Vesicular Acetylcholine Transporter Immunoreactivity in the Mesopontine Tegmentum of the Rat

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Abstract

The laterodorsal (LDT) and pedunculopontine (PPT) nuclei are composed by medium to large-sized cholinergic neurons that are implicated in behavioral state regulation. Vesicular acetylcholine transporter (VACHT) is a protein required for the cholinergic neurotransmission. Present immunohistochemical study revealed the VACHT positive neurons and terminal-like structures in the LDT and PPT nuclei.

Key Words: vesicular acetylcholine transporter, laterodorsal nucleus, pedunculopontine nucleus, cholinergic, central nervous system

The laterodorsal (LDT) and pedunculopontine (PPT) nuclei are located in the caudal midbrain and rostral pons, and composed by medium to large-sized neurons in the rat. The PPT nucleus appeared in the ventral lateral portion of the caudal midbrain tegmentum, and then shifted dorsomedially surrounding the superior peduncles at the levels of caudal end of midbrain. The LDT nucleus lies in the ventral lateral potion of the periaqueductal gray, extending from the levels of rostral end of the forth ventricle to the rostral levels of locus coeruleus. The LDT and PPT nuclei are recognized as cholinergic neuronal clusters in the rat, and each corresponds to Ch6 and Ch5 cholinergic neurons in the primate (Mesulam et al., 1983). To identify the cholinergic neurons, the cholinesterase histochemistry had been employed (Lewis and Shute, 1967; Shute and Lewis, 1967). However, this hydrolytic enzyme is present in not only the cholinergic neurons but also other neurons such as dopaminergic neurons (Butcher et al., 1975). With the development of the immunohistochemistry, the antibody for the choline acetyltransferase (ChAT) has
been thought as a reliable marker for the cholinergic neurons, and then the distribution of the cholinergic cells in the central nervous system has been well investigated in the rat (Sofroniew et al., 1982; Armstrong et al., 1983; Houser et al., 1983; Satoh et al., 1983; Tago et al., 1989), cat (Kimura et al., 1981; Jones and Beaudet, 1987), and primate (Mesulam et al., 1984). Contrary, there has been less information about the terminal site of the cholinergic neurons because of the predominantly appearing of this enzyme in the cell body. Recently, the antibody for the vesicular acetylcholine transporter (VACHT) has been developed as a cholinergic neuronal marker (Roghani et al., 1996; Ichikawa et al., 1997), in particular, for its terminal site (Arvidsson et al., 1997). Actually, the VACHT presents on the membrane of vesicles secreting acetylcholine and concentrates in nerve terminals (Weihe et al., 1996). In the LDT and PPT nuclei, VACHT immunopositive perikarya have been detected (Roghani et al., 1996; Arvidsson et al., 1997; Ichikawa et al., 1997) as like as ChAT immunohistochemistry. However, the existence of cholinergic terminal-like structures in these nuclei has not been clear. The present study has been done to clarify the cholinergic terminals in the LDT and PPT nuclei in the rat.

Male adult Sprague-Dawley rats (200-300g) were anaesthetized with sodium pentobarbital (50 mg/Kg, I.P.), and then perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffer (PB). The brains were removed from the skull and post-fixed for 2 hours in fixative solution, and then kept overnight in 30% sucrose buffer. Frozen sections were cut transversally at 30μm and collecting in 0.1 M phosphate buffered saline (PBS; pH 7.4) into six sets of serial sections. The first and second sets of sections were applied to the VACHT immunohistochemistry, and the third and forth sets were for the ChAT immunohistochemistry. The sections were soaked in a mixture of 5% normal goat serum, 0.3% Triton-X 100, and PBS for 30 min at room temperature, and then incubated for three days at 4°C in the primary antibody (diluted 1: 500 for VACHT and ChAT)(Chemicon U.S.A.). After three times rinses in PBS, sections were incubated for 15 min at room temperature in biotinylated goat anti-guinea pig immunoglobulin G (IgG) (dilution 1:200 Vector U.S.A.) for VACHT and goat anti-rabbit IgG (dilution 1:200 Vector U.S.A.) for ChAT. Following three rinses, the sections were placed for 10 min in avidin-biotin-peroxidase complex (ABC Vectastain U.S.A.), rinsed three times with PB, and then immersed for 6 min into the solution containing 0.02% diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in 0.05 M Tris-HCl buffer. After brief washing in PB, the sections were mounted on glass slides and allowed to dry overnight. The second and third sets of sections were dehydrated in alcohol, treated with xylene, and coverslipped with Entelan (Merck Germany). The first and forth sets of sections were counterstained with thionine prior to the dehydration.
Fig. 1. Photomicrograph showing VACHT immunoreactivity in the PPT nucleus. Cholinergic somata and presumptive terminals are demonstrated. Scale bar = 20 μm.

The VACHT immunoreactive neurons were observed in the LDT and PPT nuclei (Fig. 1). The location of these neurons was similar to that on the adjacent preparation in serial sections reacted by ChAT immunohistochemistry. The VACHT immuno-positive neurons showed diffuse brown stained cytoplasm with, in some neurons, dark brown particles. Swelled brown puncta were appeared on proximal dendrites in some immuno-positive neurons. These puncta were much smaller than those appeared on the brain stem cranial motor nuclei, where the immuno-positive cell body were surrounded by large immuno-positive puncta. Small preterminal and terminal like brown products were also detected in the space among immuno-positive neurons.

The VACHT immunoreactivity in the central nervous system has been investigated (Roghani et al., 1996; Arvidsson et al., 1997; Ichikawa et al., 1997; Roghani et al., 1998; Schafer et al., 1998), supporting the results obtained from the ChAT immunoreactivity. Among these studies, it is agreeable that many VACHT immunoreactive neurons are placed in the LDT and PPT nuclei as like as ChAT neurons. However, some reports indicated preterminal like structures with immuno-reactivity for VACHT (Arvidsson et al., 1997; Schafer et al., 1998), another failed to detect them (Ichikawa et al., 1997). Our results confirmed the existence of preterminal-like structures in the LDT and PPT nuclei. The reason of this discrepancy is not known. The difference of the epitope sites of antibodies may be one of the reasons explaining for uneven morphological properties.

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Concerning to the behavioral changes such as waking and sleep, cholinergic PPT and LDT neurons were well investigated physiologically (Leonard and Llinas, 1990; Kamondi et al., 1992; Koyama and Kayama, 1993). Physiological data indicated that the mesopontine tegmentum include two types of neurons showing different responses for the application of the acetylcholine mimic carbachol (Sakai and Koyama, 1996). One type of cholinceptive neuron is showing the excitatory response and thought to be a non-cholinergic, provably glutamatergic, neuron, and another type is responding the inhibition and thought to be a cholinergic neuron. The electron microscopical study revealed the symmetric synaptic junctions on the non-cholinergic PPT cells (Spann and Grofova, 1992). The terminal-like structures observed outside the cholinergic neurons may connect such non-cholinergic neurons.

The cholinergic synaptic input to the cholinergic neurons in the mesopontine tegmentum is a matter of debate. Electrophysiological experiments revealed cholinceptive cholinergic neurons within the PPT and LDT nuclei (Koyama and Kayama, 1993; Sakai and Koyama, 1996), where these neurons were hyperpolarized by acetylcholine through a muscarinic M2 receptor (Leonard and Llinas, 1994). However, the electron microscopic study detected very few cholinergic synaptic terminals on the cholinergic cells in the rat PPT nucleus (Spann and Grofova, 1992). The intracellular staining with biocytin of the cholinergic LDT neurons in the guinea-pig indicated local highly branched terminal plexus, some of which overlapped with dendritic field of the parent neuron (Surkis et al., 1996), suggesting recurrent inhibition. Due to the limitation of the present experiment with the light microscopic level, further study will be necessary to clarify if the swelled puncta appeared on the proximal dendrite are the component of the presynaptic structures or comprising postsynaptically inside of the cell body.

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