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Connections of the superior vestibular nucleus with the oculomotor and red nuclei in the rat: An electron microscopic study

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Abstract

Phaseolus vulgaris leucoagglutinin (PHA-L) was injected into the individual vestibular nuclei of the rat to study their efferent connections. One of the major differences between the connections of these nuclei was found at the level of the mesencephalon: the eye-moving cranial nerve nuclei received the densest projection from the superior vestibular nucleus (SVN). In the present electron microscopic study, we have found that terminals of SVN origin established symmetric synaptic contacts in the oculomotor nucleus. More than two-thirds of PHA-L-labeled boutons terminated on dendrites, the rest of them established axosomatic contacts. Most of the labeled terminals were GABA-positive, supporting the results of previous physiological experiments, which showed inhibitory effects. In the mesencephalon, the other termination area was found in the red nucleus. The PHA-L-labeled boutons of SVN origin were in close contact with the perikarya and proximal dendrites of the magnocellular part of the red nucleus. The types of synaptic contacts and distribution of terminals of SVN origin were similar to those found in the oculomotor nucleus. Our results indicate that the SVN can modify the activity of the cerebellorubral and corticorubral pathways, exerting inhibitory action on the neurons of the red nucleus.

Keywords: Vestibular system; GABA; *Phaseolus vulgaris* leucoagglutinin

1. Introduction

The primary afferent fibers originating in the vestibular sense organs terminate in the superior (SVN), medial (MVN), lateral or Deiters (LVN) and descending (DVN) vestibular nuclei of the brainstem. Earlier experiments performed on mammalian species suggest that individual differences exist between the vestibular nuclei *in* their roles played in the maintenance of equilibrium and orientation in three-dimensional space and their involvement in the vestibular compensation [3,4,8,9]. Numerous studies were performed to elucidate the morphological background of the functional differences, which could be associated with different afferent and efferent connections of the individual vestibular nuclei.

In a series of experiments we have studied the efferent connections of the secondary vestibular neurons located in the individual vestibular nuclei and described their projections to different parts of the brain stem and spinal cord [3,4]. One of the termination areas of the secondary vestibular fibers was found in the mesencephalon. We have found that each vestibular nucleus projects to the oculomotor and trochlear nuclei receiving the densest innervation from the SVN similarly to the results of previous experiments [8]. Earlier immunohistochemical studies demonstrated GABAergic neurons in the vestibular nuclei of the cat [10] and physiological studies described that GABA antagonists block the inhibitory postsynaptic potentials in the oculomotor nucleus after stimulation of the vestibular nerve [2]. This finding suggests a GABAergic projection from the vestibular nuclei to the oculomotor nucleus, however its origin from the SVN has not been unequivocally verified with neuronal tracing techniques. Moreover, we showed that the magnocellular part of

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the rat red nucleus receives direct projections from all vestibular nuclei [3]. Data on the excitatory or inhibitory nature of the vestibulorubral connections are not available so far.

2. Materials and methods

The experiments were carried out on adult Wistar rats in accordance with European Community guidelines. Under urethan anaesthesia, the cranial cavity was opened and a glass micropipette filled with a 2.5% solution of *Phaseolus vulgaris* leucoagglutinin (PHA-L) was introduced into the superior vestibular nucleus using the coordinates of Paxinos and Watson [6]. After 14 days, the animals were transcardially perfused with 0.9% NaCl solution and with a fixative of 2.5% glutaraldehyde, 0.5% paraformaldehyde and 0.2% picric acid. Cross sections of the midbrain were made with a Vibratome at a thickness of 60 μm and incubated with biotinylated goat-anti-PHA-L and then with avidin-biotin peroxidase complex. The immunoreaction was completed with a 3-3-diaminobenzoline chromogen reaction. The sections were postfixed in 0.5% osmium tetroxide and flat embedded in Araldite. Selected areas containing the oculomotor and red nucleus were re-embedded, serial ultrathin sections were cut and collected on Formvar-coated nickel grid. Sections on every second grid were processed for GABA immunohistochemistry according to Somogyi and Hodgson [7] using a rabbit-anti body-GABA (Sigma, 1:2000). The secondary immunogold conjugate goat-anti-rabbit IgG was connected to these complexes in order to visualize them in EM (golden granules diameter: 20 nm). The photographs of labeled terminals were taken with Jeol electron microscope.

3. Results

The PHA-L injection into the brainstem was restricted to the dorsolateral edge of the brainstem at the level of the eighth cranial nerve (Fig. 1A). This area corresponds to the localization of the SVN within the vestibular nuclear complex of the rat [6]. Following injections of the SVN, labeling was found in different structures of the nervous system, including the oculomotor nucleus and the magnocellular part of the red nucleus. The PHA-L positive fibers with varicose axon terminals were distributed throughout the extent of these nuclei. The majority of PHA-L-labeled boutons displayed very similar distribution patterns in the oculomotor and red nucleus appearing in close contact with the cell bodies and proximal dendrites (Fig. 1B and C). At ultrastructural level, the PHA-L-labeled boutons were almost exclusively in presynaptic position in both nuclei (Fig. 2A–D). The boutons were engaged in symmetrical synaptic connections, the postsynaptic profiles were either dendrites of 3.5–4 μm in diameter or cell bodies. Occasionally, the PHA-L-labeled boutons were located in postsynaptic position related to an unlabeled presynaptic axon terminal containing spherical clear and dense core vesicles. Of the 166 PHA-L-labeled boutons counted in the oculomotor nucleus, 133 established axodendritic, 29 axosomatic and 4 axoaxonic contacts. In the red nucleus these numbers were 47, 16 and 2, respectively (Table 1). The immunostaining for GABA revealed immunogold particles in the majority of PHA-L-labeled terminals of SVN origin in both the oculomotor and red nucleus (Fig. 2C and D), the postsynaptic elements were negative for GABA reaction. The GABA-positive immunoreaction was also observed in PHA-L-negative boutons. In addition, immunogold particles indicating the presence of GABA were rarely present in those dendrites and cell bodies that were not engaged in synaptic contact with the PHA-L-labeled terminals (Fig. 2D).

Table 1
Distribution of axodendritic, axosomatic and axoaxonic boutons in the oculomotor nucleus and in the red nucleus

	Oculomotor nucleus	Magnocellular part of red nucleus
Axodendritic contacts	133 (80.12%)	47 (72.30%)
Axosomatic contacts	29 (17.46%)	16 (24.51%)
Axoaxonic contacts	4 (2.42%)	2 (3.09%)
Total	166 (100%)	65 (100%)

The number in parentheses shows the ratio of different types of boutons.

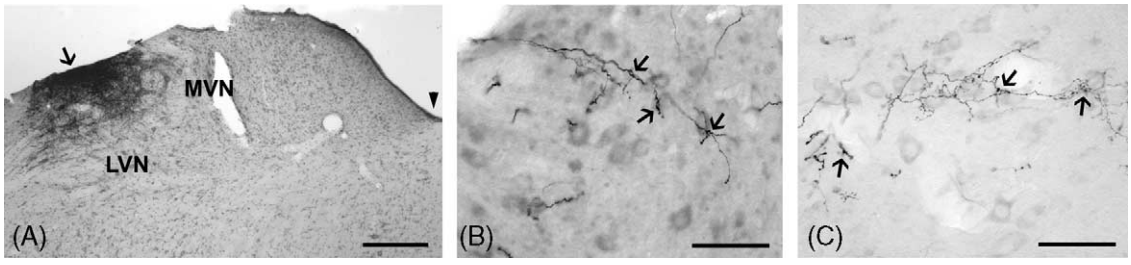

Fig. 1. (A) Cross-section of the rat brainstem showing the place of the *Phaseolus vulgaris* leucoagglutinin (PHA-L) injection into the superior vestibular nucleus (SVN, arrow). Arrowhead indicates the midline. LVN: lateral vestibular nucleus, MVN: medial vestibular nucleus. (B and C) Light microscopic visualization of the PHA-L-labeled axons and terminals (arrows) of SVN origin in the oculomotor (B) and red nucleus (C). Scale bars: 200 μm in (A) and 100 μm in the rest of figures.