Quantitative and ultrastructural study of ascending projections to the medial mammillary nucleus in the rat

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Summary. We analyzed the termination pattern of axons from the superior central nucleus and the ventral tegmental nucleus of Gudden within the medial mammillary nucleus (MM) in the rat. The neuropil of the MM consists of two classes of terminals, that is, terminals containing round synaptic vesicles and forming asymmetric synaptic contact, and terminals containing pleomorphic synaptic vesicles and forming symmetric synaptic contact. The number of axodendritic terminals with round vesicles is almost equal to that of terminals with pleomorphic vesicles. Almost all axosomatic terminals contain pleomorphic vesicles with symmetric synaptic contact. Injection of WGA-HRP into the central part of the superior central nucleus permitted ultrastructural recognition of many anterogradely labeled terminals within the median region of MM. The labeled terminals contacted mainly intermediate (1-2 μm diameter) and proximal dendrites (more than 2 μm diameter) as well as the neuronal somata. Serial ultrathin sections of neurons of the median region of the MM revealed that 37% of the axosomatic terminals were labeled anterogradely. The pars compacta of the superior central nucleus had reciprocal connections with the median region of MM. The axon terminals from this nucleus occupied 53% of axosomatic terminals, and contacted mainly intermediate dendrites. Following injection of WGA-HRP into the ventral tegmental nucleus, many labeled terminals were found in the medial and lateral regions of MM. They contacted mainly intermediate dendrites as well as neuronal somata. In the medial region, 78% of axosomatic terminals contacting retrogradely labeled neurons were labeled anterogradely. All labeled terminals from these nuclei contained pleomorphic vesicles, and made symmetric synaptic contact.

Key words: Tegmentomammillary projection – Synapses – Superior central nucleus – Ventral tegmental nucleus of Gudden – WGA-HRP study

Introduction

Autoradiographic and retrograde tracing studies have shown that the medial mammillary nucleus (MM) sends collateral fibers to the anterior thalamic nucleus, ventral tegmental nucleus of Gudden (TV), pontine nucleus, and nucleus reticularis tegmenti pontis (Cruce 1975, 1977; Watanabe and Kawana 1980; Veazey et al. 1982; Seki and Zyo 1984; Hayakawa and Zyo 1985, 1986). The descending afferents project to the MM from widespread regions such as the subiculum, prefrontal cortex, septal area, and anterior hypothalamic area (Meibach and Siegel 1977a, b; Swanson and Cowan 1977, 1979; Shibata 1989). The ascending afferents originate mainly from the TV (Bleier 1969; Briggs and Kaelber 1971; Petrovicky 1973; Hayakawa and Zyo 1984). There are also reciprocal connections between the MM and the TV. In addition, topographical projection of the TV to the MM is such that the caudal part of the TV projects to the lateral region of the MM, the rostral part of the TV to the medial region of the MM, and the pars compacta of the superior central nucleus (CC) to the median region of the MM (Shibata 1987).

Our previous study (Hayakawa and Zyo 1990) revealed the fine structure of descending afferents from the MM to the TV. The majority of terminals from the MM neurons contained round synaptic vesicles and made asymmetric synaptic contact, while a few terminals contained flat vesicles and made symmetric synaptic contact. These terminals contacted mainly intermediate and distal dendrites. Few axosomatic terminals were labeled.

Several studies have reported that the superior central nucleus (median raphe nucleus) projects to widespread areas including the MM (Bobillier et al. 1975, 1976, 1979; Azmitia and Segal 1978; Vertes and Martin 1988). The MM sends fibers to the CC, but no projection fibers to the central part of the superior central nucleus. Relatively little is known concerning the ultrastructure of ascending afferents from the superior central nucleus to the MM. Allen and Hopkins (1989) suggested that terminals of the TV contain pleomorphic vesicles in the MM.
Quantitative results concerning synaptic organization of the MM, however, have not been well established.

The present study was undertaken to examine the ultrastructure of axodendritic and axosomatic terminals of the ascending afferents within the MM. The ultrastructural anterograde tracing method of horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) was used to elucidate what kind of axon terminals the TV, CC and superior central nucleus have within the MM, where those terminals contact different portions of MM neurons, and whether termination patterns of the TV, CC and superior central nucleus are different in the MM.

Materials and methods

Twelve male Sprague-Dawley rats weighing 250–300 g were used. All surgical procedures were carried out in the animals under sodium pentobarbital (30 mg/kg, i.p.) anesthesia. A 1-μl Hamilton syringe, 0.02 to 0.03 μl of 5% WGA-HRP in 0.1 M phosphate buffer at pH 7.4 was injected stereotaxically under pressure into the TV, the CC, and the superior central nucleus. The injection was made over a period of 15 min, and the syringe was kept in situ for an additional 20 min after the injection in order to restrict the injection site. Two days after the injection, the anesthetized animals were perfused first with 100 ml of 0.9% NaCl, then 500 ml of 1% glutaraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were immediately removed and placed in the same fixative for 1 h. Serial frontal sections were cut at a thickness of 80–100 μm with a Vibratome. Every second section through the mammillary body was processed for light microscopic study with tetramethylbenzidine (TMB) at pH 3.3, and counterstained with neutral red (Mesulam 1978). Sections involving the injection sites were subsequently processed with DAB and counterstained with cresylviolet (LaVail and LaVail 1972). Although the DAB reaction was less sensitive, we employed it for determining the center of the injection site.

In order to carry out electron microscopic studies, every second Vibratome section through the mammillary body was collected and rinsed with 0.1 M phosphate buffer at pH 7.4. These sections were presoaked for 5 min with a solution containing 195 mg ammonium heptamolybdate, 4 mg TMB in 2 ml ethanol, and 78 ml of 0.1 M phosphate buffer at pH 5.8. They were processed for 30 min by adding 0.8 ml of 0.3% H2O2 per 80 ml solution every 5 min. Following a brief rinse for 10 s with 0.1 M phosphate buffer at pH 5.8, the sections were postfixed with 2% OsO4 in 0.1 M phosphate buffer at pH 6.0 for 2 h at 45°C, then dehydrated in methanol and propylene oxide (Olucha et al. 1985). Each section was embedded in a flat dish with Epon 812. In addition, osmium-fixed Vibratome sections through the MM of three normal animals sacrificed in the same manner were prepared by conventional methods and embedded in Epon 812. Ultrathin sections were collected and stained with uranyl acetate and Reynold’s solution, and examined with a JEOL 1200EX transmission electron microscope.

A total of 18,300 μm² of normal neuropil in the MM obtained from six sections of three animals was surveyed from electron micrographs at a final magnification of ×13,000 in order to carry out quantitative analyses. Axon terminals were classified according to diameter, vesicular shape, and symmetry of the pre- and postsynaptic densities. Dendrites were classified into distal (less than 1 μm diameter), intermediate (1–2 μm diameter), and proximal (more than 2 μm diameter). We counted the number of terminals in the normal neuropil, and obtained the ratio of terminals attached on the three classes of dendrites. Similar measurements were carried out on WGA-HRP labeled terminals. The chi-square goodness of fit test was applied for comparison of the distribution of labeled terminals on three classes of the dendrites with those for normal neuropil. In addition, serial ultrathin sections were made through the MM neuronal somata in order to obtain the precise number of labeled axosomatic terminals. The sections were collected on Formvar-coated single-slot grids, and stained with uranyl acetate and lead citrate. Electron micrographs of every five sections were taken at a final magnification of ×6,700. The number of all labeled and non-labeled terminals contacting the neuronal somata was then counted, and the ratio of labeled axosomatic terminals was calculated.

Results

The MM is divided into median, medial and lateral regions. The median region includes the pars medianus, the medial part of the pars medialis, and the median part of the pars posterior of the MM (Paxinos and Watson 1986). The medial region corresponds to the medial half of the MM excluding the median region, and includes the principal mammillary tract, the lateral part of the pars medialis, and the medial half of the pars posterior. The lateral region is the lateral half of the MM (Shibata 1987).

The axon terminals were classified into two groups. The first consists of terminals containing round synaptic vesicles (about 40 nm), and forming asymmetric synaptic contact (Fig. 1A). The second consists of terminals containing pleomorphic synaptic vesicles (20–50 nm), and forming symmetric synaptic contact (Fig. 1B, C, D). These terminals contacted distal and proximal dendrites as well as neuronal somata. On occasion, a terminal synapsed on both a spine and its parent dendrite, two different dendrites (Fig. 1C), or two different neuronal somata (Fig. 1D). A terminal often surrounded a distal dendrite (Fig. 1A, B). There were no terminals containing flat vesicles (about 60 nm long) with symmetric synaptic contact.

In order to compare the distributions of normal and labeled terminals on different portions of dendrites, quantitative analysis of the normal neuropil was carried out in the median and medial regions of the MM. Since the boundary between the medial and lateral regions is not clearly visible in osmium-fixed sections, it was difficult to trim only the lateral region from embedded blocks. We did not analyze the lateral region in this study. The percentage of terminals with round vesicles was similar to that of terminals with pleomorphic vesicles in both the median and medial regions of the MM (Tables 1, 2). Comparing the percentages of terminals with round vesicles and terminals with pleomorphic vesicles on different portions of the dendrites, the terminals with round vesicles decreased from 58.1% in the distal dendrites to 10% in the proximal dendrites in the median region of the MM (Table 1). In contrast, the terminals with pleomorphic vesicles increased from 41.9% in the distal dendrites to 90% in the proximal dendrites. There was a similar tendency in the medial region (Table 2), that is, the percentage of the terminals with round vesicles decreased from the distal dendrites to the proximal dendrites, and the terminals with pleomorphic vesicles increased from the distal dendrites to the proximal dendrites. Because the cells in the medial region were smaller...