NEW DATA ON THE FINE STRUCTURAL ORGANIZATION OF THE SUBSYNAPTIC ZONE OF THE NERVE CELL

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The fine structural organization of the subsynaptic zone of the nerve cell was studied by electron microscopy. This zone of the neuron was shown to be directly connected with special intracellular organelles or microtubules. Their peripheral ends, expanded like funnels, are in close contact with the postsynaptic membrane and apparently merge with its inner osmiophilic layer. These observations definitely show that direct structurally organized connections exist between the synaptic region of the neuron and the deep layers of its cytoplasm. A hypothesis to explain the functional role of these connections is put forward.

KEY WORDS: *neurons*; *synapse*; *postsynaptic membrane*; *microtubules*.

In the last two decades much progress has been made toward the understanding of the structure and physiological functions of interneuronal synapses, yet much still remains unexplained. This is particularly true of the subsynaptic zones of the neuron, with which ideas of the possible mechanisms of perception and subsequent processing of the information reaching the neuron through the synapses are naturally connected. Recent alternative explanations of this process simply confirm the existing view [1, 2]. It was accordingly decided to study some of the principles governing the structural organization of the subsynaptic zone of the nerve cell.

EXPERIMENTAL METHOD

Neurons of the ventral horns of the spinal cord of sexually mature albino mice were used as the test object. The material was fixed in solutions of glutaraldehyde and osmium tetroxide and embedded in a mixture of Araldite and Epon-812 resins. Sections were examined in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

After the well-known work of de Robertis [6] and Gray [7, 8], followed by the investigations of Taxi [14], Pappas et al. [10, 11], and others it gradually became realized that the subsynaptic zone of the nerve cell is a formation of complex construction. These workers found that special neurofibrillary or finely granulated structures, described by English-speaking workers as the "subsynaptic web," descend from the membrane into the depths of its cytoplasm. Often in this area structures of a special type — dense bodies, cisternae, etc, — can also be seen.

These morphological observations, demonstrating definite structural connections between the postsynaptic membrane of the nerve cell and the internal areas of its cytoplasm, strongly suggest that the action of mediators during synaptic transmission is evidently not confined to the membrane itself, and that the mediators themselves or some of their derivatives can also penetrate into the depths of the nerve cell. The possibility likewise cannot be ruled out that they may reach the center of the neuron containing the nucleus, at least the results of some experimental studies would appear to indicate that this is so [3, 9].

It is logical to suggest that some sort of drainage mechanism, enabling certain biologically active substances to be moved rapidly in the direction from the postsynaptic membrane into the depths of the nerve cell, must exist in the subsynaptic zone of the neuron.

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Fig. 1. Axo-dendritic synapse in neuropil of ventral horns of mouse spinal cord. Arrow points to microtubule beginning as a distinctive frontal-shaped structure from the postsynaptic membrane and running along a dendrite deep into the nerve cell; 190,500×.

In the modern view, the most probable mechanism of this type could be the method of transport of materials that is connected with special intracellular organelles or microtubules [4, 5, 12, 13]. However, to confirm this hypothesis, it was first necessary to show that the microtubules are in direct communication with the subsynaptic region of the nerve cell. Since these organelles proved to be unusually sensitive to the action of many of the factors most commonly used in histology, and readily disintegrated during such treatment, it was a long time before such communications could be reliably demonstrated. Now, however, a sufficient number of electron micrographs are available to enable it to be confidently concluded that the microtubules communicate directly with the subsynaptic zone of the neuron. Some of these micrographs have already been published [1], and in this paper new observations confirming and developing the ideas already put forward are given.

An ordinary axodendritic synapse from the neuropil from a ventral horn of the mouse spinal cord is illustrated in Fig. 1. In its presynaptic part (the axon terminal) a cluster of many circular, pale vesicles can be seen close to the membrane. It will be noted that one of them is depicted in the act of emptying its contents into the synaptic space. In the postsynaptic part of the dendrite, one of the microtubules which aims directly from the depth of the cytoplasm to the active zone of the synapse was well preserved. After almost reaching the postsynaptic membrane, this microtubule widens considerably and terminates in a distinctive funnel-shaped structure. The wide edges apparently rest on the membrane and fuse with the layer of osmiophilic material covering it.

This is thus the picture of a special organelle which communicates directly through the wide end (funnel) with the postsynaptic membrane, whereas the narrow end is continuous with a typical microtubule. This is evidence that direct structurally organized connections in fact exist between the subsynaptic zone of the nerve cell (in particular, its membrane) and the internal, deep layers of its cytoplasm.

A similar picture was repeatedly observed in our electron micrographs, not only in neurons of the CNS, but also in those of the peripheral ganglia (ganglia of the sympathetic chain).

These observations necessitated a more careful study of the fine structural organization of those parts of the subsynaptic zone of the nerve cell which are directly adjacent to the postsynaptic membrane. It was immediately found that, on disappearance (disintegration) of the microtubules their wide funnel-shaped terminal structures still remained as before, and they were easily found in the sections (Figs 1 and 2). They are undoubtedly the most stable

Fig. 2. Synapse of neuron from ventral horn of mouse spinal cord. Arrows point to system of special funnel-shaped structures on subsynaptic membrane; 144,400×.

formations of the subsynaptic region. The microtubules themselves, being unusually labile, appear and, in the writer's view, form a single combined entity with the funnel-shaped structures only under certain conditions. Very probably the narrow end of these funnel-shaped structures, stretching in the direction of the cytoplasm, under these conditions becomes the organizing center from which polymerization (self-assembly) of the microtubule from its precursor proteins is initiated. As a result of this process, in the writer's view, a direct pathway is created for the transport of biologically active substances from the subsynaptic membrane into the depths of the nerve cell. These funnel-shaped formations, incidentally, are found as a rule only in the active zone of the synapse, which they fill completely.

These investigations thus require substantial modification of previous views on the fine structure of the subsynaptic zone of the nerve cell. This zone is not simply a collection of fibrous or granulated material near its postsynaptic membrane, but it is a region of the neuron with a strict and orderly structural organization. It seems to the writer to be an agglomeration of regularly arranged and distinctive organelles, resembling a funnel in shape, which are closely connected by their wide ends with the subsynaptic membrane, whereas their other, narrow, end may under certain conditions be continued as a microtubule. Taken together, they form, it is considered, a special type of drainage mechanism, through which biologically active substances (mediators, their derivatives, or other substances connected with them functionally somehow) may be transported in the direction from the postsynaptic membrane of the neuron into its inner zones.

The principles of the structural organization of the subsynaptic region thus revealed suggests that a special mechanism for the oriented movement of mediators or of other biologically active substances connected with them functionally from the subsynaptic membrane into the depths of the nerve cell must exist in precisely this region of the neuron.

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