QUANTITATIVE ULTRASTRUCTURAL CHARACTERISTICS OF GABAERGIC SYNAPTIC TERMINALS ON NEURONS OF THE RETICULAR PORTION OF THE SUBSTANTIA NIGRA

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The reticular portion of the substantia nigra is one of the two most significant outputs of the basal ganglia to the structures of the pons and the diencephalon. The nigrothalamic, nigrotectal, and nigroreticular pathways use GABA as a mediator. In their turn GABAergic synaptic inputs from the striatum and the globus pallidus converge on the reticular portion of the substantia nigra. Thus, an important physiological process is effected at the level of the substantia nigra, the "inhibition of inhibition," or the disinhibition of the efferent influences of the basal ganglia. The first studies devoted to the description of the ultrastructural characteristics of GABAergic synaptic terminals on identified nigroretical [4] and nigroreticular [2] neurons have appeared recently. However, a detailed quantitative analysis of the various structural parameters of the synapses was not carried out in these studies.

The object of the present investigation was the study of the ultrastructural characteristics of the synaptic terminals on the nigrothalamic and unidentified neurons of the reticular portion of the substantia nigra.

The study was carried out on five adult rats. The standard methods of the fixation and embedding of the material in araldite or durcupan was utilized [1]. The nigrothalamic neurons were identified by the microinjection of a 10% solution of horseradish peroxidase into the ventromedial nuclei of the thalamus. The dorsolateral division of the reticular portion of the substantia nigra, in which collections of horseradish peroxidase-labeled neurons were identified in semithin sections, was selected for analysis. The synaptic terminals were tested for the presence of GABA in them by immunohistochemical staining using secondary antibodies conjugated with colloidal gold [1, 5]. Those instances in which the density of the disposition of the particles of colloidal gold per unit area of the synapse cross-section was not less than four times greater than the background level were considered positive.

In total 195 synaptic terminals were analyzed. Of these, 25 were found on horseradish peroxidase-labeled nigrothalamtic neurons. The type of contact, as well as the form, size, and character of the distribution of the synaptic vesicles, were the principal criteria of the division of the synapses into groups. According to the previously described classification [3], three groups of neurons can be distinguished in the reticular portion of the substantia nigra.

According to our data, 66% of the synaptic terminals belong to group I. They are characterized by symmetrical contact, polymorphic (elongation coefficient 0.83), agranular vesicles, which often occupied the entire area of the cross-section, imparting a dark coloration to it. The average sizes of the vesicles were 42.7 and 34.7 nm along the major and minor diameters, respectively. About 60% of such synapses were found on the distal dendrites.

The remaining synapses were distributed equally on the soma and the proximal dendrites; further, 63% of the synapses of this type were GABAergic (see Fig. 1, 1, 2). Comparison of the structural parameters of these group I synapses with the remaining 37% of the cross-sections of unclara neurochemical nature did not reveal differences in the size of the terminals, their form, the extent of the contact with the postsynaptic membrane, or in the size and form of the synaptic vesicles as compared with the entire population of synaptic terminals which we have classified as group I.

The group II synapses (29% of the total number) had an asymmetric type of contact. The round (elongation coefficient 0.94), agranular vesicles were distributed as a fairly small group, mainly close to the zone of contact (see Fig. 1, 3). The average sizes of the vesicles in this case were 34.7 and 32.1 nm along the major and minor diameters, respectively. Of these synapses 79% were located on the distal dendrites, 19% on the proximal dendrites, and only 2% on the soma of the neurons. The group II terminals were on the average larger and had a rounder form than the group I endings. In this group...
Fig. 1. Immunohistochemical demonstration of GABA in the axon terminals of the reticular portion of the substantia nigra. 1, 2) Group I GABAergic synaptic terminals on the body of horseradish peroxidase-labeled nigrothalamic neurons; 3) group II nonGABAergic synaptic terminal on distal dendrite. AT) axon terminal. Black dots) very fine particles of colloidal gold conjugated with secondary antibodies, which demonstrate the binding sites of anti--GABA serum. Labels are indicated by arrows. Calibration: 0.5 μm.

30% (11 out of 37) of the synapses investigated were GABAergic. Synapses with the asymmetric type of contact and small, round synaptic vesicles are traditionally classified as excitatory; this is apparently not always justified. The quantitative analysis of the structural parameters of the synaptic endings showed that the group II GABAergic synapses were larger in size, flattened in form, and contained larger vesicles than the remaining 70%, non-GABAergic synapses of the same group.

The group III synapses, with symmetrical contact and flattened vesicles, made up 5% of the total number. Somewhat more than 60% of the synapses of this type were also GABAergic.

The ratios of the synapses of the different types on the horseradish peroxidase-labeled nigrothalamic neurons mainly coincided throughout the entire population of cross-sections analyzed.

The data obtained make it possible to obtain a clearer notion of the morphologic basis of the process of disinhibition at the level of the substantia nigra. At the same time it should be noted that attempts to link the features of the ultrastructural organization of the synaptic terminals to their function without the necessary identification can lead to erroneous notions regarding the neurochemical organization and integrative processes in different brain structures.