PHYSIOLOGY

MICROVESICLES IN DEVELOPING SYNAPSES

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During formation of the membranous components of developing synapses new morphological subunits, of still uncertain nature, are revealed. These structures are known as microvesicles [2]. They differ from the other vesicular structures of developing synapses (synaptic vesicles, growth cone vesicles, etc.) by their minimal size, their location, and their structure. The aim of the present investigation was to determine the ultrastructural characteristics of the microvesicles and to discuss their possible role in synaptogenesis.

EXPERIMENTAL METHOD

The sensomotor cortex and caudate nucleus of embryonic (14-22 days) and newborn rats were studied. Pieces of brain from embryos were obtained by the method in [1]. Newborn animals were killed by decapitation without preliminary anesthesia. After craniotomy the brain was fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.2-7.4, for 24 h at 4°C. The pieces were then removed, washed in phosphate buffer, and postfixed in a 2% solution of osmic acid for 2 h, dehydrated in alcohols of increasing concentration, and embedded in Epon 812. The material was photographed in a "Hitachi" H-600 and H-11E electron microscope. The diameter of the microvesicles was measured on electron micrographs by means of a Tesla RMD85 computer, using a program written at the Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Czechoslovakia.

EXPERIMENTAL RESULTS

Two types of microvesicles were found: those with a smooth outer surface (SMV) and those with a rough surface (RMV; Fig. 1). The SMV were round and the RMV oval or rectangular in shape. The diameter of both types of microvesicles was 10-20 nm, but the diameter, for example, of synaptic vesicles is 40-50 nm. The RMV were attached to the outer surface of one or both contacting membranes to form a chain in which a free surface of presynaptic membrane remained between individual microvesicles. RMV were detected only in the period of formation of the mixed synapse, i.e., at the time when membrane specialization of symmetrical or asymmetrical junctions was beginning to form from a desmosomelike junction. The number of RMV therefore depends on the degree of differentiation of the membranes. The greater the area occupied by the specialized membrane, the more RMV are formed. However, no RMV were found in a polarized synapse.

A different picture was observed with SMV. They first appeared in the region of membrane specialization of an undifferentiated junction, and with the appearance of the first synaptic vesicles their localization became very varied. Often they formed focal concentrations of three to ten vesicles, located near the presynaptic membrane or in the axoplasm of a presynaptic terminal. In the latter case, SMV could be single. They could be observed in the synaptic cleft. An important fact is that SMV in the presynaptic outgrowth may be connected with other structural units. For instance, they could be found on the outer surface of agranular and granular synaptic vesicles. In the latter, SMV were frequently arranged around

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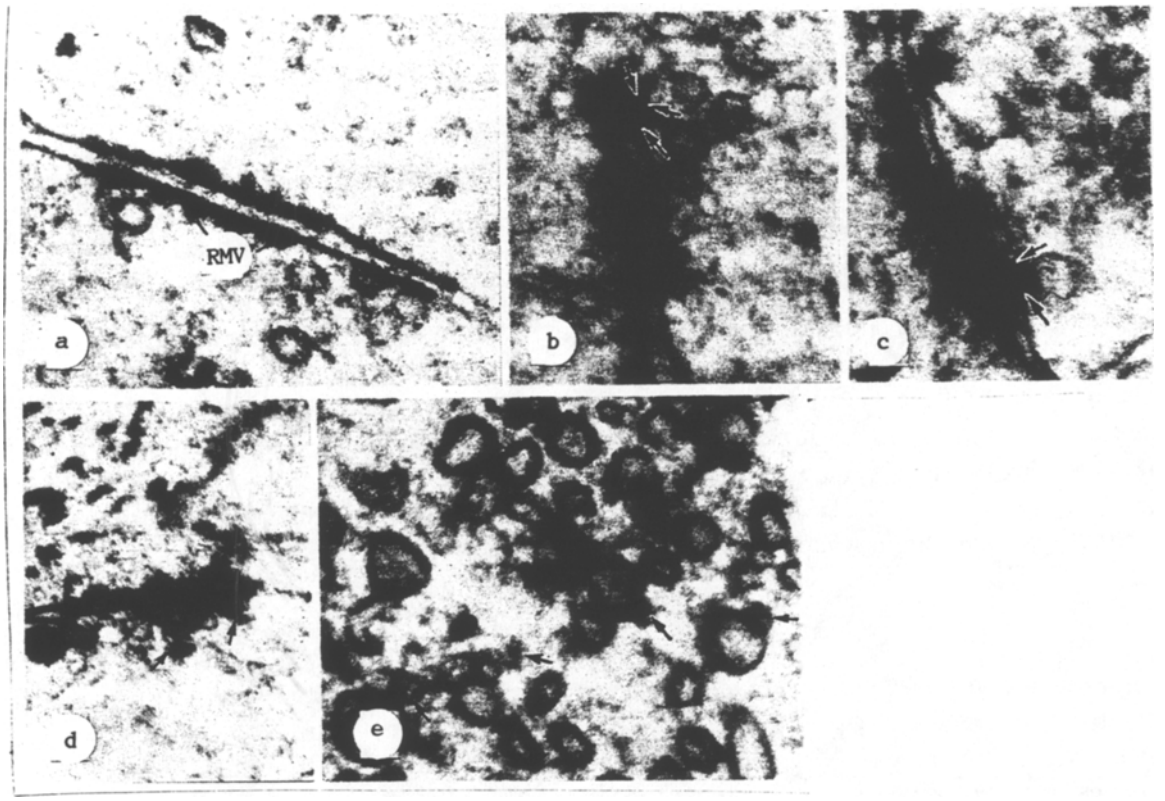

Fig. 1. Microvesicles in synaptic endings of sensomotor cortex and caudate nucleus of newborn rats: a) RMV, 49,400×; b-e) SMV (arrows): in region of presynaptic membrane (b), 90,000×; between presynaptic membrane and synaptic vesicle (c), 90,000×; resembling a bunch of grapes (d), 41,600×; on surface of synaptic vesicles (e), 90,000×.

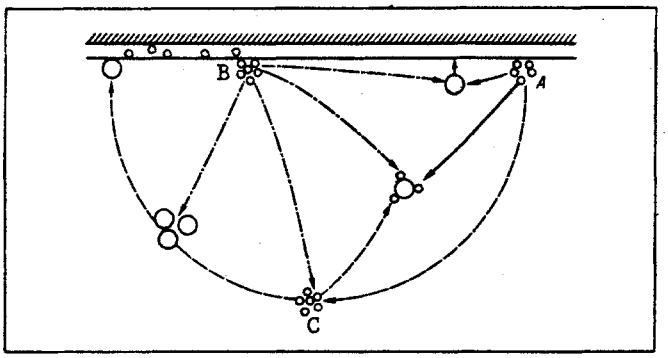

Fig. 2. Possible involvement of SMV in mediator secretion by developing synapses. A) Embryonic form of synaptic vesicles, B) inactivated form of synaptic vesicles, C) mixed (embryonic) and/or inactivated form of synaptic vesicles.

a dense rod. The number of microvesicles attached to the surface of the synaptic vesicles varied from one to three, but could probably be even greater. In addition, a small group of SMV was found firmly applied to the presynaptic membrane on one side and to the synaptic vesicles on the other side. Less frequently SMV were seen in the zone of postsynaptic thickening of the junction, and also in the neuron soma in the immediate vicinity of the cytolemma.

It thus follows from the results of this investigation that SMV and RMV differ from each other not only morphologically, but also in their location. RMV are found only in the region of differentiating membranes of the synapse during its development. SMV have a wider spectrum of localization. They were found in the cytoplasm of the neuron, near the

presynaptic membrane of the synapse, in the axoplasm, and on the surface of synaptic vesicles. Hence it follows that their functional significance is different. RMV, formed as a result of twisting of the fibrillar threads of the desmosomelike junction [2], evidently later become inserted into the developing synaptic membranes and assist in their condensation. The possibility likewise cannot be ruled out that RMV serve as a barrier preventing interaction of synaptic vesicles with the presynaptic membrane. When the synapse is functionally active, a redistribution of RMV may perhaps take place with the formation of a smooth surface or of channels where complementary interaction takes place between membranes of the synapse and the synaptic vesicles, and mediator is released. Another possibility is that RMV, on the other hand, may help to secure the synaptic vesicles to the presynaptic membrane on account of the contractile proteins of this system [5].

As regards the functional role of SMV it can be tentatively suggested that they are connected mainly with mediator secretion in the synapses (Fig. 2). In particular, they can be regarded as an inactivated or embryonic form of synaptic vesicles which, when filled with mediator, are converted into mature synaptic vesicles. It can be tentatively suggested that some SMV are filled with mediator actually on the presynaptic membrane and are converted into synaptic vesicles ready for secretion (Fig. 2A). Meanwhile, those SMV which lie freely in the axoplasm, and also those attached to the surface of the synaptic vesicles, can evidently receive mediator from the axoplasm or from these vesicles themselves, in which case they are converted into mobilizable synaptic vesicles, which approach the presynaptic membrane and release mediator into the synaptic space (Fig. 2C). As a result of exocytosis SMV are formed, and constitute the inactivated form of synaptic vesicles (Fig. 2B). Some of these vesicles return by means of the retrograde flow of axoplasm into the cytoplasm of the cell, and there they are replenished with mediator. Thus transformation of the SMV into synaptic vesicles and back again into SMV is observed, like the mechanism of renewal of synaptic vesicles [4]. The more SMV are located near the presynaptic membrane and in the cytoplasm of the axon, the more intensively the process of formation of synaptic vesicles develops and the higher the degree of functional activity of the synapse. It can also be postulated that SMV are structures in which acetylcholinesterase is concentrated, and that the concentrations of SMV which we observed near the presynaptic membrane are evidently acinous form of acetylcholinesterase, as revealed electron-microscopically [3].

The heterogeneous structure of the microvesicles of developing synapses is thus evidently a feature of their functional specificity at certain periods of synaptogenesis [6].

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