SYNAPTOGENESIS IN THE ANTERIOR HORNS OF THE SPINAL CORD OF HUMAN EMBRYOS

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UDC 611.822.1-018.83-013

The beginning of synaptogenesis is one of the most critical periods in the development of the nervous system, for with the appearance of the first synaptic junctions between differentiating precursors of nerve cells its formation as a system truly begins. It is at this period that the first of its functional interneuronal junctions, which significantly affect the prospective development both of the CNS itself and of the organism as a whole, appear. For that reason a careful and systematic study of processes of synaptogenesis, especially in the early period of development of the nervous system, provides an essential basis for the understanding of the mechanisms of its formation and maturation. Furthermore, since the organism is exceptionally sensitive to the action of various harmful factors on the formation of its nervous system at this critical period of ontogeny, when the first synaptic junctions are formed, it will be clear that determination of exact times of the synaptogenic period in human ontogeny is of considerable practical importance, for this is one of the important conditions for the development of scientifically based programs for protection of the health of the future generation. On this assumption, it was decided to undertake a systematic study of processes of synapse formation on differentiating nerve cells in early prenatal human ontogeny. The writers showed previously [1] that synaptogenesis in the anterior horns of the brachial region of the spinal cord is already distinctly observable in human embryos at the 7th-8th week of intrauterine life. This fact suggests that the synaptogenic period in this particular region may evidently have a much earlier beginning.

Almost simultaneously with our own observations on synapse development, a similar study of the same region of the spinal cord in human embryos was carried out by Japanese workers [2]. In their material the earliest manifestation of synaptogenesis was found in 14-mm human embryos, which compared with those which we studied belonged to a rather later age of antenatal development.

EXPERIMENTAL METHOD

Two 6-week human embryos (crown-rump length 13 mm) were studied by electron microscopy in the usual way. To study structures of the embryonic spinal cord sections were stained by Nissl's method.

EXPERIMENTAL RESULTS

During light-opitcal study of transverse sections through the brachial region of the spinal cord in the 6-week human embryos the central canal was clearly visible, stretching widely in the dorsoventral direction. It was surrounded by a stratified layer of ventricular neuroepithelium which, by this time, is considerably reduced in the ventral half of the spinal cord compared with the alar half, and mitoses in this region are seen relatively rarely.

In the anterior horns separation of the cells into two future motor nuclei — lateral and medial — is hardly visible. In this region, in its ventrolateral areas, quite large developing nerve cells can be distinguished, with the characteristic pale cytoplasm, surrounding the large oval nuclei in a narrow border. These cells are usually arranged in groups, most frequently at the boundary with the marginal layer, but they may also penetrate relatively deeply into the layer.

On electron micrographs the cytoplasm of the cells appears translucent; it envelops the long, oval, or round nucleus, with its dispersed chromatin, in a narrow layer and contains a

KEY WORDS: synaptogenesis; embryonic spinal cord; early prenatal human ontogeny.

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 0007-4888/81/9104-0546$07.50

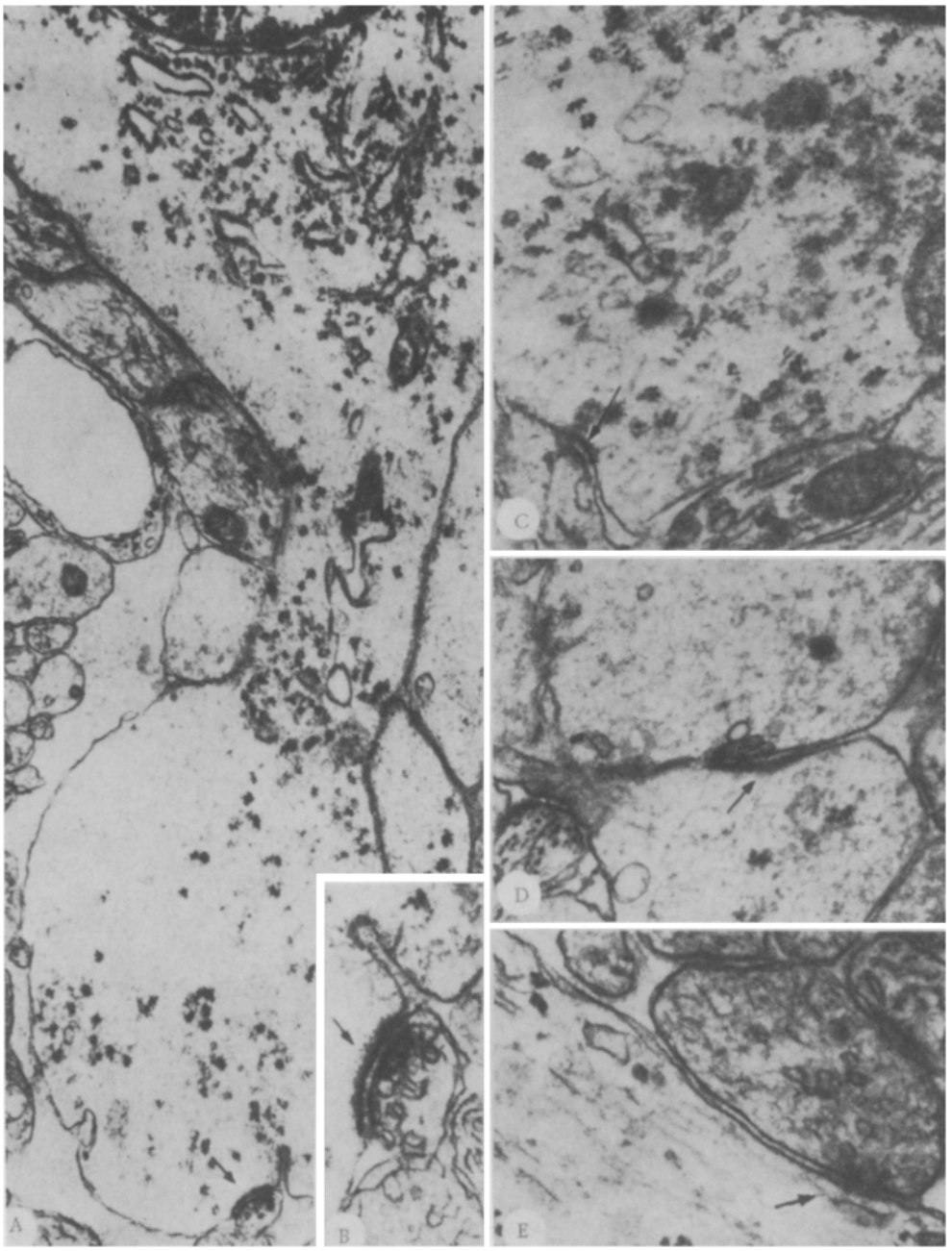

Fig. 1. Synaptic junction (arrows) on developing nerve cells and their processes in anterior horns of brachial segment of spinal cord of 6-week human embryos. Magnification: A) 25,000; B) 62,500; C) 42,000, D) 50,000; E) 57,500×.

very few ordinary cell organelles. However, ribosomes are located here mainly in the form of polysomal complexes. Scattered throughout the cytoplasm, they are concentrated in larger or smaller numbers only near the distal pole of the nucleus, and in this same region of the cell the first cisterns of its rough endoplasmic reticulum begin to form (Fig. 1A).

The perinuclear cytoplasm of these cells changes without visible boundaries into a wide (leading) cell process, running radially through the marginal layer toward the periphery of the spinal cord, to the region of its anterior roots. In some cases it could be seen how such a process, narrowing sharply, changed into the typical profile of an axon, entering the system of root fibers.

On the surface of the cells described above morphologically fully developed synaptic junctions were constantly found. They were located most frequently either on the cell body or on the proximal segments of its processes, not far from the nucleus (Fig. 1A, C).

As a rule these synapses were asymmetrical, with clearly defined subsynaptic membrane specializations characteristic of them. Under high power, distinctive structures, considered to be specific receptor formations, connected with the postsynaptic membrane could be seen in their slightly widened synaptic space and penetrating into its lumen.

In the presynaptic region of these junctions well-marked differentiation of the membranes also took place, as shown particularly by the formation of conical condensations resembling the characteristic Gray–Akert presynaptic ending, and this, in the writers' opinion, is definite evidence of the sufficiently high degree of functional maturity of these synapses. At this age as a rule only a small concentration of synaptic vesicles is observed in the active zone of their presynaptic profiles. Usually they are transparent, round, and relatively small vesicles (Fig. 1A, B, E), but in some cases definite polymorphism was observed, when together with synaptic vesicles as described above there were also fairly large, pale vesicles and also others with a dense core (Fig. 1D).

Axon profiles participating in the formation of the synapses described above may be of two types: Some, of small diameter, run through, united into whole groups, whereas others, on the other hand, are of very large caliber and often run singly. The former evidently belong to the system of axons of true spinal interneurons [3], whereas the latter are probably of different origin. Very possibly they are formed by axon processes of primary sensory neurons, which at that time begin to reach the region where differentiating motor nerve cells are located.

Besides asymmetrical junctions, in this region of the spinal cord so-called symmetrical forms also were observed, with morphologically indistinct specialization of their postsynaptic membrane. Our observations showed that they lie mainly in the depth of the marginal layer, on different types of cell processes, some of which, especially on longitudinal section, were easily identified as leading processes of developing nerve cells (Fig. 1E).

All interneuronal synaptic junctions described here, from the presumptive anterior horns of the spinal cord of 6-week human embryos, on the basis of their principal morphological parameters can be classed definitely as functionally competent synaptic forms; consequently, these interneuronal junctions must be already capable of transmitting a certain quantity of information from cell to cell. This naturally suggests a possible functional role of these synaptic junctions even at this early period of development of the nervous system in the human embryo.

It is of course difficult to give a definite answer to this question until fundamental investigations have been made of the developing activity of the nervous system in such embryos. Nevertheless, considering that in human embryos of the age studied no signs of spontaneous or reflex motor activity have yet been found [4], it can be tentatively suggested that these synapses are evidently not yet intended for direct participation in nervous activity, and that they must perform some other role. It is possible that these primary synaptic junctions transmit oriented influences responsible for the differential development of future nerve cells.

Transsynaptic influences are known to exert a profound effect on the cell, by causing definite changes in the differential activity of its genome and, consequently, transforming ultimately its entire morphological and functional state. The appearance of the first synaptic junctions on the prospective nerve cell must therefore be a critical stage in its development — the beginning of its conversion from neuroblast into functionally competent young neuron. Since later it is under the continuous and ever-increasing influence of an unbroken flow of specific information reaching it through these synapses, this young nerve cell gradually becomes prepared or, more expressively speaking, "trained" for its future special and strictly determined activity in the created functional systems of the developing human organism.

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