gelatinosa, the external shell, and as diffuse plexus in the magnocellular internal core (Fig. 3C).15

In cases with RCA application to the supraorbital nerve, degenerating axons in passage were seen within the most ventral part of the spinal tract of the fifth nerve throughout its rostrocaudal extent. There was a small terminal field in the ventral aspect of the principal nucleus and a compact area of terminal degeneration, which could be traced caudally as far as the C-2 vertebral level, was found in the spinal tract nucleus of the fifth nerve (Fig. 3D).
In rats with RCA injected into the sciatic nerve, degenerating fibers were identified as dense bundles in the tract of Lissauer, between L-4 and L-6, and in the ipsilateral Goll’s tract. Terminal degeneration was also seen within the substantia gelatinosa and the nucleus proprius of the dorsal horn as a heavy accumulation of fine silver grains. Some degenerating axons passed through the dorsal horn to form terminal plexuses in the intermediate gray matter and around the motoneuron columns. The structures distal to the root entry zone of L4–6, ipsilateral Clarke’s column, and the nucleus gracilis (also known to be targets of primary afferents) contained plexuses of heavy terminal degeneration.

**Histochemical Demonstration of RCA Transport**

As early as 19 hours after the injection of RCA-HRP conjugate into the sciatic nerve, numerous HRP-react...
The toxic effects on neurons of several lectins transferred by way of axoplasmic flow was first demonstrated by Harper, et al. They observed chromatolytic changes in neurons in the superior cervical ganglion of the rat after injections of toxic lectins into the submandibular gland and the anterior chamber of the eye. They thought, however, that the toxic effects were transient and that the majority of the neurons might survive. Wiley, et al., reported more recently that intraneural injection of toxic lectins into the vagal nerve trunk gave rise to degeneration and ultimate death of neurons in the nodose ganglion and the dorsal motor nucleus of
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Fig. 4. Immunohistochemical demonstration of retrogradely transported RCA within the dorsal root ganglion cells, 20 hours following RCA injection into the sciatic nerve. Peroxidase-antiperoxidase method of Sternberger, et al.12 A: Many immunoreactive darkly stained cells are scattered in the ganglion of L-5. × 80. B: A larger magnification of the enclosed area in A. The immunoreactivity is found within the perikarya of ganglion cells, where reaction product is not diffuse but granular in appearance. This may imply that RCA is attached to subcellular organelles and is not present as a diffusible form in the cellular matrix. × 300.

the vagus by way of "suicide transport" of the lectin. The present studies clearly demonstrated the selective death of sensory ganglion cells and their primary afferents after the application of a minute amount of toxic lectins to their counterparts in the sensory and mixed peripheral nerve.

Ricinus communis agglutinins are plant lectins extracted and purified from castor beans: RCA 60 has a molecular weight of 60,000 and is known to be extremely toxic to animal cells by interfering with their ribosomal functions9,10 whereas RCA 120 is less toxic and stronger in hemagglutinating activity in vitro.9 Our preliminary study on rabbit anti-RCA 60 antibodies by a double immunodiffusion method showed strong cross-reactivity between the two lectins. Concerning toxic effects of these two lectins on sensory neurons, RCA 120 and RCA 60 were equally potent, but the former exhibited less systemic toxicity. Thus, RCA 120 seems to be the drug of choice for this purpose. The toxicity of RCA's on sensory ganglion cells was extraordinary. Only a minute amount of RCA's transported by means of a retrograde axoplasmic flow caused severe degeneration of the parent neurons within a matter of a few days, as revealed by routine histological and ultrastructural examinations. Terminal degeneration of their primary afferents was evident within 3 days on ultrastructural observation and by 4 to 5 days with the Fink-Heimer impregnation method5 for degenerating axons and their terminals.

Studies with RCA-HRP conjugate and RCA-PAP methods confirmed that RCA's were incorporated into the perikarya of the target cells by means of a retrograde axoplasmic transport. Many substances are known to be taken up by the nerve terminals or through the axonal stump and to be sent back to the perikarya of their parent neurons.3,4 Nerve growth factor, which is vital for the development of sympathetic and sensory ganglion cells and essential for the maintenance of neuronal functions in mature ganglion cells,7,16 is one of those substances. The present study provided additional evidence that retrograde axoplasmic flow not only conveys substances beneficial to parent cells, but also those noxious to them.

It is of interest that sensory ganglion cells were more vulnerable to RCA's than supporting cells such as Schwann cells. This might be due to the higher content of ribosomes in neurons, reflecting a higher rate of protein synthesis when compared with other cell types. In any case, sensory ganglion cells were the first to die, and the function of axoplasmic flow was apparently well sustained until the neural machinery in the parent cells ceased functioning. Successful transport of toxic lectins and selective destruction of parent cells have an important bearing on clinical neuroscience for a number of reasons, one of them being pain control.

As a means of pain control, peripheral neurectomy and dorsal rhizotomy have met with a varying degree of success.17 Peripheral neurectomy or neurotomy (the simplest procedure) may provide temporary relief from pain. Frequent recurrence, painful amputation neuroma formation, and disagreeable anesthesia dolorosa, however, have rendered this method less popular. Retrogasserian rhizotomy and dorsal rhizotomy are associated with a fair degree of good results. In particular, differential radiofrequency coagulation of the root via percutaneous radiologically controlled settings is now a common procedure. Tew and Mayfield14 used this approach in a large number of cases, and reported a recurrence rate of 22%, while Sweet13 had a 28% recurrence rate. The reasons for recurrence with this procedure could be diverse, including regeneration of primary afferent fibers and/or sprouting of surviving axons within the spinal tract of the fifth nerve.

Dorsal rhizotomy has often failed to relieve pain in
patients suffering from a variety of disorders. One explanation for this failure may be the presence of intradural anastomoses between dorsal roots. Another possibility stems from the fact that sensation also reaches the spinal cord through the ventral root. Coggeshall, et al., demonstrated that human as well as mammalian ventral roots contain a large number of unmyelinated afferent fibers; they advocated that dorsal root ganglionectomy might be the procedure of choice for patients for whom rhizotomy was contemplated. Aberrant ganglion cells scattered in the ventral roots may also contribute to ventral root afferents, as shown by a retrograde HRP method.

The method of sensory ganglionectomy presented in this communication may alleviate the problems of ventral root afferent fibers and intradural anastomotic channels as well, since at least theoretically the loss of ganglion cells after RCA application also implies the abolition of processes extending from those cells both peripherally and centrally. Regeneration and sprouting cannot take place in the absence of ganglion cells, which are well preserved in neurectomy and dorsal rhizotomy procedures but are sacrificed in RCA ganglionectomy.

Another important aspect of this method is that it can be a powerful tool to establish one-to-one correlation between peripheral-central axons. If the correct peripheral nerve or branch is chosen, it should be possible to destroy its central counterpart selectively without damaging adjacent or unrelated nerve components.

Several problems need to be resolved before further application of RCA ganglionectomy. The nature and extent of the systemic toxicity of the drug should be carefully determined. Our preliminary study has shown that massive hepatoceular necrosis may occur with a lethal dose of RCA’s, in the range of 5 to 10 µg of RCA 60 and around 20 µg of RCA 120 for rats weighing approximately 200 gm. A study is under way to counteract the systemic toxicity of RCA’s by active or passive preimmunization with RCA’s and anti-RCAs antibodies in hope of building up a tolerance to a higher dose of RCA’s without interfering with “suicide transport” within the peripheral nerve. Another problem is the degeneration of motoneurons when RCA’s are applied within the peripheral nerve. Another problem is the nature and extent of the systemic toxicity of the drug should be carefully determined.

References

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