FORMATION OF LINEAR SYSTEMS OF AGGREGATES IN DISSOCIATED EMBRYONIC BRAIN CELL CULTURES

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UDC 612.822:612.647]-085.23

KEY WORDS: nerve tissue culture; dissociated and reaggregated cultures of nerve tissue; growth of nerve fibers; formation of interneuronal connections.

Cultures of dissociated and reaggregated embryonic brain cells have been widely used in investigations of intercellular interactions participating in cell differentiation processes and the histogenesis of the developing nervous system [2, 3, 7, 9, 10]. Reaggregation of dissociated brain cells and the formation of tissue glioneuronal aggregates are determined by the specific properties of cytoplasmic cell membranes, responsible for recognition and selective adhesion of cells [7, 8, 10]. Nerve and glial cells forming aggregates develop and achieve a high degree of morphofunctional differentiation, which is expressed as the formation of the histotypical structure of the aggregates, growth of dendrites and axons of the nerve cells, the formation of synaptic connections, and myelination of nerve fibers [4, 6, 11-14]. Electrophysiological investigations of glio-neuronal aggregates attached to a collagen substrate have shown that the formation of neuronal connections between aggregates is accompanied by the development of functional synaptic connections [5].

In the investigation described below processes of spontaneous reaggregation of dissociated brain and spinal cord cells from mouse embryos, and the subsequent development of the aggregates and the formation of systemic connections between them were studied. An original method of culture of dissociated and reaggregated brain cells in a collagen well was used for the investigation.

EXPERIMENTAL METHOD

C57BL mouse embryos were used. The spinal cord (12-16-day embryos) or the cerebral cortex (16-20-day embryos) was cut into pieces measuring not more than 1 mm^3, washed with Simms' salt solution, and incubated for 10-15 min at 37°C in 0.25% trypsin solution, made up in Eagle's minimal medium (pH 7.0). After washing in a mixture of equal volumes of minimal Eagle's medium, Simms' salt solution, and placental serum the pieces of tissue were transferred to nutrient medium (30-40% placental serum, 40% Eagle's minimal medium, 20-30% Simm's salt solution, 800 mg% glucose, 0.2 unit/ml insulin, 10^{-2} M HEPES buffer) and dissociated by repeated pipeting. After centrifugation the cells were resuspended in a fresh batch of nutrient medium of the same composition. One or two drops of suspension of dissociated cells (2×10^6 to 1.5×10^7 cells/ml) were placed in a collagen well on a coverslip mounted in a Maximow's chamber [1]. The specimen was cultured at 36°C for 2-3 min. The technique of preparation of the collagen well was as follows: With a curved Pasteur pipet a ridge of solution of collagen acetate was applied around the edge of a coverslip (18 × 18, 24 × 24 mm), and was hardened in ammonia vapor. After careful washing in distilled water, one drop of collagen solution was applied in the center of the coverslip and spread over the whole surface of the glass, until it reached the collagen ridges. After treatment with ammonia vapor a well of solidified collagen was formed. The coverslips with the collagen well were washed in distilled water and with Simms' salt solution until all traces of ammonia had disappeared and were kept in Simms' salt solution with 5% serum.

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Laboratory of Functional Synaptology, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 9, pp. 353-356, September, 1980. Original article submitted January 25, 1980.

0007-4888/80/9009-1283$07.50

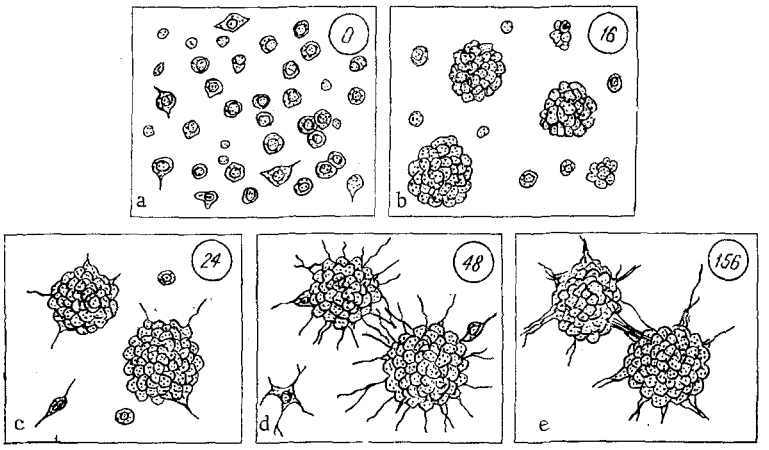

Fig. 1. Formation and development of aggregates in suspension of dissociated embryonic brain and spinal cord cells cultured in collagen well. Scheme based on data of intravital time-lapse microfilming of cultures. Time of observation (in h) shown in circles. a) Suspension of brain cells immediately after dissociation; b) formation of cellular aggregates in suspension; c) adhesion of glioneuronal aggregates to collagen substrate. Beginning of growth of fibers and migration of gliocytes; d) active growth of fibers oriented radially. Formation of primary connections between adjacent aggregates; e) culmination of formation of bundles (fasciculation) of nerve fibers and formation of glioneuronal bridges between aggregates. Gliocytes migrating from aggregates are not shown.

EXPERIMENTAL RESULTS

Formation and Development of Cell Aggregates. The principal stages of formation and development of aggregates are shown schematically in Fig. 1, based on the results of time-lapse microfilming of living cultures. Immediately after seeding into the collagen wells, isolated cells remained suspended in the medium (Fig. 1a). During the first 20 h of culture reaggregation of the majority of cells took place and the newly formed aggregates adhered to the collagen substrate (Fig. 1b, c). On the 2nd-3rd days of culture migration of undifferentiated gliocytes and active growth of nerve and glial fibers were observed from the marginal zones of the aggregates (Fig. 1d). These fibers as a rule radiated from the aggregates and were uniformly distributed around their perimeter. In the same period primary connections formed between neighboring aggregates. When such cultures were impregnated with silver, nerve fibers connecting the aggregates were rarely seen, evidence of the predominantly glial nature of the primary connections between the aggregates. In the later stages of development of the reaggregated cultures (5th-7th days) bundles and glioneuronal bridges connecting individual aggregates formed as a result of fasciculation of the growing fibers (Figs. 1e and 2a). On impregnation with silver, these bundles could be seen to contain numerous parallel nerve fibers (Fig. 2c). In the second week of culture of the reaggregated spinal cord cells, myelinated nerve fibers appeared in the aggregates and, later, in the glioneuronal bridges connecting them.

Formation of Systems of Aggregates. During the first day in culture newly formed aggregates, adhering to the collagen substrate, already formed systems with random or oriented arrangement of their aggregates. As a rule, the type of spatial distribution of aggregates which appeared was maintained in each culture, and changed only slightly on account of proliferation and migration of the gliocytes, spreading of the aggregates, and their merging. If the distribution of the aggregates was random, no permanent geometric pattern was obtained, and later, after the formation of connections between aggregates, the formation of similar systems of interconnections, reproduced from one experiment to another, was not observed. The ability of the dissociated brain cells to form linear systems of reaggregates on the surface of the collagen substrate (Fig. 2a, b), discovered for the first time during

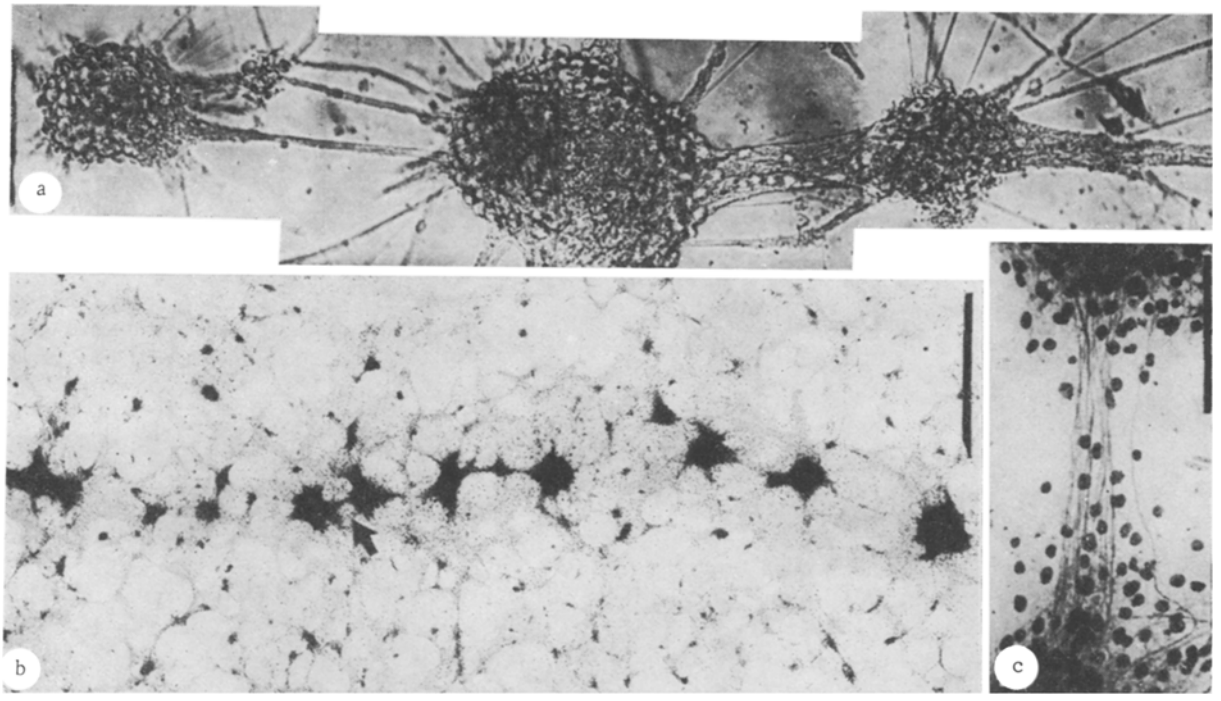

Fig. 2. Linear systems of aggregates formed in cultures of dissociated embryonic spinal cord cells grown in collagen wells. a) Cellular aggregates and glioneuronal bridges connecting them, 6 days of culture. Living, unstained culture. Light ground. Scale 100 μ; b) part of a chain of aggregates formed in a culture of dissociated spinal cord cells of a 12-day mouse embryo. Eleven days of culture. Impregnation with silver by Holmes' method. Scale 1000 μ; c) bundle of neurons connecting aggregates, indicated by arrow on photomicrograph b. Scale 100 μ.

culture in collagen wells, was of much greater interest. The primary linear arrangement of the aggregates determined the character of development of the connections between them. The initial radial growth of the fibers without any marked orientation was replaced in the later stages of development by the formation of glioneuronal bridges between adjacent aggregates (Fig. 2a), as a result of which chains of interconnected aggregates formed on the surface of the collagen well (Fig. 2b). The dimensions of these chains and the number of aggregates composing them varied considerably. For instance, in reaggregated spinal cord cultures from 12-14-day mouse embryos chains about 20-24 mm long consisting of over 30 aggregates were formed. The number of isolated aggregates, not composing such chains, was very small. In reaggregated cultures of dissociated cortical cells short chains of aggregates, running among randomly arranged aggregates, were more frequently formed. It should be noted that during culture in collagen wells almost complete reaggregation of the cells took place. Isolated neurons were infrequently found outside aggregates, but after 2 weeks in culture virtually none were present. Meanwhile the number of glial cells, mainly astrocytes, not included in the aggregates increased in the later stages of culture, due to migration of these cells from aggregates and to their proliferation.

The results obtained by culture of dissociated embryonic brain cells in collagen wells thus showed that processes of reaggregation of the cells and subsequent development of aggregates on the surface of the collagen substrate were similar in both features with those already described in the literature [4, 5, 15]. Meanwhile, in these cultures, the phenomenon of formation of regular linear systems of aggregates, connected together by nerve fibers, not previously described, was shown for the first time in these cultures. Data in the literature on synapse formation within aggregates [12-15] and on functional synaptic connections between them [5] suggest that these chains of aggregates can be regarded as newly formed systems of synaptically interconnected nerve centers. There is no doubt that reaggregated cultures of dissociated embryonic brain cells, grown in collagen wells, can serve as a model for the study of processes of nerve tissue histogenesis and of the principles governing the formation of synaptic connections within the nervous system.

Factors determining the formation of linear chains of aggregates during culture of dis-

sociated brain cells in collagen wells are not clear. The fact that characteristic chains are formed more frequently in cultures of dissociated spinal cord cells of 12-14-day mouse embryos suggests that the processes described above may perhaps be dependent on the stage of embryonic differentiation of nervous and glial cells in the spinal cord. It is thus likely that the formation of linear chains of aggregates reflects the organ specificity and histogenetic powers of dissociated nerve tissue cells.

LITERATURE CITED

1. I. V. Viktorov, in: Textbook of Nerve Tissue Culture [in Russian], Moscow (1976), pp. 21-28.
2. I. V. Viktorov, in: Textbook of Nerve Tussue Culture [in Russian], Moscow (1976), pp. 30-38.
3. I. K. Svanidze and E. V. Didimova, Tsitologiya, No. 1, 90 (1979).
4. M. B. Bornstein and P. Model, Brain Res., 37, 287 (1972).
5. S. M. Crain and M. B. Bornstein, Science, 176, 182 (1972).
6. G. R. DeLong, Dev. Biol., 22, 563 (1970).
7. B. B. Garber, in: Cells, Tissue and Organ Cultures in Neurobiology, S. Fedoroff and L. Hertz, eds., New York (1977), pp. 515-537.
8. B. B. Garber and A. A. Moscona, Dev. Biol., 27, 217 (1972).
9. J. -M. Matthieu and P. Honegger, in: Models for the Study of Inborn Errors of Metabolism, edited by F. A. Hommes, Amsterdam (1979), pp. 259-278.
10. A. A. Moscona, in: Cells and Tissues in Culture, edited by E. N. Willmer, Vol. 1, New York (1965), pp. 489-529.
11. E. B. Palacios, B. B. Garber, and L. M. N. Larramendi, Brain Res., 66, 173 (1974).
12. N. W. Seeds, in: Tissue Culture of the Nervous System, G. Sato, ed., New York (1973), pp. 35-53.
13. N. W. Suburo and R. Adler, Cell Tiss. Res., 182, 407 (1977).
14. B. D. Trapp, P. Honegger, E. Richelson, et al., Brain Res., 160, 117 (1979).
15. E. Trenkner and R. L. Sidman, J. Cell Biol., 75, 915 (1977).

CIRCADIAN RHYTHM OF MAST CELL FUNCTION IN THE RAT DURA

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UDC 612.112.93"52"

KEY WORDS: dura mater; mast cells; biogenic amines.

Processes taking place in the body at the cell, tissue, and organ levels are characterized by a definite rhythm [7]. Mast cells, which contain biogenic amines, are local regulators of tissue homeostasis [5].

The object of the present investigation was to study the diurnal activity of mast cells in the dura mater of rats and changes in their content of biogenic amines during the 24-h period.

EXPERIMENTAL METHOD

The dura of sexually mature noninbred albino rats weighing 180-200 g was studied. The method of Falk and Hillarp was used. The animals were kept under identical conditions and fed twice a day; material was taken every 3 h. The dura, straightened out on slides, was dried at room temperature for 15 min, then treated with gaseous formaldehyde at 80°C for 1 h. The specimens were studied in light in the blue-violet region of the spectrum with a wavelength of 410-480 nm and photographed on highly sensitive RF-2 film. Biogenic amines

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 9, pp. 356-357, September, 1980. Original article submitted December 12, 1979.

0007-4888/80/9009-1286$07.50