HUMAN BRAIN SYNAPSES DURING EARLY PRENATAL DEVELOPMENT

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The principles governing development of the human brain, especially in the early period of its antenatal ontogeny, have recently assumed particular importance. The undoubted key factor in individual formation of the human brain is early synaptogenesis, which leads to the appearance of the first neuronal nets, responsible for the formation of the specific functions of this developing organ. However, the process of formation of synaptic connections in the presumptive human brain and, in particular, in its cerebral cortex, has received very little study. We could find only one reference [1] in which true synaptic interneuronal junctions were described in the cerebral cortex of human fetuses, the earliest of which was aged 9 weeks.

The aim of this investigation was to study synaptogenesis in the cerebral cortex of human embryos at an earlier stage of intrauterine development, before any true synapses are considered to exist [3, 4].

EXPERIMENTAL METHOD

The investigation was carried out by methods of conventional electron microscopy on six human embryos aged from 6 to 8 weeks of intrauterine life. The age of the embryos was determined on the basis of morphometric data. All the embryos studied were obtained from clinically healthy women by artificial termination of pregnancy.

EXPERIMENTAL RESULTS

In the modern view [2] the wall of the cerebral vesicle in the region of the presumptive cerebral cortex in man, as in other mammals, consists of only one layer of elongated cylindrical cells which, together, have been called the germinative, matrix, or ventricular neuroepithelium. The apical ends of these cells face the cavity of the brain vesicles (primary ventricles), and their elongated basal segments, in close contact with each other by their terminal pedicles, form the outer or pial surface of the developing brain, separated from the surrounding mesenchyme by a clearly defined basal membrane. The nuclei of the matrix cells lie at various distances from their apical end, to give the impression of pseudostratification of the ventricular neuroepithelium.

During subsequent development of the brain the basal segments of the neuroepithelial cells, which grow rapidly in length, form as if it were a second superficial layer, not containing nuclei, in the wall of the brain vesicle which has been called the marginal layer. According to our observations, this is already clearly visible in electron micrographs of the region under investigation by the end of the 2nd month of intrauterine life of the human embryo. It is this marginal layer which, soon after its formation, begins to be colonized from the germinative zone by the first cells of the neuronogenetic series, which give rise to primary neurons of the developing cerebral cortex. This event in man, according to our data, takes place not later than the 6th week of its antenatal ontogeny, for in electron micrographs of this region obtained from human embryos at the age of 6-7 weeks this process is in full swing [1]. By the same time, the first tangential nerve fibers are penetrating into the marginal layer, and some of them run toward the first young neurons of the cerebral cortex — the Cajal—Retzius cells. These nerve fibers run right up to them, spread out over their bodies and processes, but form morphologically distinguishable interneuronal junctions only occasionally, in one or two places. By their structural organization these initial

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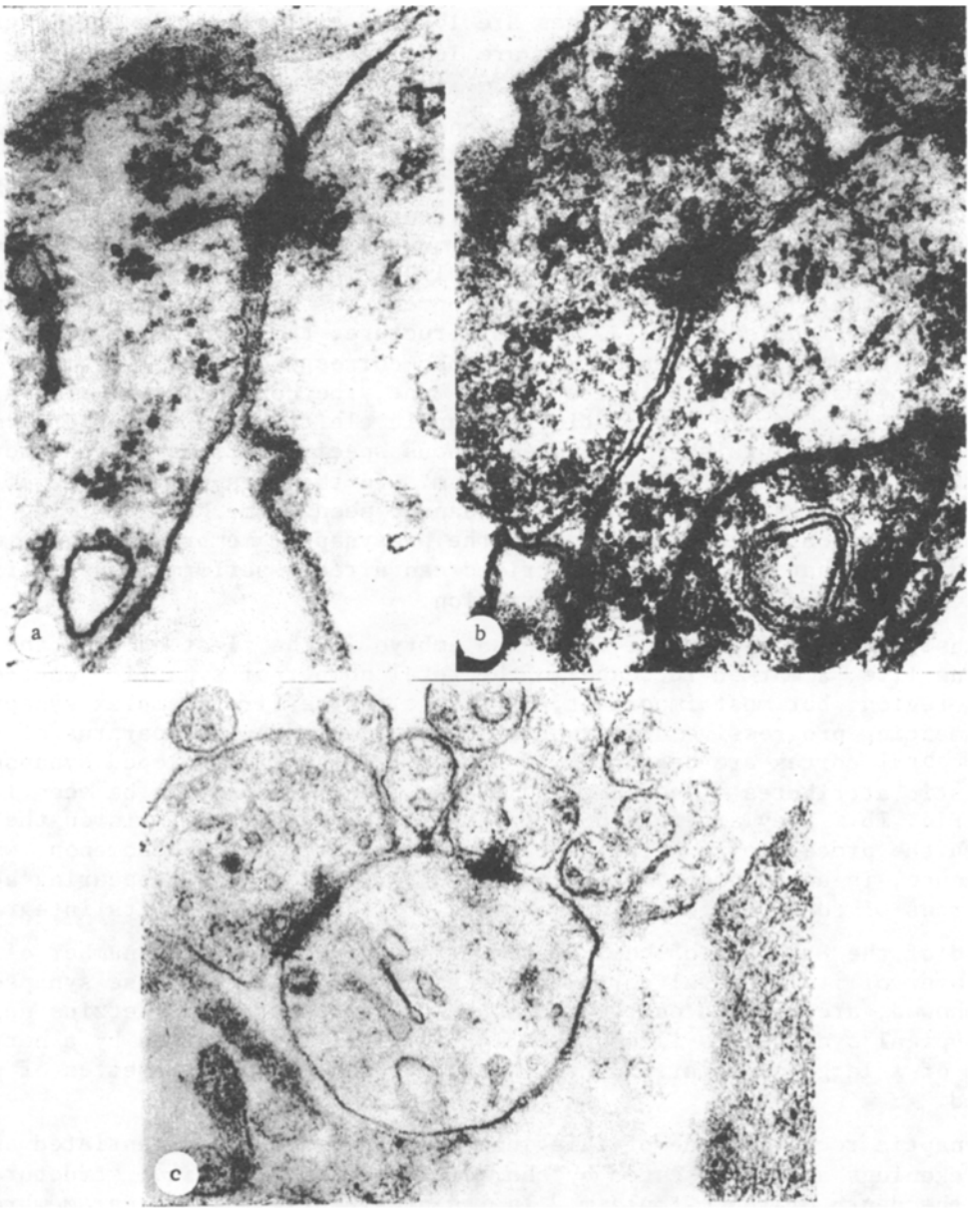

Fig. 1. Interneuronal synapses in marginal layer of developing cerebral cortex of human embryos aged 7 (a) and 8 (b, c) weeks of intrauterine life. Magnification: a) 60,000, b) 64,000, c) 40,800×.

junctions resemble in many of their features the structure of interneuronal synaptic connections, and this is particularly true in their receptor (subsynaptic) zone. However, unlike true synapses, these junctions, which are generally called synaptoid, do not contain synaptic vesicles. Although in the contemporary literature a discussion on whether information is transmitted in the interneuronal junction even without the participation of synaptic vesicles is still going on, it must be pointed out that nearly every pathology of the synapse is reflected somehow or other primarily in the state of its vesicles. We therefore cannot accept the view that only their presence in the newly formed synapse can serve as a reliable criterion of its functional maturity.

At the same period of development we observed how separate, often single vesicles appeared in these synaptoid junctions, in which they lay close to the presynaptic membrane. Pictures of this kind, in our view, indicate a unique kind of transitional stage from synaptoid junctions to true synaptic forms.

However, the first true interneuronal synaptic connections possessing all the attributes of a functionally competent synapse were not found in the developing human cerebral cortex until the end of the 7th and beginning of the 8th week of intrauterine ontogeny (Fig. 1).

Our observations showed that these synapses are located exclusively in the marginal layer of the presumptive cerebral cortex, where they are localized on the surface of the bodies or processes of its primary neurons.

As an example of the structural organization of these early interneuronal synapses let us examine the structure of the synapse illustrated in Fig. 1a. This synapse was found in the marginal layer of the dorsolateral region of the presumptive cerebral cortex of a 7-week human embryo. The marginal layer in the cerebral cortex of these embryos is already quite clearly defined. Besides peripheral pedicles of neuroepithelial cells, profiles of tangentially running nerve fibers and also primary Cajal–Retzius nerve cells can be seen in it, at different stages of their own differential development. A synapse which we identified (Fig. 1a) lies on the soma of one such cell. In its structure, this axosomatic interneuronal synapse, as can be seen in the electron micrograph, corresponds exactly to the structure of a functionally competent synapse. In its active zone, the contacting membranes are clearly differentiated and are separated by distinct synaptic clefts. The subsynaptic membrane is equipped with clearly distinguishable paramembranous specializations, in the form of thin conical formations running deep into the cytoplasm. In the presynaptic zone of this junction a collection of typical synaptic vesicles can be seen, some of them lying immediately next to the paramembranous specializations of the presynaptic membrane. These morphological pictures are evidence that the synapse described can already perform its specific function, that of interneuronal communication of information.

During subsequent development of the human embryo in the first half of the 8th week of its intrauterine life, a marked increase in the total number of synaptic junctions is observed in this region, but most important, by this time the first complex synaptic forms indicating commencing progressive differentiation of the synaptic apparatus of the presumptive human cerebral cortex are beginning to appear. Two well developed synapses with all the characteristic attributes of a functionally competent synapse can be seen in the electron micrograph in Fig. 1b. They are formed by two different nerve terminals on the same cross-section through the process of a nerve cell. This characteristic phenomenon, which has been called convergence, is unequivocal morphological proof of the newly appearing ability of the developing neurons of the human embryonic cerebral cortex to perform its integrative activity.

By the end of the 8th week of human intrauterine development the number of synapses in the marginal layer of its cerebral cortex increases steadily. All these synapses, as our observations showed, are located on the bodies or processes of Cajal–Retzius nerve cells. One of these typical synapses is illustrated in Fig. 1c. It is formed by a nerve terminal on the surface of a large cellular process, where mitochondria and rosettes of polysomes are still preserved.

The subsynaptic receptor zone of this junction is clearly differentiated and its paramembranous thickenings are represented by characteristic small conical structures, whose apices run in the depth of the cytoplasm. In its presynaptic region, paramembranous specializations beginning to form the grids described by Gray and Akert, to which synaptic vesicles are clearly drawn, can be clearly seen also. It must be noted that some of these vesicles, in close proximity to the presynaptic membrane itself, were recorded at a time when it is beginning to empty its contents into the synaptic cleft. This morphological picture, apparently fixing the moment of trans-synaptic transmission, is evidence of the high functional activity of the interneuronal synapse illustrated.

The discovery of interneuronal synapses which are fully mature in their morphological criteria in the developing human cerebral cortex in embryos at the end of the 7th and beginning of the 8th week of intrauterine ontogeny thus suggests that even at this early period of development the first neuronal mechanisms are appearing in their presumptive cerebral cortex, and are capable of logical processing of incoming information. In other words, we consider that the morphological and functional bases of higher nervous functions in man are laid down extremely early — not later than the 2nd month of intrauterine life.

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MORPHOMETRY OF SYNAPTIC VESICLES OF THE NEUROMUSCULAR JUNCTION UNDER DIFFERENT CONDITIONS OF TRANSMITTER RELEASE

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The axon terminal of the neuromuscular junction is the classical object for study of the structural and functional aspects of neurotransmitter secretion and its disturbances [3]. The abundant experimental data on which the quantum vesicular theory is based [4] have been obtained mainly on this object. Meanwhile there is evidence that is incompatible with this hypothesis [5]. Investigations of the vesicular apparatus of the axon terminal of the intact neuromuscular synapse in different functional states have also yielded contradictory results.

The aim of this investigation was to study the ultrastructure of the neuromuscular junction when functioning under different conditions, by means of a morphometric method.

EXPERIMENTAL METHOD

Experiments were carried out on August rats weighing 100-120 g. During the experiments the rats were anesthetized with ether. The diaphragm muscle was fixed in situ by injecting the cold formol-sucrose fixative into the peritoneal and both pleural cavities. Fixation was carried out 20 min after unilateral division of the phrenic nerve, i.e., in the "resting" state, and also during supramaximal electrical stimulation (square pulses, frequency 50 Hz) of the nerve isolated in the neck. Stimulation began 20 min after division of the nerve. A method of fixation preceded by freezing in situ [2] also was used both "at rest," during supramaximal electrical stimulation of the phrenic nerve, and also during physiological contraction and relaxation of the diaphragm during breathing. Postfixation of the material was carried out in buffered OsO_4 solution, followed by dehydration in ethyl alcohol and acetone, and embedding in Araldite. Ultrathin sections were cut on the LKB-III Ultrotome and examined in the JEM-7A electron microscope. The electron micrographs thus obtained were analyzed by means of a modified Leitz ASM apparatus for semiautomatic image analysis (West Germany). The perimeter of the synaptic vesicles (SV) was measured; for statistical analysis this was converted into volume as being more appropriate for the physiological significance of the parameter. Since, as was shown previously, formaldehyde during fixation causes release of synaptic transmitter [1], the number of SV was counted in material fixed after preliminary freezing, in the zone 200 nm wide adjacent to the presynaptic membrane. Student's and the Kolmogorov–Smirnov tests were used for statistical comparisons.

EXPERIMENTAL RESULTS

Analysis of the electron micrographs showed that supramaximal electrical stimulation of the phrenic nerve causes changes in the ultrastructure of the neuromuscular junction (Fig. 1a, b). These changes were manifested in the axon terminals as widening of elements of the smooth endoplasmic reticulum, displacement of the mitochondria closer to the presynaptic membrane, an increase in the number of SV in contact with the presynaptic membrane (Fig. 1d), marked tortuosity of that membrane, and the formation of invaginations (Fig. 1b). The synaptic folds were shortened and thickened, the synaptic cleft was irregular in width, and the electron density of its material was reduced.

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