Autoradiographic Evidence for Dopaminergic Innervation in Guinea Pig Spinal Cord

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Abstract—In studies of the localization of the dopaminergic nerve terminals in the cervical cord of guinea pig, autoradiographic analysis of the spinal cord loaded with [3H]dopamine ([3H]DA) was done under conditions that prevented the nonspecific uptake of [3H]DA. There was specific labeling in the gray matter and a high density of [3H]DA was present in the dorsal horn (DH). Moderate labeling was observed in the neuropil in the vicinity of the central canal. There were grain concentrations in close approximation to the cell bodies of numerous neurons in the DH and to the cell bodies of a few of the motoneurons in the ventral horn (VH). These dopaminergic terminals are possibly linked to sensory transmission and somatic motor function.

There is histochemical and neurochemical evidence for dopaminergic innervation into the spinal cord from the substantia nigra (1). diencephalon (2–4) and hypothalamus (5). Nguyen-Legros et al. (6) reported an in vitro method for achieving the radiographic visualization of DA axons in the brain, based on in vitro labeling with [3H]DA, under conditions that prevented the nonspecific uptake of [3H]DA. Using this method, we attempted to visualize cellular localization of the dopaminergic nerve terminals in guinea pig cervical cord.

Guinea pigs of both sexes, weighing 350–450 g, were treated with α-methylparatyrosine (150 and 200 mg/kg, respectively, 12 hr and 2 hr before sacrifice) to deplete the endogenous stores of CA and were then decapitated, and tracheotomy was done to identify the segments. The segment (C5) was rapidly excised after exposure by complete laminectomy. From the tissue block, transverse sections of about 100 μm in thickness were sliced with a razor blade. Under bubbling with 95% O₂ and 5% CO₂, the sections were preincubated at 37°C in MEM tissue culture medium for 15 min, in the presence of pargyline (10⁻⁴ M), ascorbic acid (5×10⁻⁴ M) and desmethylimipramine (DMI, 5×10⁻⁸ M, a NE uptake inhibitor (7)). [3H]DA was then added to a final concentration of 10⁻⁷ M for an additional 15 min. The sections were fixed in 3.5% glutaraldehyde in Millonig buffer for 30 min, dehydrated in ethanol (70% for 15 min, 95% for 15 min, 100% for 1 hr), cleared, embedded in paraffin, sectioned at 6 μm, mounted on microscope slides, coated with Sakura NR-M2 nuclear emulsion and stored over desiccant at 4°C for 8 weeks. These slides were then developed and stained with toluidine blue.

The labeled structures were found in the gray matter, especially in the DH and in the neuropil surrounding the central canal (Fig. 1). These findings corresponded with observations obtained by the histofluorescence technique (4). This distribution is similar to that of endogenous DA in the cord (8–10) in that the DA contents in the DH were two to three times higher than in the VH—a different distribution to NE, found in much higher concentrations in more ventral regions.

On the other hand, noradrenergic terminals, localized by means of dopamine-β-hydroxylase (DBH) immunocytochemical staining, were heavily concentrated in the VH and in the marginal zone of the DH in the cervical cord (11). As these distributions of
noradrenergic terminals were clearly different from those of the labeled terminals. [3H]DA was assumed to be taken up by dopaminergic nerve terminals in the cervical cord.

Fig. 1. Schematic mapping of the density (not number) of dopaminergic nerve terminals on the transversal section of guinea pig cervical spinal cord (C5), as visualized autoradiographically.

Fig. 2. Light microscopic autoradiograph of guinea pig cervical spinal cord incubated with [3H]DA (10^{-7} M) in the presence of DMI (5x10^{-6} M); exposure, 8 weeks. There are grain concentrations (1) in close approximation to the soma of neurons in the DH. Scale: 10 μm.

noradrenergic terminals were clearly different from those of the labeled terminals. [3H]DA was assumed to be taken up by dopaminergic nerve terminals in the cervical cord.

There were grain concentrations in close approximation to the cell bodies of numerous neurons in the DH (Fig. 2) and to the cell bodies of a few of motoneurons in the VH. We observed grain concentrations in the neuropil in the vicinity of the central canal. The spinal dopaminergic afferents to the DH and VH seem to make direct contact with the sensory and motor neurons as well as with their dendrites and axon terminals.

In immunocytochemical studies of DBH, most DBH-positive terminals seemed to be associated with processes, most likely dendrites, in the spinal cord (12). Autoradiographic studies with [3H]NE indicated that noradrenergic terminals were concentrated in neurophils (13). Moreover, noradrenergic terminals in the DH of the rat cervical cord were present throughout the substantia gelatinosa, particularly in its outer layer, and contacts were formed with the dendrites (44.8%), axon terminals (28.4%), unidentified components (26.5%) and, most rarely, the neuronal perikarya (0.3%) (14). Thus, there is a considerable difference between the localization of dopaminergic and noradrenergic terminals in the spinal cord.

As we obtained autoradiographical evidence for the localization of dopaminergic terminals in the spinal cord, the spinal dopaminergic nerve system probably plays some roles in sensory transmission and somatic motor function.

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