79. *Nucleus raphe pallidus of Rat Medulla oblongata*

A Possible Contact Point of Serotonergic and Catecholaminergic Systems

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Nucleus raphe pallidus (NRP) which is a caudal component of the raphe nuclear groups is a single mass of relatively densely packed neurons situated in the median region adjoining the dorsomedial edges of the pyramidal tracts. Within this nucleus, a group of serotonin (5-HT) neurons which show yellow fluorescence by the Falck-Hillarp method have been identified as B1 group by several investigators (Dahlström and Fuxe, 1964; Yanagisawa, 1975). Numerous green fluorescent catecholamine terminals converging upon these B1 neurons have also been demonstrated (Dahlström and Fuxe, 1964; Fuxe, 1965a, b). In addition, possible participation of the sensorimotor cortex and the spinal cord in the afferent systems to the NRP has been suggested, but the major neural afferents to this nucleus are thought to come from the brain stem areas (Brodal *et al.*, 1960).

In the present study, as one step to obtain evidence for the possible association between the catecholaminergic and serotonergic systems, the fine structure of the neuronal cell bodies and neuropil in the NRP was studied with the electron microscope and by the Falck-Hillarp method.

Materials and methods. Nine Wistar rats weighing 200–400 g were perfused with 1% glutaraldehyde-1% paraformaldehyde fixative in 0.12 M phosphate buffer *via* the left ventricle under Nembutal anesthesia. Slices of the medulla oblongata were immersed in the fixative for 2 hr. After rinsing in the buffer, the samples were post-fixed with 1% OsO₄, dehydrated in a graded series of ethanol and embedded in epoxy resin. Thin sections cut on an LKB 8800 ultramicrotome were doubly stained with uranyl acetate and lead citrate and examined in a Hitachi H-500 electron microscope.

Five of 9 rats were given 5-hydroxydopamine (5-OHDA) for identification of CA terminals (Richards and Tranzer, 1970). Approximately 600 µg of 5-OHDA dissolved in 70 µl of 0.9% saline containing 0.1% ascorbic acid was infused for 30 min into the fourth ventricle. Thirty min later, they were killed and the sections for elec-

tron microscopy were prepared as described above.

In addition, a number of rats with or without monoamine oxidase inhibitor (MAOI) treatment (Nialamide 400 mg/kg or Iproniazid 200 mg/kg i.p.) were used for the fluorescent microscopic examinations. Blocks of the medulla oblongata were taken out from decapitated animals, processed following the Falck-Hillarp method (Dahlström and Fuxe, 1964) and examined in an Olympus FLM fluorescence microscope equipped with HBO 200/4 super pressure mercury lamp (Osram).

Results and discussion. As reported by earlier investigators (Dahlström and Fuxe, 1964; Yanagisawa, 1975), considerable numbers of yellow fluorescent cell bodies were easily identified in the NRP of MAOI-treated rats by the Falck-Hillarp method. Most of them were small to medium in size and round to oval in shape. These were frequently found in the elongated oval-shaped region of the NRP adjoining dorsomedial edges of the pyramidal tracts. Medium-sized multipolar or fusiform yellow fluorescent cells were also found in the same region. In the rostral portion of the NRP, large multipolar yellow fluorescent neurons were observed. A number of similar large multipolar neurons could also be traced up to the region of the nucleus raphe magnus (NRM) (B3 group) which situated dorso-rostral to the NRP. However, it is difficult to detect a distinct border between these two groups (Taber *et al.*, 1960; Dahlström and Fuxe, 1964).

In these nuclear areas, numerous green fluorescent fine varicose fibers (Fig. 1) which are thought to be catecholaminergic components were found around yellow fluorescent cell bodies of the NRP or the NRM. Some of them seemed to make close contact with these neurons. There were less green fluorescent fibers in the caudal half of the NRP.

The ultrastructural morphology of the neurons in the NRP was very similar to that of the kitten reported by Fox *et al.* (1976). At least three types of neurons were recognized in the NRP in our materials. The first type was the small neurons (Fig. 2) found mostly in the ventral portion of the nucleus in which region a number of small round yellow fluorescent neurons were observed by the Falck-Hillarp method. This type of neuron had a round to oval cell body, being 10–15 μm in diameter in transverse section. An eccentric nucleus with extensive indentations of the nuclear membrane occupied a major portion of the cell body. Generally, the perikaryon of this type of neuron appeared “clear” because of sparsity of cytoplasmic organelles. On the soma of this type of neuron one or two synapses were usually found. The second type of neuron was medium-sized (15–25 μm in diameter) multipolar or fusiform one containing a centrally or eccentrically positioned oval nucleus with various degrees of

Figs. 1-3. 1: Fluorescence micrograph of the rostral part of the Nucleus raphe pallidus (NRP) of the nialamide-treated rat. Numerous green fluorescent varicose fibers are present surrounding the yellow fluorescent cell bodies ($\uparrow$). P, pyramidal tract. $\times 200$. 2: A small-sized neuron from the ventral part of the NRP. $\times 9,000$. 3: Large granular vesicles ($\blacktriangle$) found near the Golgi apparatus (G) in the medium-sized neuron. $\times 17,000$.

indentation of the nuclear membrane. The cytoplasm contained numerous well developed Golgi apparatus which were mostly present in the perinuclear region and at the base of dendrites. In association with the well developed Golgi apparatus, a number of agranular vesicles (50–100 nm in diameter) were present. It was not unusual that one or two large granular vesicles (LGV, 100–120 nm in diameter) were found near the Golgi apparatus (Fig. 3). Rough endoplasmic reticulum appeared as clumps of short non-oriented membranes or laminar stacks of membranes. Pleomorphic mitochondria, free ribosomes and lysosomes were also abundant. On this type of neuron three to five axosomatic synapses were seen in one profile of a neuron. On the surface of the proximal part of the dendrites just extending from the soma of this type of neuron more frequent axo-dendritic synapses were found. The synaptic terminals contained small spherical vesicles (50 nm in diameter) and/or flattened vesicles. LGV (100–120 nm in diameter) were also coexisted with these small spherical synaptic vesicles. In addition, larger multipolar neurons (25–45 μ m in diameter) containing well developed cytoplasmic organelles were also present in the rostradorsal portion of the NRP. On these neurons numerous axo-somatic and axo-dendritic synapses could be identified. Although it is still hard at present to conclude whether each type of neuron observed electron microscopically corresponds exactly to the 5-HT neuron, the localization and cellular characteristics—size, shape and Nissl body distribution— of neurons of each type observed by electron microscopy closely resemble those of yellow fluorescent neurons shown by the Falck-Hillrp method. The fine structure of medium or large-sized neurons in the NRP is similar to that of the 5-HT neurons of the nucleus raphe dorsalis described by Fuxe *et al.* (1966). Furthermore, considering dense-packed distribution of yellow fluorescent neurons in the same area of the NRP, it is probable that the other type of neuron observed with the electron microscope also is the 5-HT neuron.

In the neuropil of the NRP numerous nerve endings containing different types of synaptic vesicles mentioned above were found. Intraventricular infusion of 5-OHDA resulted in the differentiation of two types of axon terminals (Fig. 4). One type contained 5-OHDA-labelled small granular vesicles (SGVs) about 50 nm in diameter which were regarded as catecholaminergic terminals (Richards and Tranzer, 1970). LGVs were found to coexist with SGVs in almost all terminals of the labelled type, and the electron density of such LGVs appeared to be enhanced markedly. The other type of terminal contained agranular vesicles with or without LGVs of which electron density of the contents showed no apparent change. The labelled axon terminals

Fig. 4. The neuropil of the NRP of a 5-OHDA-treated rat. 5-OHDA-labelled (▲) and unlabelled (△) axon terminals are shown. D, dendrite. $\times 16,500$.

were at most 5% of all terminals of the neuropil of this region. The labelled axo-somatic synapses were scarcely found. Their incidence seemed to be less than 1% of that of labelled axo-dendritic synapses. The presence of labelled axo-somatic and axo-dendritic synapses in the NRP well corresponds to the observations by histochemical method that green fluorescent terminals were abundant in the neuropil and near the neuronal somata of the NRP.

Fuxe and his associate reported that the intimate contacts of CA terminals with 5-HT neurons exist in B1, B2 and B3 groups in the medulla oblongata and B7 group in the mesencephalon. Most of these CA terminals are thought to be noradrenergic (Dahlström and Fuxe, 1964; Fuxe, 1965a, b). The demonstration of 5-OHDA-labelled synapses in the NRP in the present study may provide an electron microscopic evidence for the synaptic contacts between CA and 5-HT neurons. The possible role of CA inputs to the 5-HT neurons of the NRP is really not known. However, they may modulate the neuronal activity of the spinal neurons through these 5-HT neurons in the NRP, because the NRP neurons are considered to send axons down to the spinal cord (Dahlström and Fuxe, 1964; Fuxe, 1965a, b; Leichnetz *et al.*, 1978).

In the cell bodies of the NRP, no increase in the number of LGVs and no enhancement of the electron density of their contents were found after intraventricular infusion of 5-OHDA. Recently, coexistence of substance-P and 5-HT in some neurons of the NRP have

been demonstrated by immunohistochemical and/or radioautographical methods (Chan-Palay *et al.*, 1978; Hökfelt *et al.*, 1978). The presence of LGVs in the perikarya of the NRP neurons may correlate with both 5-HT and this neuropeptide. Further studies are required to clarify these points.

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