MICROTUBULES AND THEIR INTERRELATIONS WITH SUBSYNAPTIC STRUCTURES AND THE NUCLEAR MEMBRANE IN HUMAN NEUROBLASTS

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The dynamics of appearance of microtubules and the development of their connections with synapses and the nuclear membrane was studied by electron microscopy in differentiating neuroblasts from the anterior horns of the human spinal cord during the first half of antenatal development. A hypothesis is put forward to explain the function of the connections discovered between the microtubules and subsynaptic zone of the neuroblast and its nucleus.

KEY WORDS: microtubules; synapse; nucleus of the neuroblast; spinal cord; human prenatal development.

It was observed previously that after the appearance of synaptic contacts on developing neuroblasts their functional maturation and differentiation are considerably accelerated [5, 6, 8]. These processes are known to be accompanied by profound changes in the internal cell organization and, consequently, they are connected with increased synthesis of enzyme and structural proteins in the cell, i.e., in other words, with high activity of its genetic apparatus. It was accordingly suggested that synaptic interneuronal contacts have a stimulating influence on the activity of the genome of the developing nerve cell.

At present, however, hardly anything is known about the nature of this stimulating factor or of the ways and methods of its transport to the cell nucleus. If it is assumed that this factor is a substance formed in the synaptic zone of the nerve cell, the most likely explanation would be that it moves toward the nucleus through the participation of special cell organelles (microtubules), whose role in the rapid and oriented intracellular transport of materials is becoming increasingly more evident [7, 9, 10]. Direct connections between microtubules and the subsynaptic zone in mature neurons have been discovered on more than one occasion [1-4, 11]. However, it is not yet known how they form in the developing neuroblasts, or when and how direct relations are established between the microtubules and subsynaptic region of the nerve cell and also with its nucleus, although knowledge of these processes is extremely essential for the understanding of the mechanisms of development and differentiation of neurons.

In the investigation described below the dynamics of appearance of microtubules and of development of their connections with maturing synapses and the cell nucleus was studied with particular reference to neuroblasts of the human spinal cord during the first half of prenatal development (the 8th-21st weeks of pregnancy).

EXPERIMENTAL METHOD

Material for the investigations was obtained after abortions carried out in gynecological clinics in Moscow. Altogether 32 human embryos and fetuses aged from 8 to 21 weeks of gestation were studied by electron microscopy. Their age was determined on the basis of clinical and morphological data.

EXPERIMENTAL RESULTS

Microtubules were clearly found in developing neuroblasts from the anterior horns of the human spinal cord in 8-week embryos. They must certainly have been there also at earlier stages of intrauterine development, but they were difficult to detect because of their extreme lability and their instability toward many of

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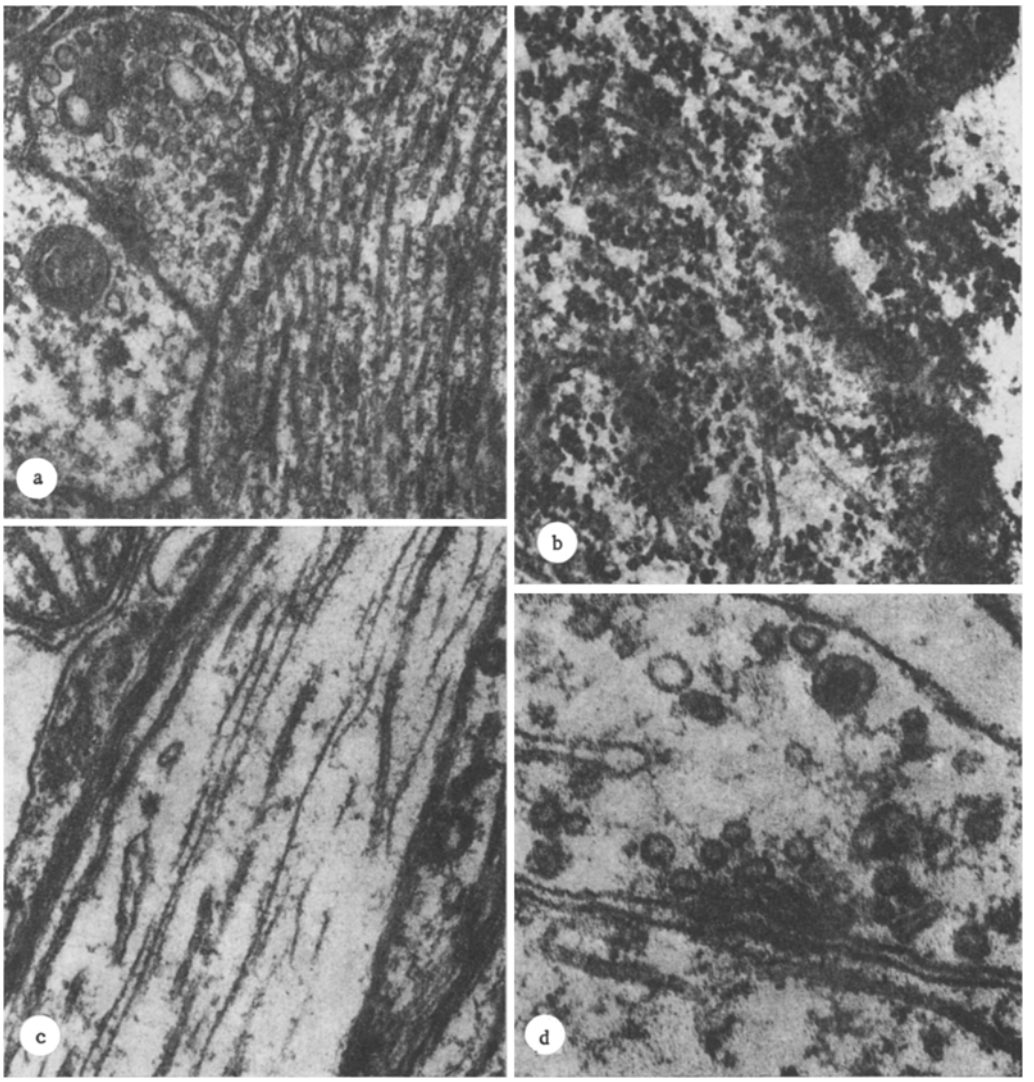

Fig. 1. Microtubules (arrows) in developing neuroblasts from anterior horns of brachial portion of spinal cord of 12-week (a, b) and 21-week (c, d) human fetuses. Magnification: a) 38,500×, b) 52,500×; c) 67,800×, d) 105,800×.

the factors arising during histological preparation of nerve tissue. This is evidently the reason for the scarcity of information in the literature on these organelles in developing nerve cells.

At the 8th week of prenatal development, as also at later stages (9-12 weeks), microtubules were found in the cytoplasm of the body of the neuroblasts and also in their growing dendrites and axons. In the latter they formed well-developed bundles, usually filling their entire thickness (Fig. 1a). They are "packed" particularly densely in the thin branches of the growing axons. In this region, along the microtubules, accompanying vesicles of varied caliber could frequently be seen, some of them indistinguishable from synaptic vesicles in their structure.

Microtubules were located less densely in the dendrites than in the axons, but there too they were also arranged longitudinally and were sometimes accompanied by vesicles and by small electron-dense granules.

In this early period of ontogenetic development of the neurons microtubules were found exceptionally in the cytoplasm of the growing axons and dendrites (Fig. 1a). No other similar structures and, above all, microfilaments could be found.

In the later period of development (18-21 weeks), when growth of the axons and dendrites was slower and, in some neuroblasts, was largely complete, the number of microtubules in them was considerably reduced and it appeared that they were beginning to be replaced by microfilaments (Fig. 1c). This process is evidently con-

nected with the fact that in the differentiating neurons the need for structural and enzyme proteins to be transported to their growth cones was naturally reduced after the end of the period of rapid growth of their axons and dendrites and, consequently, there was no longer any need for the well-developed microtubular transport systems that are characteristic of the earlier stages of ontogenetic development of the nerve cell.

Microtubules appeared in the cytoplasm of the body of the neuroblasts from this region of the spinal cord at the same times as in the axons and dendrites and the pattern of their development was most marked in fetuses aged 10-12 weeks. At that time microtubules ran through the cytoplasm of the cell body in large numbers, sometimes singly, sometimes in small bundles. Very often they ran in the immediate vicinity of organelles such as mitochondria and Golgi complexes. They always appeared to surround the Golgi complexes, where they were in close contact with the vesicles formed in them.

To understand the processes of development of the neuroblasts, relations between the microtubules and nuclei are important. The electron micrographs frequently showed how microtubules ran toward the nucleus of the neuroblast from all sides and in large numbers. Many went right up to the nucleus and joined the nuclear membrane with their slightly expanded end, most frequently by the nuclear pore itself (Fig. 1b).

The function of these connections has not yet been adequately studied. One thing is certain, however: if the microtubules in fact serve as a transport system in the cell, their attachment to the nuclear membrane by the nuclear pores would be most in harmony with this function.

Particular efforts were made to study the dynamics of relations between microtubules and synaptic structures developing during synaptogenesis. Having studied many electron micrographs on which the microtubules were seen most clearly, the writers concluded that in all probability they connect with the subsynaptic zone only in the final stages of synaptogenesis, when the synapse, to judge from the morphological picture, reaches a high degree of maturity.

One of the initial stages of synaptogenesis when the growth cone of the axon, although it had not yet lost its characteristic features (groups of vesicles of different caliber – the so-called mound area, glycogen granules, and so on), forms a synaptic contact with the dendrite of a neuroblast is shown in Fig. 1a. The concentration of typical synaptic vesicles in the active zone of this contact, in close apposition with the presynaptic membrane, and the clear asymmetrical thickening of the opposed membranes are evidence of its functional competence.

At the same time, however, the growing microtubules have not yet reached the synapse. This can be concluded with certainty, for in the section these organelles were well preserved on fixation. This is shown by the presence of well-developed bundles of microtubules running through into a cell process located alongside the synapse (Fig. 1a).

Even in the later periods of synaptogenesis, when in its morphological picture the synapse appeared to correspond completely to mature forms, the microtubules still did not reach it. Only in the latest stages of this process, when all the morphological features of the functionally mature interneuronal contact had been acquired, did the growing microtubules gradually begin to reach the synapse (Fig. 1d). Their end facing the postsynaptic membrane under these circumstances widened into a funnel, the edges of which merged with the osmiophilic substance on the inner surface of the subsynaptic membrane. The opposite end of the microtubule sank into the depth of the cell cytoplasm in the direction of its center, containing the nucleus. With the establishment of direct connections between these organelles and the subsynaptic zone the process of functional maturation of the interneuronal synaptic contact can be regarded as complete.

By studying the ontogenetic development of nerve cells in the anterior horns of the human spinal cord it was thus shown that their special organelles – microtubules – establish direct connections with cell structures such as synapses and the nuclear membrane. This suggests that rapid and oriented transport of biologically active substances from the subsynaptic region of the neuroblast toward its nucleus can take place through the participation of these organelles.

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EFFECT OF VAGOTOMY ON MORPHOLOGY AND FUNCTION OF THE ADRENAL MEDULLA IN RATS

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Marked dilatation of the venous sinusoids, a decrease in area of the chromaffin tissue, and a stronger chromaffin reaction in the adrenal medulla were found 7 and 45 days after bilateral subdiaphragmatic vagotomy in male albino rats. Considerable changes were found in the ultrastructural organization of the secretory cells and endothelium. Injection of glucose into these animals caused only slight fluctuations in the adrenalin content in the chromaffin cells.

KEY WORDS: vagotomy; adrenals; chromaffin tissue; hyperglycemia.

Previous investigations showed that bilateral subdiaphragmatic vagotomy depresses functional activity of the insulin system of the pancreas [1] and of the adrenal cortex [3]. The object of this investigation was to study the effect of bilateral subdiaphragmatic vagotomy on the adrenal medulla.

EXPERIMENTAL METHOD

Altogether 90 male albino rats weighing 120-140 g were used. Under ether anesthesia bilateral subdiaphragmatic vagotomy was performed on the animals. The rats were killed 7 and 45 days after the operation after starvation for 24 h and 1, 2, 3, and 6 h after administration of 20% glucose solution by gastric tube in a dose of 2 g/kg body weight. Glucose was chosen because of the need to synchronize the secretory process of the chromaffin cells of the adrenal medulla to some degree, for hyperglycemia inhibits catecholamine secretion. At each time of the investigation five control and five experimental animals were studied. The adrenal medulla was studied by Yaglov's combined histochemical method [2], whereby adrenalin and noradrenalin can be detected simultaneously in the chromaffin cells, unsaturated phospholipids in the adrenocorticocytes, and DNA in the cell nuclei. To compare the areas of the chromaffin tissue and venous sinusoids of the adrenal medulla in the control and vagotomized animals, a gravimetric method was used, with sections obtained from the middle part of the organ. The adrenalin and noradrenalin content was judged from the intensity of the chromaffin reaction. Material for electron-microscopic investigation was taken from three experimental and three control animals at each time.

EXPERIMENTAL RESULTS

In all experimental animals considerable dilatation of the venous sinusoids was observed 7 days after the operation, amounting to 92%. Homogeneous contents were seen in their lumen and stasis of erythrocytes, giving a positive reaction for catecholamines, was observed. The area occupied by the chromaffin tissue was 20.8% smaller than in animals of the control group. Analysis of the state of the chromaffin cells in the experimental animals showed that most cells in the medulla were in the phase of accumulation of secretion and gave a strong chromaffin reaction. Vacuolation of the cytoplasm was observed in many noradrenocytes and some adrenocytes. Administration of glucose caused changes in the catecholamine content in the chromaffin cells

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