The Ultrastructure of Somatostatin-Immunoreactive Cell Bodies, Nerve Fibers and Terminals in the Dorsal Horn of Rat Spinal Cord*

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Summary. The distribution and ultrastructure of somatostatin-immunoreactive neurons, nerve fibers and axon terminals in the dorsal horn of rat thoracic spinal cord were studied by immunohistochemistry at both the light and electron microscopic levels. Somatostatin-immunoreactive neurons were predominantly observed in Rexed laminae I and II of the dorsal horn of spinal cord. Somatostatin-immunoreactive electron dense peroxidase material was concentrated in the Golgi apparatus and rough endoplasmic reticulum of the somatostatin-immunoreactive neurons, and was characteristically sparse in other regions of the cytoplasm. In the somatostatin-immunoreactive fibers and terminals, immunoreactive electron dense material was concentrated in the microtubules and large synaptic vesicles.

The tetradecapeptide somatostatin was first identified in the hypothalamus (Brazeau et al., 1973). Somatostatin-immunoreactivity has been demonstrated through immunohistochemistry in many regions of the central nervous system, including the spinal cord (Johansson et al., 1984; Vincent et al., 1985), where it is predominantly found in nerve fibers and terminals of Rexed laminae I and II of the dorsal horn at all levels (Hökfelt et al., 1975). Somatostatin-immunoreactive neurons have also been demonstrated in the spinal cords of the guinea pig, rat and monkey (Hökfelt et al., 1975; Burnweit and Forssmann, 1978; Forssmann, 1978; Forssmann et al., 1979; Dalsgaard et al., 1981; Senba et al., 1982; Ho, 1983; Johansson et al., 1984; Schröder et al., 1984; Vincent et al., 1985; Krupoff et al., 1986). Identification of somatostatin-immunoreactivity in a population of small cells in the spinal ganglia and in a dense terminal field in the substantia gelatinosa provided the initial evidence suggesting that this peptide may be involved in sensory transmission (Hökfelt et al., 1975). The present study demonstrates the distribution and ultrastructure of somatostatin-immunoreactive cell bodies, fibers and terminals in the dorsal horn of rat spinal cord by electron microscopic immunohistochemistry. These findings provide additional information concerning the involvement of somatostatin in the sensory pathways.

Materials and Methods

Five adult male Wistar rats (weighing 200–250 g) were used in this study. The animals were anesthetized with pentobarbital and perfused transcardially with 50 ml ice-cold physiological saline followed by 200 ml of fixative consisting of 4% paraformaldehyde and 0.4% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) (PB) at 4°C. The spinal cords were promptly removed and post fixed at 4°C for 24 h in 4% paraformaldehyde in the PB. The thoracic spinal cords were sectioned in the frontal plane on a Vibratome at 30 μm for light microscopy and at 100 μm for electron microscopy.

The sections were collected in saline buffered to pH 7.4 with phosphate buffer (PBS) and then immunostained using the mouse ABC system (Vector Labora-

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The free-floating sections were incubated in the primary antibody (monoclonal somatostatin antibody Ab 8) diluted 1:1000-5000 in PBS containing 0.1 mg/ml bovine serum albumin and 2% normal horse serum (NHS) for 72 h at 4°C. Following this incubation, the sections were rinsed and incubated in biotinylated horse anti-mouse IgG diluted 1:100 in PBS for 2 h at room temperature. After further rinsing, the sections were incubated in avidin-biotinylated horseradish peroxidase complex diluted 1:100 in PBS for 2 h at room temperature. Following another rinse, the sections were treated with 0.025% 3,3-diaminobenzidine (DAB) solution containing 0.01% H2O2 for 10 min at room temperature. Each rinsing was in three changes of PBS over 2 h.

For light microscopic investigation, the sections were mounted and dehydrated and coverslips were applied with Permount.

For electron microscopy, the sections were fixed in 1% OsO4 for 2 h at 4°C, dehydrated through alcohol and propylene oxide and then embedded in Epon 812 on a flat plastic slide. Ultrathin sections were cut on an MT 5000 Ultramicrotome, and examined under a JEM 1000X electron microscope with or without staining with uranyl acetate and lead citrate. The preparation and specificity of the monoclonal somatostatin antibody (Ab 8) used in this study have previously been documented in detail (BUCHAN et al., 1985; VINCENT et al., 1985). The specificity of this monoclonal antibody (Ab 8) was examined with a preabsorption test with synthetic somatostatin at 10 n mol/mL concentration; this blocked all specific staining (VINCENT et al., 1985).

RESULTS

Axon terminals containing intense somatostatin-immunoreactivity were recognized to be gathered in the marginal regions of the dorsal horn, especially in Rexed laminae I and II. The terminals were more densely distributed in Rexed lamina I than in Rexed lamina II. Furthermore, in the laterodorsal funiculus, very large networks of varicosities with the immunoreactivity were noted around the nerve fiber bundles (Fig. 1A). Without pretreatment with colchicine, a few somatostatin-immunoreactive cell bodies were observed among these dense terminal fields in Rexed lamina II. These cells were usually small in size (5 × 15 μm) and spindle-shaped by light microscopy (Fig. 1B). In other areas of the spinal cord, i.e., the anterior horn, lateral horn and pericentral canal regions, only very sparse fibers and few neurons were observed to be immunoreactive.

By electron microscopy, strongly somatostatin-immunoreactive electron dense materials were observed in the Golgi apparatus and rough endoplasmic reticulum in the cytoplasm of somatostatin-immunoreactive neurons (Figs. 2A, B). The large somatostatin-immunoreactive dendrites also contained immunoreactive electron dense materials (Figs. 2A, B).

Large somatostatin-immunoreactive dendrites contained large amounts of an electron dense product attached to the membranes of the Golgi apparatus and to some associated in the microtubules (Fig. 3A). In some cases, the somatostatin-immunoreactive cell process received both symmetrical and asymmetrical synapses (Fig. 3B).

There were many somatostatin-immunoreactive nerve fibers in the marginal region of the dorsal horn. Somatostatin-immunoreactivity was observed mainly in nonmyelinated nerve fibers where it was associated with microtubules and the outer membranes of mitochondria (Fig. 4A). In thin myelinated axons, the somatostatin-immunoreactivity was also associated with the microtubular system (Fig. 4B). Somatostatin-immunoreactive nerve fibers contained great numbers of small, clear electron-lucent vesicles and the outer membranes of these vesicles and mitochondria were heavily deposited with a somatostatin-immunoreactive electron dense product. These nerve fibers did not show typical synapses (Fig. 4A).

In the Rexed laminae I and II, there were many somatostatin-immunoreactive axon terminals. These contained large numbers of small clear synaptic vesicles; the outer membranes of these vesicles were heavily deposited with somatostatin-immunoreactive electron dense material. These terminals usually formed asymmetric contacts with an immunonegative dendrite (Fig. 5).
Fig. 1. Low (A) and high (B) magnification light micrographs of the dorsal horn (DH) of the rat spinal cord. Very dense networks of somatostatin-immunoreactive nerve fibers and axon terminals can be seen in the Rexed laminae I and II, and less dense networks and large numbers of varicosities of the somatostatin fibers can be observed in the lateral spinal nucleus. Some somatostatin-immunoreactive cell bodies (arrowheads) can be seen among the dense networks of the terminal fields in the inner part of the Rexed lamina II. FL funiculus lateralis. A: ×270; B: ×450
Fig. 2. Low (A) and high (B) magnification electron micrographs of somatostatin-immunoreactive cell bodies in the dorsal horn. 

A. Note the small size and spindle-shaped somatostatin-immunoreactive cell. The cell has a large clear nucleus (N) and a narrow cytoplasm. In this cytoplasm, electron dense materials are concentrated in the Golgi apparatus and rough endoplasmic reticulum (arrows). ×15,000.

B. Note electron dense somatostatin-immunoreactive products concentrated in the Golgi apparatus and rough endoplasmic reticulum (arrowheads). Somatostatin-immunoreactive dendrite (D) is also observed. Electron dense materials are seen in this dendrite. ×35,000
that the ultrastructural characteristics of the somatostatin systems in this spinal cord are different from those in the cortex and striatum of the rat brain (Difiglia and Aronin, 1982; Takagi et al., 1983; Mizukawa et al., 1986, 1987). The somatostatin-immunoreactive neurons in the dorsal horn of the rat spinal cord revealed that the immunoreactivity was concentrated in the Golgi apparatus and rough endoplasmic reticulum. This electron microscopic findings of ours agrees with the light microscopic result by Johansson (1978) who demonstrated somatostatin immunoreactivity within the Golgi apparatus of central and peripheral neurons. It is well known that somatostatin-immunoreactive neurons in the spinal cord, especially in the dorsal horn, can be easily demonstrated by pretreatment with colchicine. In this study, which was performed without colchicine treatment, somatostatin-immunoreactive products were only found concentrated in the Golgi apparatus and rough endoplasmic reticulum. This result suggests that somatostatin-immunoreactive cells in the dorsal horn may have a very rapid turnover of somatostatin, differing from the somatostatin neurons in areas such as the cerebral cortex and striatum where neurons are easily revealed without pretreatment with colchicine. In the cerebral cortex, somatostatin has an excitatory physiological effect (Phillis and Karpf, 1980) and somatostatin-positive neurons contain large amounts of somatostatin in the cytoplasm (Mizukawa et al., 1987). These neurons thus seem to contain enough amounts of somatostatin to obviate pretreatment with colchicine in order to reveal them. The somatostatin-positive neurons in the spinal cord, on the other hand, seem to have only a small store of somatostatin in the cytoplasm.

In their light microscopic immunohistochemical studies on the spinal cord, Hökfelt et al. (1975) suggested that the somatostatin-immunoreactive nerve terminals in the dorsal horn arise both from intrinsic (dorsal horn) and extrinsic (spinal ganglion) neurons. It is possible that myelinated somatostatin-immunoreactive nerve fibers of small diameter may come from the spinal ganglion neurons and extend longitudinally while the nonmyelinated somatostatin-immunoreactive nerve fibers might be very short axons from the intrinsic dorsal horn neurons.

Somatostatin-immunoreactive axon terminals contained electron dense immunoreactive products attached.
Fig. 3, A and B. High-magnification electron micrographs of somatostatin-immunoreactive cell process in the dorsal horn. Very strong somatostatin-immunoreactive electron dense materials are contained in this cell process, and can be easily recognized as somatostatin-positive cell process. ×38,000. A. The somatostatin-immunoreactive electron dense products are deposited with the Golgi apparatus (G) and microtubules in this cell process. B. This somatostatin-immunopositive process is clearly receiving two different types of synapses, i.e., symmetrical and asymmetrical synapses. The presynaptic terminal contains a large number of clear synaptic vesicles forming an asymmetrical synapse (arrow) with the somatostatin-positive cell process. In contrast, the presynaptic terminal contains a large number of flattened, clear synaptic vesicles forming a symmetrical synapse (arrowhead) with the somatostatin-positive cell process.
Fig. 4, A and B. High-magnification electron micrographs of somatostatin-immunoreactive nerve fibers in the dorsal horn. ×33,000. A. A large number of somatostatin-immunoreactive nerve fibers are observed among the large myelinated non somatostatin-immunoreactive nerve fibers. Somatostatin-immunoreactive electron dense materials are observed in the somatostatin-positive nerve fibers. In these somatostatin-positive nerve fibers, there are many small clear synaptic vesicles, and the outer membranes of these vesicles are deposited with somatostatin-immunoreactivity. M mitochondria. B. Somatostatin-immunoreactivity in the microtubules observed not only in small non-myelinated nerve fibers but also in some small diameter, myelinated nerve fibers (*).
Fig. 5, A-D. High-magnification electron micrographs of somatostatin-immunoreactive axon terminals. In these axon terminals, there are many small, clear synaptic vesicles; the outer membranes of these vesicles and mitochondria are deposited with somatostatin-immunoreactivity. The large synaptic vesicles (80–100 nm in diameter) (arrows) also display somatostatin-positive staining. These axon terminals show mainly asymmetric synaptic contacts with unlabeled dendrites. A: × 32,000, B: × 32,000, C: × 40,000, D: × 50,000
to the outer membranes of small clear synaptic vesicles, although a few heavily immunoreactive, large granular vesicles were usually intermingled. These findings are similar to those reported by other authors investigating the somatostatin-positive axon terminals of the striatum (Difiglia and Aronin, 1982; Takagi et al., 1983) and spinal cord (Foster and Johansson, 1985).

In conclusion, somatostatin-immunoreactivity is found in the Golgi apparatus and rough endoplasmic reticulum of the somatostatin-immunoreactive dorsal horn neurons. It seems therefore stored in these cells in very little amounts, being transported rather rapidly to the axonal terminals. The turnover of somatostatin metabolism in these neurons is thus presumed to be much faster than that in somatostatin neurons of the cerebral cortex and striatum.

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REFERENCES


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