Distribution of substance P in the spinal cord of patients with syringomyelia

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CENTRAL pain characterized by burning dysesthesia and hyperpathia is a common and sometimes disabling symptom in patients with syringomyelia. A similar syndrome is reported by patients with traumatic paraplegia and other intrinsic spinal cord lesions such as intramedullary tumors and multiple sclerosis. Although the phenomenon of central pain is usually attributed to deafferentation hyperactivity, there is limited information concerning the role played by pain-modulating neuropeptides.

Previous studies in animals and humans have established that substance P, a putative pain-related peptide, is found in high concentration in the dorsal horn and, to a lesser extent, in the intermediate gray and ventral gray matter. There is convincing evidence that this peptide is localized in axons and terminal-like processes of primary afferents and is concentrated preferentially in nociceptive afferents. Among the physiological effects of substance P is its ability to potentiate the excitation of nociceptive neurons after cutaneous afferent stimulation. The role of substance P in the genesis and maintenance of central pain remains speculative.

In this study, we examined the immunohistochemical localization of substance P in the spinal cords obtained from autopsy of 10 patients with syringomyelia and 10 neurologically normal individuals. As part of a larger study on the pathology of syringomyelia, the sections used for immunohistochemical staining were obtained from the same paraffin blocks used for histopathology in the original study. A particular advantage of substance P is that it is an extremely stable peptide and can be identified in paraffin-embedded material after many years.

Materials and Methods

Study Protocol

Protocols for the use of human autopsy material were approved separately by the institutional review boards of the University Hospital of Brooklyn, Kings County Hospital Center, and the Office of the Medical Examiner, Brooklyn, New York.

Autopsy Material

Paraffin blocks of the spinal cord obtained from 10 patients with syringomyelia and 10 individuals with no known neurological disease were selected from a permanent autopsy collection of 105 and 232 cases, respectively. The details of tissue collection and preparation were reported previously. Clinical data concerning patients with syringomyelia are provided in Table 1. Histological findings in these cases have been recently reported as part of a larger study of the pathology of syringomyelia.
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Staining Methods of Immunohistological Examination

Paraffin blocks obtained from seven sites (the medulla oblongata, C-2, C-5, T-2, T-5, T-8, and the conus medullaris, defined here as the lumbosacral segment receiving dorsal roots L2–S3) were cut serially with a microtome to a thickness of 10 μm. The sections were placed on slides coated with 1% poly-L-lysine and dried in an oven at 55°C for 48 hours. Thereafter, the sections were treated with xylene to remove the paraffin, then rehydrated and incubated in the following solutions: 0.25% Triton X-100 for 30 minutes at room temperature; Tris-buffered saline (TBS), 0.5 M, (pH 7.4), for 30 minutes at room temperature; 3% hydrogen peroxide (to block endogenous peroxidase) for 5 minutes at room temperature; 3% normal goat serum in TBS for 30 minutes at room temperature; rabbit anti–substance P, 1:500 in TBS containing 1% normal goat serum, for 24 hours at 4°C; goat anti–rabbit serum, 1:20 in TBS containing 1% normal goat serum, for 30 minutes at room temperature; rabbit peroxidase–antiperoxidase complex, 1:200 in TBS containing 1% normal goat serum, for 30 minutes at room temperature; 0.05% diaminobenzadine hydrochloride in TBS (pH 7.4), and 0.01% hydrogen peroxide for 10 minutes at room temperature; and distilled water for 10 minutes at room temperature. Between each incubation step, the sections were rinsed twice for 5 minutes in TBS, except after incubation in 3% normal goat serum, which was removed by tilting the slide. The sections were dehydrated in a graded ethanol and xylene series and secured under a coverslip with Permount.

For use as a control for immunohistochemical specificity, rabbit anti–substance P was replaced on adjacent sections by normal goat serum. No staining was seen in these negative controls. Cross-reactivity testing of substance P antisera by the supplier determined that there was no reaction with the following: neurokinin A, neurokinin B, eledosin, cholecystokinin-8, serotonin, somatostatin, leucine enkephalin, methionine enkephalin, neurotensin, and vasoactive intestinal peptide.

Preparations were viewed through a microscope and photographed at magnifications ranging from ×10 to ×1000. The gray matter laminae were identified according to the classifications of Rexed and Schoenen.

Sources of Supplies

Rabbit anti–substance P was obtained from Incstar, Stillwater, MN. Goat anti–rabbit serum, rabbit peroxidase–antiperoxidase complex, and diaminobenzadine hydrochloride were provided by Sigma Chemical Co., St. Louis, MO.

Results

Normal Spinal Cords

Serial sections through seven levels of 10 normal spinal cords revealed substance P–like immunoreactivity in long varicosities and punctate processes in close apposition to neurons in the dorsal horn (Fig. 1), the intermediate gray matter, and the ventral horn. At all levels of the spinal cord, the largest amount of peroxidase-positive staining was found in the first, second, and third laminae of the dorsal horn. Less intense immunoreactivity, reflecting a smaller number of stained profiles, was present in the fifth lamina of the dorsal horn, the intermediolateral nucleus, the intermediomedial nucleus, and the ventral horn.

Spinal Cords From Patients With Syringomyelia

Syrinx cavities were associated with consistent abnormalities in substance P staining. In nine of 10 spinal cords, there was a marked reduction or absence of substance P immunoreactivity in the dorsal horn, the intermediate gray matter, and the ventral horn involving segments of the spinal cord occupied by the syrinx (Fig. 2). Symmetrically enlarged central cavities were found to produce bilateral decreases in staining whereas eccentric or lateralized cavities produced an ipsilateral decrease, often with no reduction contralaterally (Fig. 3). An absence of substance P staining was observed with gross expansions (see Fig. 2 left) and with cavitations that extended into specific quad-

![Fig. 1. Photomicrographs of axial sections of the normal human spinal cord at T-8 demonstrating substance P–like immunoreactivity. Left and Center: Peroxidase–antiperoxidase reaction product (arrows) is present in the first, second, and third laminae of the dorsal horn and, to a lesser extent, in the fifth lamina. Original magnifications ×20 (left) and ×100 (center). Right: High-power magnification of the first through third laminae reveals substance P staining in long varicosities and terminal-like processes. Original magnification ×400. Roman numerals are used to designate the laminae.](image-url)
rants of the spinal cord (see Fig. 2 right). Rostral to syrinx cavities, the distribution of substance P–like immunoreactivity was essentially the same as that in normal sections at comparable levels.

Immediately caudal to syrinx cavities, there was a striking increase in substance P staining in the first, second, third, and fifth laminae of the dorsal horn (Fig. 4). The intensity of staining was attributable to a markedly increased number of long varicosities and terminal-like processes (Fig. 5), which were found on serial sections extending one to five levels below the lesion. Central cavities were associated with a bilateral increase in dorsal horn staining (see Fig. 4); lateralized cavities that were confined to one hemicord tended to produce an increase in dorsal horn staining on the ipsilateral side as compared to the contralateral side (Fig. 6). The intensity of the staining in the intermediate gray matter and the ventral horn of the spinal cord immediately caudal to the syrinx cavities was similar to that observed on control sections at comparable levels.

No abnormalities were observed in the distribution of substance P–like immunoreactivity in the spinal cord of one patient with a small central syrinx of unknown etiology (Case 10). The lesion was lined entirely by ependyma, occupied no more than 25% to 30% of the transverse diameter of the spinal cord, and was found incidentally at the time of autopsy. A previous report of this case (cited therein as Case 1) indicated that the lesion had been asymptomatic during life.

Discussion

Substance P is an oligopeptide that has attracted considerable attention because of its apparent role in the perception and modulation of pain. The peptide is distributed widely throughout the nervous system including the brain, brainstem, spinal cord, dorsal root ganglia, and autonomic ganglia. There is strong biochemical and physiological evidence that substance P is released from primary afferent terminals that synapse on dorsal horn neurons and produce excitatory postsynaptic potentials (EPSPs) and membrane depolarization. It is generally agreed that substance P is an excitatory or pain-upregulating transmitter of nociceptive information. Substance P may also be involved in antidromic vasodilatation and appears to play a role in synaptic transmission in autonomic ganglia. Experimental studies have suggested that substance P is released from axon collaterals of primary visceral afferent neurons in prevertebral ganglia where

![Fig. 2. Photomicrographs demonstrating a marked reduction or an absence of substance P staining at the level of spinal cord cavitation in syringomyelia. Peroxidase–antiperoxidase. Left: Axial section at C-5 through an extracanicular syrinx (Case 8). Original magnification × 20. Center and Right: Axial sections at T-5 and C-5 through a central syrinx with paracentral dissection (Case 3). Original magnification × 40. DH = dorsal horn region, which is outlined by arrows; S = syrinx.](image)

![Fig. 3. Case 3. Photomicrographs displaying axial sections through the upper cervical spinal cord at the level of a lateralized syrinx. Left: There is a marked reduction of substance P staining in the dorsal horn ipsilateral to the syrinx (S). Right: No reduction in substance P staining is evident in the contralateral dorsal horn at the same level. Peroxidase–antiperoxidase, original magnification × 100. DH = dorsal horn region, which is outlined by arrows.](image)
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it generates noncholinergic slow EPSPs in principal cells.  

The distribution of substance P in the human spinal cord has been described previously by LaMotte and de Lanerolle and by others. In normal individuals, the peptide is localized in the first, second, third, and fifth laminae of the dorsal horn, the intermediolateral nucleus, the intermediomedial nucleus, and the ventral horn. The concentration of the peptide, as reflected by the number of peroxidase-positive stained profiles, is significantly greater in the first, second, and third laminae than in other areas and this disparity is present at all levels of the spinal cord. The distribution of substance P in the spinal cords of the 10 normal subjects in the current study was consistent with these data. We observed that excellent results could be achieved using peroxidase–antiperoxidase immunohistochemical preparations of formalin-fixed, paraffin-embedded materials as old as 31 years (2–31 years; mean

FIG. 4. Photomicrographs demonstrating an increase in substance P–like immunoreactivity in the dorsal horn caudal to the level of spinal cord cavitation. Peroxidase–antiperoxidase. Upper Left and Right: Case 2. Axial sections of the lumbar spinal cord below a symmetrically enlarged central syrinx. Original magnifications × 20 (upper left) and × 100 (upper right). Lower Left and Right: Axial sections through the normal lumbar spinal cord. Original magnifications × 20 (lower left) and × 100 (lower right).

FIG. 5. Case 2. Photomicrographs displaying high-power views of substance P immunoreactivity in the dorsal horn caudal to spinal cord cavitation. The intensity of staining is apparently due to an increase in the number of terminal-like processes and long varicosities (arrow). Peroxidase–antiperoxidase, original magnification × 400.
loss of immunoreactivity (see Fig. 2), which is consistent with pathological evidence that such lesions produce widespread neuropathic pain and wallerian degeneration. Another explanation for the reduced concentration of substance P at the level of cavitation is that the lesion destroys an intrinsic source of substance P or interferes with axoplasmic flow of the peptide in the spinal cord.

The observation that substance P–like immunoreactivity was increased in the dorsal horns caudal to the level of cavitation is of particular interest. Naftchi, et al.,39 have reported that following transection of the spinal cord, substance P accumulates in the dorsal horns below the lesion and is depleted in the substantia gelatinosa above the lesion. These and other data have suggested that substance P moves in a rostral direction along an ascending pathway, in contrast to the downward movement of monoamine transmitters from the brainstem. The current study is the first to demonstrate an accumulation of substance P in the human spinal cord. The increase was observed below the level of cavitation and extended caudally for only one to five spinal segments. Assuming that substance P is concentrated predominantly in afferent terminals, this finding is consistent with the proposition that substance P moves in a rostral direction by axoplasmic flow and that this movement can be impaired by structural lesions of the spinal cord.

There was insufficient information in the autopsy records of 10 patients with syringomyelia to correlate clinical findings with abnormalities in substance P staining. No abnormalities were present in one patient with an asymptomatic syrinx found incidentally at the time of autopsy (Case 10). Because substance P is a putative pain-related peptide, its role in central pain syndromes is of clinical importance. Pain is a complaint in 50% to 90% of patients with syringomyelia,12,15,17,53 and segmental dysesthesias, characterized by burning pain, hyperesthesia, and trophic changes, are present in up to 40% of patients with post-traumatic syringomyelia.5,34,66,67 It is important to emphasize that the alterations in substance P distribution reported in the current study may represent nonspecific findings. Decreased staining of the peptide at the level of the syrinx is most likely due to the local destruction of spinal cord tissue, whereas the significance of increased staining below the level of cavitation cannot be established without detailed information about the presence or absence of pain syndromes in these patients. Future studies are warranted to examine the distribution of substance P and other biologically active peptides in patients with structural lesions of the spinal cord who have well-documented clinical findings.

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References

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