The Problem of Neuronal Syncytical Connection in Disease

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The membranes of neuron profiles adjacent to each other in the caudal mesenteric ganglion were examined electron microscopically during the first 3 days after crossing of the preganglionar or postganglionar branches. The contacting membranes were thinned and perforated, and neuron-to-neuron syncytial connections were forming. Multiple connections were formed between the nerve processes and terminals in the synapse. The pre- and even postsynapses were clear in pronounced perforations.

Key Words: *neuron-neuron syncytial bonds; membrane perforation; autonomic ganglion; degeneration*

Along with analysis of the cytoplasmatic organelles, nucleus, and common optical density of the neuroplasma, the integrity of the neurolemma focussed special attention of scientists in modern ultrastructural studies of pathological changes in the nervous system. These observations bring unexpected discoveries concerning the philosophy of the nervous system structure. It seems that chemical synaptic and contact electric relationships can be supplemented in case of disease by the formation of a new type of communication: cytoplasmatic syncytial connection (syncytium-cytoplasmatic relationship between two and more cells; the extreme variant of this relationship is cell fusion with the formation of a multicaryon (symplast). Another probability is direct fusion of nerve cells with the formation of hybrid cells. In 1968 C. Jacobson revealed that the green monkey neurons formed the syncytium and fused with fibroblasts. The probability of neuron-glia hybrid cell formation was also demonstrated later [8]. Due to unfolding studies on stem cell transplantation, the phenomenon of Purkinje's neurons fusion with bone marrow mesenchymal cells was discovered [9]. It was found that this capacity of Purkinje's cells to fusion was higher in type C Niemann-Pick mice with pathological changes of the cerebellum [11]. Fusion of the hemispheric cortical pyramidal cell dendrites with the microglia cells in viral infection was revealed [8]. Studies of AIDS infection revealed heretofore unknown fusion of many "brain cells" with the formation of multinuclear giant cells in encephalitis and dementia, in tuberous sclerosis, leukoencephalitis [13], and other diseases. Hence, it was persuasively shown that the neurons were in principle capable of forming the syncytium and fusing with cells of other types.

Results of ultrastructural studies of pathological changes in the nervous system indicate that the neuron-neuron syncytial relationship is an actual phenomenon of pathological changes in the neuronal membranes. It was detected in morphine intoxication [3] and brain hypoxia [5]. Overdosage of L-glutamate mediator became the cause of emergence of a syncytial bond between the frog cerebellar granular cells during an experiment [4]. The

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probable mechanism of measles virus propagation via the syncytial neuron-neuron communication was discussed [12]. Presumably, the formation of syncytial relationships and cell fusion in disease is a common biological cellular phenomenon, intrinsic of the neurons as well as of other cells.

The counts of syncitially linked and fused cells other than neurons constantly increases in various diseases [2,14]. The number of giant multinuclear cells increases during tumor formation. Methods for artificial cell fusion induction by excessive exposure to electric current [15] or pathological exposure to laser have been developed. Fusion of "brain cells" in viral diseases have been described previously [10,13]. It seems that the membranes acquire capacity to fusion and molecular instability, paralleled by perforations of fused membranes (metastable membranes) in many local and common pathological processes [7]. If these properties are common biological and manifest in many diseases, the emergence of the syncytial relationship can be expected in its most prevalent form (nervous system degeneration). We verified this hypothesis.

MATERIALS AND METHODS

Experiments were carried out on 10 cats in 2 series of experiments. In experimental series I all preganglionar nerves of the caudal mesenteric sympathetic node (CMG) and in series II the postganglionar nerves of this ganglion (hypogastric nerves) were crossed. The animals were sacrificed in accordance with ethical principles presented in the European Convention for Protection of Vertebrates used in Experiments. The blood system of animals was perfused by Ringer's solution and then by 2.5% glutar aldehyde solution in 0.1 M phosphate buffer (pH 7.2-7.4). Fragments of the ganglion were fixed in cold 2.5% glutar aldehyde solution during 1.5 h and then in 1% osmium tetroxide during 2 h at 4°C. The fragments of the ganglion were then dehydrated in 70%, 96% (twice), and 100% ethanol (three 20-min exposures). The material was then embedded in araldite [2] and incubated in a thermostat at 37°C and then at 56°C. The sections prepared with an ultratome were contrasted by lead citrate and uranyl acetate after Reynolds. For electron microscopy the ganglia were fixed during the first 2-3 days after neurotomy.

RESULTS

Changes in the neuroplasma of myelin-free autonomic nerve fibers have been described not once [1]: clear matrix, aggregation of synaptic vesicles, changes in the mitochondria, *etc.* All these signs of degeneration were present in many preparations (Fig. 1). We paid special attention to the unstable structural status of contacting membranes and emergence of perforations in them, particularly in the synapse zone. For example, a clear-cut violation of the structure of contact membranes, their multiple vesiculation were seen in the axosomatic synapse zone, under conditions of more or less normal compactness of the matrix and destruction of just solitary mitochondria on day 2 after the operation (Fig.

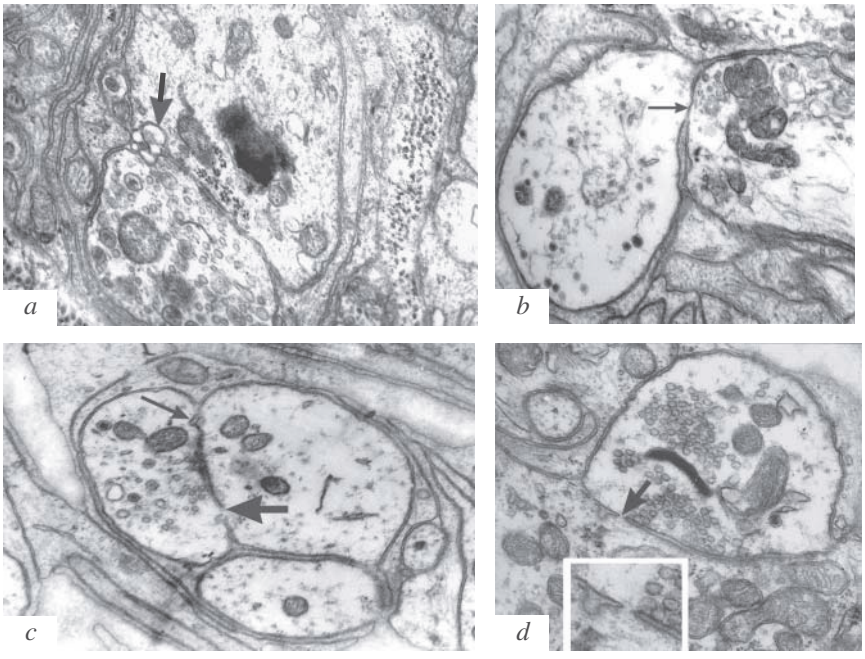

Fig. 1. Early reactive changes in contact membranes and their perforation. *a*) multiple vesicle-like protrusions in contact membranes (arrow); *b*) thinning of fused membranes (arrow); *c*) forming perforations of contact membranes (thick arrow); *d*) a solitary pore against the background of modified contact membranes (arrow); *a*, *b*) after crossing of preganglionar CMG branches; *c*, *d*) after crossing of postganglionar CMG branches. Magnification: *a-d*: 30,000; insert at *d*: 3-fold.

1, *a*). On the other hand, fusion of contacting membranes and their thinning were noted in clarification of the profiles' matrix (Fig. 1, *b*). Modified deformed contact membranes can be later lyzed, forming interneuronal pores (anastomoses; Fig. 1, *c*, *d*). Sometimes characteristic perforations formed in synapses with clarified profiles of presynapses and aggregations of synaptic vesicles; the edges of these perforations were formed by membranes which stuck to each other; residual membrane bodies could be detected in the perforation lumen (Fig. 2, *a-c*). The size of such an anastomosis is rather small (40-120 nm) and presumably not constant (Fig. 2, *a*, *b*), but proteins, toxins, viruses, *etc.* can penetrate through it. They can be multiple and fuse (Fig. 2, *d*). On day 3 after the operation the neighboring contact membranes can be largely destroyed, with just their fragments left at the interface of the pre- and postsynaptic profiles of the synapse (Fig. 2, *e*). Signs of degeneration were rather often seen in the postsynapse (matrix clarification, mitochondrial injury; Fig. 1, *c*, 2, *c-e*) after crossing of the presynaptic fibers. This can explain the well-known phenomenon of degeneration migration from the pre- to postsynapse. Numerous perforations of contact membranes in the synapse zone in the operated on animals is worthy of note. Their number and appearance differed significantly from the picture of perforated synapses in the control and in normal animals, in which such profiles were also sometimes detected, but were less incident, with normal electron density of their neuroplasm matrix [6]. Other authors also noted the syncytial relationship between the normal neuron structures [11]. Hence, our new data on the perforation of contact membranes in degeneration of presynaptic myelin-free fibers and synapses extend the range of the known structural signs, characteristic of degeneration of these structures, and supplement published data on the formation of the syncytial relationships between the neurons in disease. There are good grounds to say that degeneration of nerve structures is responsible for the regular emergence of this phenomenon in nervous system diseases.

It seems that membrane fusion in the form of close contacts is stage I of syncytial bond formation [7]. Similarity between mechanisms of membrane fusion and formation of close contacts was noted not once. The relationship between membrane fusion and the formation of contacts have been demonstrated in special studies [2] and was observed for cells other than nervous and for neurons [4]. The number of close contacts and incidence of cell

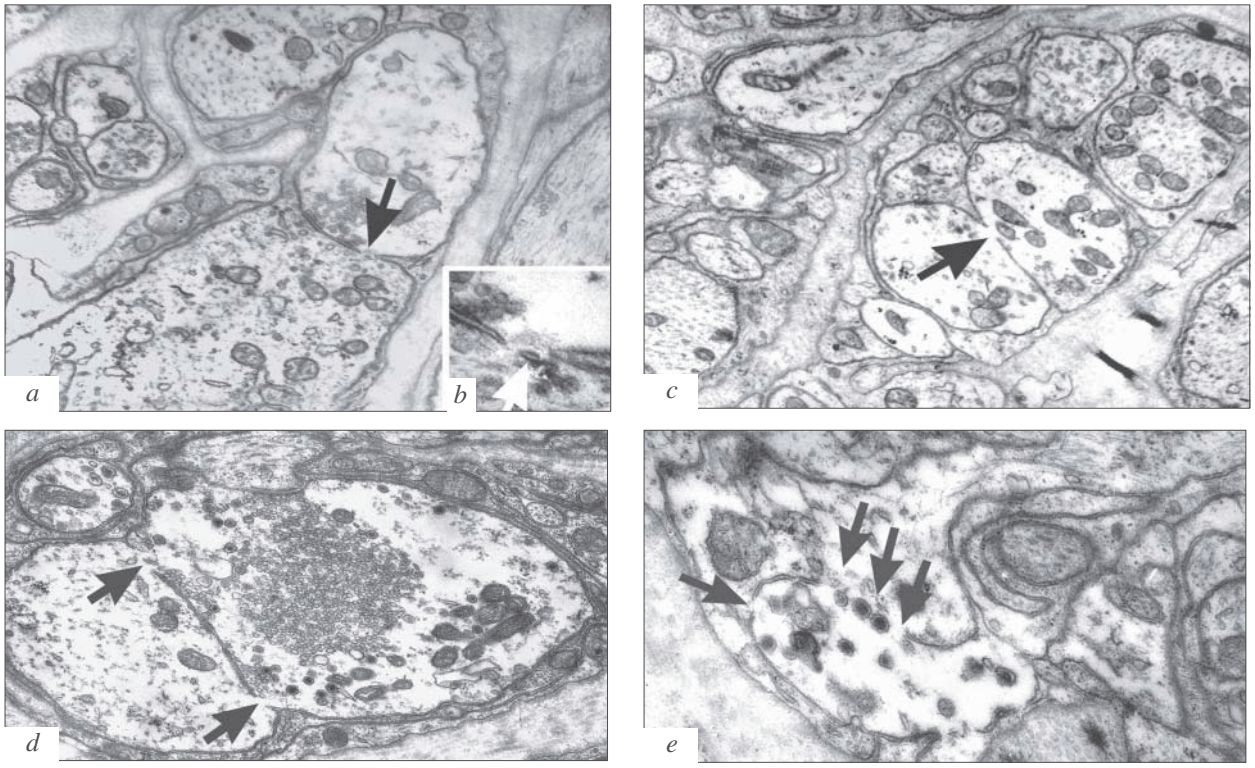

Fig. 2. Widening pores (*a-c*) and fusion of profiles constituting the synapse: beginning of the pre- and postganglionar fusion (*d*); complete fusion of the synapse profiles (*e*). *a*, *b*) after crossing of postganglionar CMG branches; *c*, *d*) after crossing of preganglionar CMG branches. Dark arrows: perforation of synaptic membranes; light arrow: residual membrane body. Magnification: *a*, *c*: 30,000; *b*: 40,000; *d*, *e*: 35,000.

syncytium formation increase in parallel under conditions of disease [14]. We showed previously that the number of syncytial bonds in the nervous system increased in disease; they formed on the base of thinned contacting, presumably fused membranes. The process is similar and characteristic of all cells, including the neurons; the problem of neurosyncytial bond is a part of the common biological problem. However this assumption is disputable as regards the nervous system. Many scientists think that the acknowledgement of neuronal theory by S. Ramon-i-Kajal and G. Valdeyer *vs.* the reticular theory (suggesting the syncytial structure of the nervous system) implies the absolute impossibility of the syncytium formation in the nervous system. However our data indicate that the formation of bonds between neurons in disease is not only possible, but justified. This conclusion is of paramount importance, as it means that a nervous disease is based on not only neuronal (cellular) organization of the nervous system, but partially on a principally different continuous cytoplasmatically related system of neurons. This implies a different essence of pathophysiological manifestations of injury. Summation of subthreshold membrane potentials and their transformation into extra (pathological) pulses, synchronization and uncommon increase in the amplitudes of action potentials or emergence of unnatural cyclic reverberation are probable for syncytially bound neurons. Hence, the problem deserves attention and further studies.

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