Electron Microscopic Investigation of Monoaminergic Terminals to α-Motoneurons in the Anterior Horn of the Cat Spinal Cord

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Abstract

In the anterior horn of the cat thoracic cord, networks of the monoaminergic fibers surrounding the alpha-motoneurons were investigated by fluorescent microscopy and submicroscopically. Monoaminergic terminals were recognized by the administration of 5-OHDA electron microscopically. These terminals could be classified morphologically into three types. The physiological significance of monoaminergic control of alpha-motoneurons was discussed. Type I of the labeled terminals did not show any typical synaptic specialization, such as aggregation of synaptic vesicles or thickening of the pre- and postsynaptic membranes. This type did not have synaptic contact with the alpha-motoneurons. Type II showed typical synaptic contact and asymmetrical synaptic type membranous thickening. A large number of small dense-cored vesicles were accumulated in the vicinity of the presynaptic membranes. Type III contained a large number of small and large dense-cored vesicles and a few flattened small vesicles. This type had synaptic contact with the presynaptic nerve ending in which a large number of agranular vesicles were contained. This study demonstrated that alpha-motoneurons in the anterior horn receive supraspinal monoaminergic control in three ways: modulator control through Type I, monosynaptic direct control through Type II, and inhibitory control through Type III.

KEYWORDS: monoaminergic terminals, cat, spinal cord, motoneuron, electron microscope

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ELECTRON MICROSCOPIC INVESTIGATION OF MONO-AMINERGIC TERMINALS TO α-MOTONEURONS IN THE ANTERIOR HORN OF THE CAT SPINAL CORD

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Abstract. In the anterior horn of the cat thoracic cord, networks of the monoaminergic fibers surrounding the α-motoneurons were investigated by fluorescent microscopy and submicroscopically. Monoaminergic terminals were recognized by the administration of 5-OHDA electron microscopically. These terminals could be classified morphologically into three types. The physiological significance of monoaminergic control of α-motoneurons was discussed. *Type* I of the labeled terminals did not show any typical synaptic specialization, such as aggregation of synaptic vesicles or thickening of the pre- and postsynaptic membranes. This type did not have synaptic contact with the α-motoneurons. *Type* II showed typical synaptic contact and asymmetrical synaptic type membranous thickening. A large number of small dense-cored vesicles were accumulated in the vicinity of the presynaptic membranes. *Type* III contained a large number of small and large dense-cored vesicles and a few flattened small vesicles. This type had synaptic contact with the presynaptic nerve ending in which a large number of agranular vesicles were contained. This study demonstrated that α-motoneurons in the anterior horn receive supraspinal monoaminergic control in three ways: modulator control through *Type* I, monosynaptic direct control through *Type* II, and inhibitory control through *Type* III.

Key words : monoaminergic terminals, cat, spinal cord, motoneuron, electron microscope.

Carlsson *et al.* (rat) (1), Dahlström and Fuxe (rat) (2), Fuxe (rat) (3), Konishi (dog) (4), Nygren and Olson (rat) (5, 6), Cruthcher and Bingham (primate) (7) and Mizukawa (cat) (8) have studied the distribution of monoaminergic fibers in the spinal cord of various mammals by fluorescence histochemistry. Motoneurons in the anterior horn are surrounded by a dense network of monoaminergic fibers, however, the fine structure and functional significance of synapses between monoaminergic fibers and motoneurons are still not clear.

Richardson (9), Hökfelt (10) and Ochi (11) developed the method of using potassium permanganate as a fixative. Tranzer and Thoenen (12) and Ibata *et al.* (13) developed the method of using false neurotransmitters such as 5-OHDA and 6-OHDA. These methods have made it possible to identify monoaminergic terminals electron microscopically and many investigators have been

studying the monoaminergic terminals in the central and peripheral nervous system (12-14). However, the ultrastructure of monoaminergic terminals in the cat spinal cord has not been investigated using this false neurotransmitter method.

The aim of the present study was to clarify the distribution and ultrastructure of the monoaminergic axonal terminals in the anterior horn of the cat thoracic cord by means of glyoxylic acid and paraformaldehyde gas fluorescence histochemistry and electron microscopic investigation using the false neurotransmitter 5-OHDA (3, 4, 5-trihydroxy-phenyl-ethylamine).

MATERIALS AND METHODS

Ten cats of both sexes were used in this study. Five cats were prepared for fluorescence histochemistry and five cats were treated with 5-OHDA and prepared for electron microscopy.

For fluorescence histochemistry. The animals were treated with an MAO inhibitor (Nialamide, 100 mg/kg i.p.) 5-6 h before being sacrificed, anesthetized with Nembutal and perfused through the left ventricles with chilled 2℃, 2% glyoxylic acid phosphate buffer solution buffered at pH 7.0. Tissues of the thoracic spinal cord (T_{7-8}) were rapidly removed and placed in isopentane cooled by liquid nitrogen. After freeze-drying, the blocks were reacted with paraformaldehyde gas at 80℃ for one hour, then embedded in paraffin in vacuo. Transverse sections (10 μm) were made, mounted in Entellan, and examined by fluorescence microscope.

For electron microscope. Under light Nembutal anesthesia, a laminectomy was performed at the T_{7-8} level in order to expose the spinal cord. A microsyringe fixed to a 26-gauge needle was introduced and five cats were given 5-OHDA (5-50 mg) dissolved in 0.5 ml saline into the subarachinoideal space of the thoracic cord. After survival periods of 6-8 h, the cats were anethetized with Nembutal and perfused via the left ventricles with a mixture of 1% glutaraldehyde and 1% paraformaldehyde buffered with phosphate buffer to pH 7.4. The thoracic cord (T_{7-8}) was removed immediately. Blocks were fixed in the same fixative for two hours, rinsed with buffer solution, and postfixed in 1% osmium tetroxide for two hours. The blocks were dehydrated through a graded series of acetone and finally embedded in EPON 812 mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100 B electron microscope. As far as possible serial ultrathin sections were studied.

RESULTS

In the anterior horn of the thoracic cord, many fine varicosities of green noradrenergic fluorescence were observed as networks surrounding the anterior large motoneurons, apparently in intimate contacts with α-motoneurons. Yellowish serotonergic fluorescence were also observed as the varicosities in the intermedial region of the anterior horn (Fig. 1).

Following the subarachinoid administration of 5-OHDA, several axon terminals containing vesicles with electron dense-cores were observed near the α-motoneurons. The diameters of these vesicles were 80-100 nm or 40-60 nm, and both types of vesicles were present in the monoaminergic terminals. The small
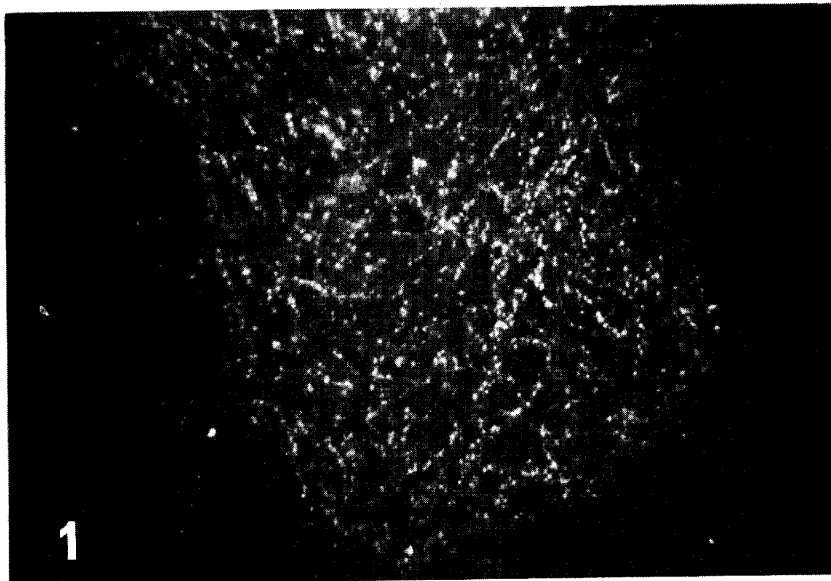
Fig. 1. Fluorescent photograph of the anterior horn in a transverse section of the cat thoracic cord: many fine varicosities of green noradrenergic fluorescence are observed as networks surrounding the α-motoneurons.   magnification: $\times 130$

synaptic vesicles, which contained the eccentrically located electron dense-cores, were gathered near the presynaptic membranes in many cases. These labeled monoaminergic nerve terminals could be classified into three types according to the shape and size of the synaptic vesicles, and the synaptic specialization *i.e.* type of junction, pre- and postsynaptic membranous thickenings and width of the synaptic cleft.

Type I. This type of axonal terminal contained numerous large vesicles (80-100 nm in diameter) with electron dense cores and a few small vesicles (40-60 nm in diameter) with dense cores. The terminals had no membrane thickening or narrow synaptic cleft. This type was often observed to be bouton en passant and had relatively wide extracellular space (40-200 nm in width) surrounding the terminals (Fig. 2a, 2b).

Type II. This type contained a large number of small vesicles (40-60 nm in diameter) with electron dense cores. These vesicles were accumulated in the vicinity of the presynaptic membrane. There was also a small number of the large vesicles with dense cores. This type showed asymmetrical synaptic type membranous thickening (approximately 50 nm in width) and formed axo-dendritic and axo-somatic contacts (Fig. 3a, 3b).

Type III. This type contained a large number of the small vesicles (40-60 nm in diameter) with electron dense cores, a small number of the large vesicles (80-100 nm in diameter) with electron dense cores, and a few flattened small
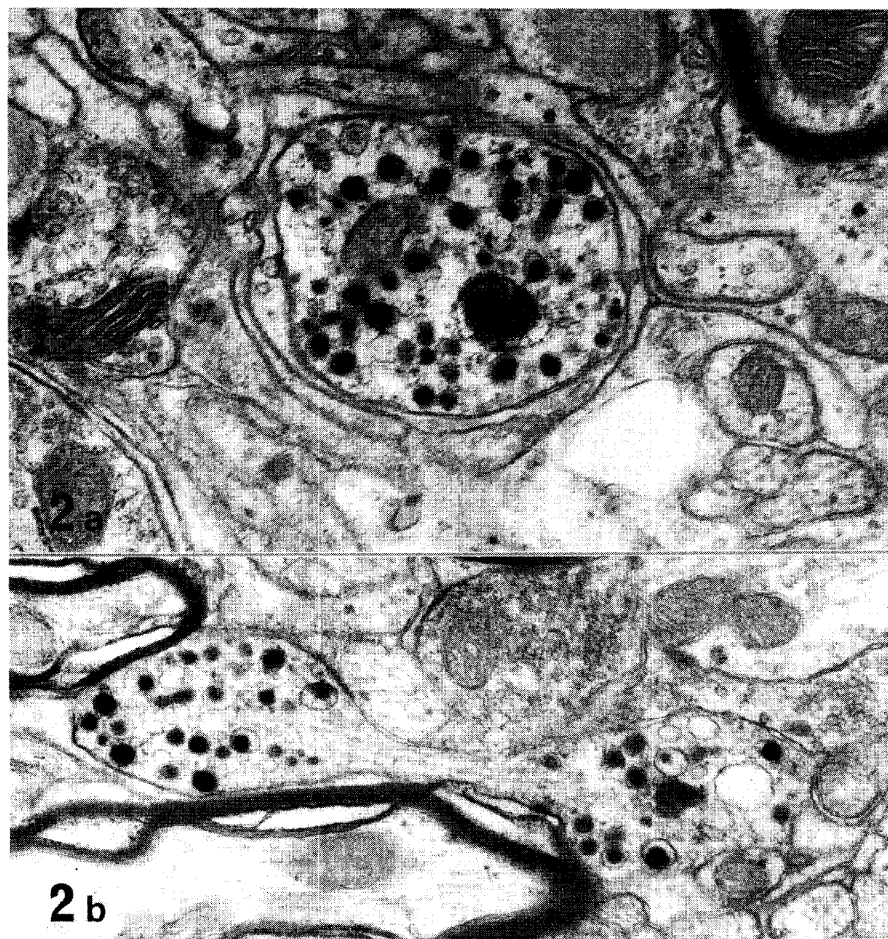
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Fig. 2. Electron micrograph of *Type* I labeled monoaminergic terminals. a. This terminal containing many large vesicles with electron dense-cores does not show synaptic specialization and exists in wide extracellular space. magnification: ×33,000 b. This type is observed to be bouton en passant. magnification: ×30,000

vesicles (10×40 nm in length). This type showed presynaptic membrane thickening (approximately 40 nm in width) and formed the axo-dendritic synaptic contact with presynaptic nerve endings which contained a large number of small agranular vesicles (50-70 nm in diameter) (Fig. 4).

Finally, semiquantitative analysis of labeled axonal terminals was performed in the anterior horn. In five animals studied, 393 of these labeled axonal terminals were observed: 40% were Type I, 55% were Type II, and 5% were Type III (Fig. 5).
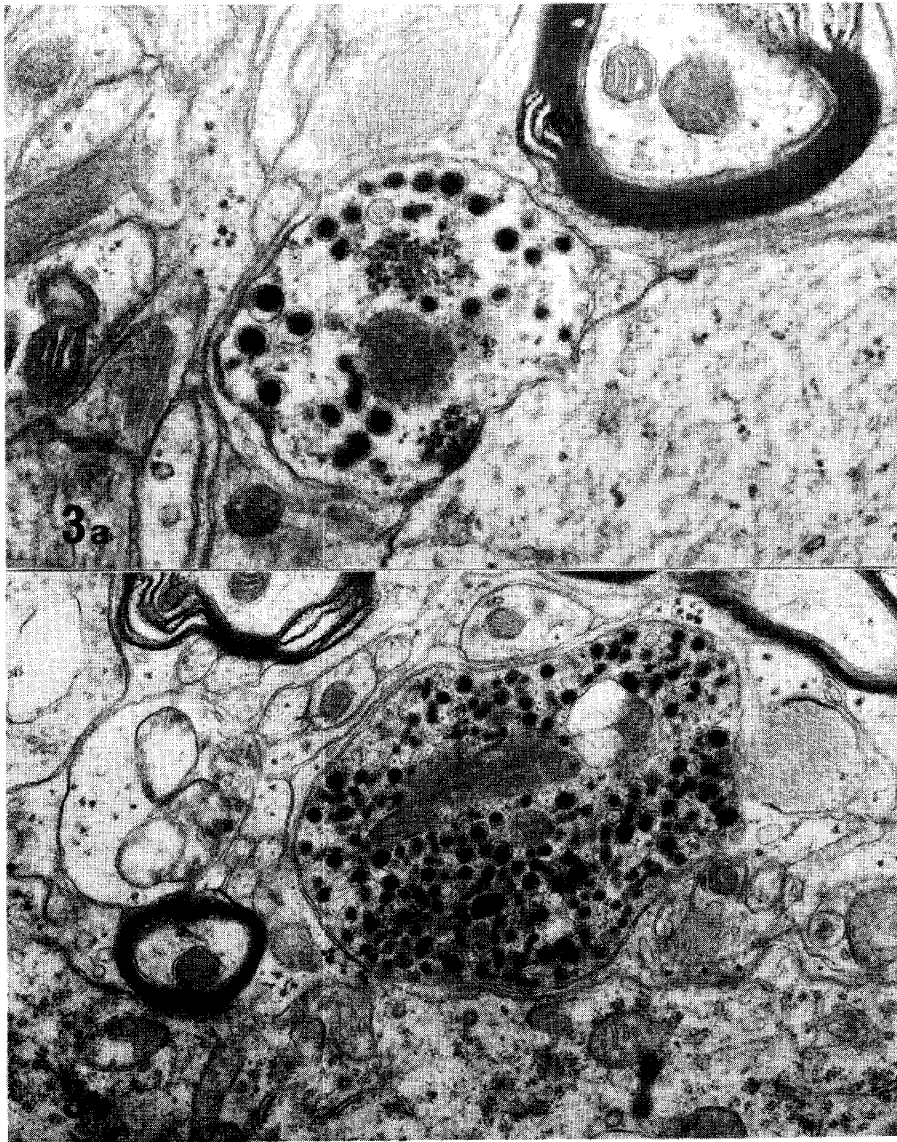
Fig. 3. Electron micrograph of *Type* II labeled monoaminergic terminals. a. Axo-dendritic type: many small and large vesicles with electron dense-cores are gathered in the vicinity of the presynaptic membrane. Pre- and postsynaptic membranous thickenings are evident. magnification: ×30,000 b. Axo-somatic type: axo-somatic synaptic contact is also observed. magnification: ×23,000

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Fig. 4. Electron micrograph of *Type* III labeled monoaminergic terminal: this type of synapse contains not only many small and large vesicles with electron dense-cores but also a few flattened synaptic vesicles and shows pre- and postsynaptic membrane thickenings and has axo-dendritic synaptic contact with the presynaptic nerve ending.   magnification: × 40,000

A SCHEME OF THE ULTRASTRUCTURAL MORPHOLOGY OF MONOAMINERGIC
TERMINALS LABELED BY 5-OHDA

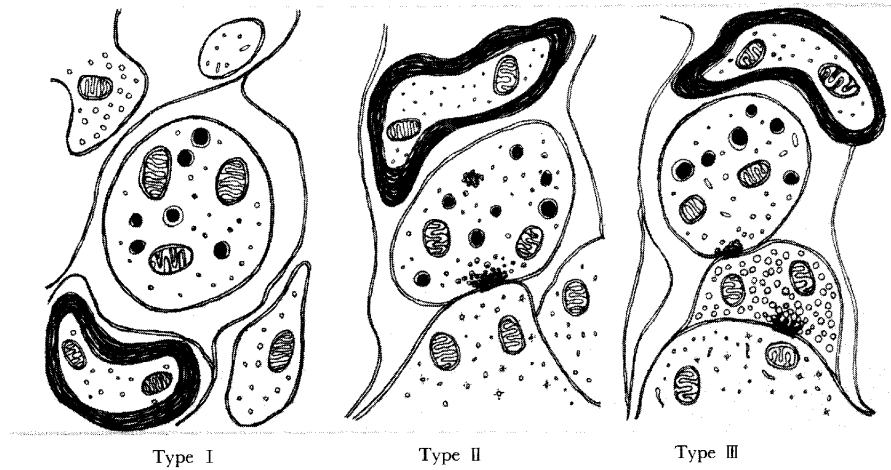Type I   Type II   Type III

Fig. 5. Scheme of the ultrastructural morphology of monoaminergic terminals labeled by 5-OHDA.

DISCUSSION

By formaldehyde fluorescence histochemistry, it has been shown that the noradrenergic neurons (A1, A2, A6), adrenergic neurons (C1, C2), and seroto-nergic neurons (B1, B2, B3) which exist in the brain stem send descending fibers through the spinal cord and terminate at the motoneurons in the anterior horn. In this area, monoaminergic fibers are distributed as networks surrounding the large α-motoneurons and seem to transmit supraspinal information to the spinal cord (1-8).

Physiologically, the α-motoneurons in the anterior horn are known to be important in the control of somatic musculature and locomotion. The mono-aminergic supraspinal controls play an important role in the smooth functioning of the α-motoneurons (15).

In order to investigate the ultrastructure of the monoaminergic axonal ter-minals, Richardson (9) developed the potassium permanganate fixation method for electron microscope visualization of peripheral autonomic nerve endings. However, this method of fixation does not give good preservation of the ultra-structure of the axonal terminals in the central nervous system. Methods using false neurotransmitters such as 5-OHDA were developed in order to investigate the ultrastructure of monoaminergic axon terminals in the central nervous sys-tem. By this method, many of the monoaminergic terminals take up 5-OHDA and large and small synaptic vesicles are labeled as electron dense-cores. How-ever, it was unclear whether the terminals containing the large and small vesicles with electron dense-cores were noradrenergic or serotonergic in this study, be-cause 5-OHDA labels both noradrenergic and serotonergic terminals. Apart from this demerit, good preservation of the ultrastructure was obtained enabling the ultrastructure of the monoaminergic terminals to be investigated in detail.

In the past, it was considered that all of the monoaminergic axonal ter-minals made synaptic contacts with the α-motoneurons by fluorescence micros-copy. By this electron microscopic study, many types of labeled axon terminals were observed surrounding the α-motoneurons, which receive supraspinal infor-mation from three different types of monoaminergic axon terminals. *Type* I of the labeled axonal terminals were often observed but did not have distinct syn-aptic membrane specialization *i.e.* pre- and postsynaptic membranous thickenings and existed in relatively wide extracellular space in serial ultrathin sections. Several investigators using permanganate potassium fixation have remarked that monoaminergic endings do not show synaptic membrane specialization and this finding is considered to be characteristic of monoaminergic axon terminals (16, 17). Swanson (18) investigated monoaminergic terminals in the hypothalamus using a false neuro-transmitter and reported that distinct synaptic membrane specialization most often asymmetrical in appearance was seen in 19 % of the labeled propils. The findings suggested that monoaminergic fibers are not in intimate contact with the target nerve cells but often may act as a modulator to

maintain the active state level.

Type II had asymmetrical synaptic contact with the dendrites of the α-motoneurons. Through this type terminal, supraspinal monoaminergic neurons control the α-motoneurons monosynaptically. The α-motoneurons are in intimate contact with the supraspinal monoaminergic fibers and smooth and segmental rhythmic function of the motoneurons is rendered possible. Finally, a small number of *Type* III were observed and showed synaptic contact with the pre- synaptic nerve endings in which large number of agranular synaptic vesicles were contained. This type of terminal contained not only small and large vesicles with dense-cores but also a few flattened synaptic vesicles. This type of synapses seems to be inhibitory morphologically. Some of the monoaminergic fibers may cause presynaptic inhibition of the α-motoneurons. On the other hand, Weight and Salmoiraghi (15) and Phillis *et al.* (19) reported that, physiologically, motoneurons in the cat spinal cord were depressed by electrophoretically administered norepinephrine. Our morphological findings correspond with these physiological results. Although it is unclear whether these three different types of the terminals shift to other types or not, it is interesting that the descending monoaminergic terminals exist as three different types in the anterior horn.

In conclusion, α-motoneurons in the anterior horn were shown to receive supraspinal monoaminergic control by three ways *i.e.* modulator control through *Type* I, monosynaptic direct control through *Type* II and inhibitory control through *Type* III.

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