A STUDY OF SOME OXIDO-REDUCTASES IN NERVE TISSUE CULTURE

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Changes in the activity of succinate dehydrogenase and NAD- and NADP-diaphorases under the influence of immune serum from animals with experimental allergic whooping-cough encephalomyelitis were studied in a dissociated culture of neonatal rat cerebellum. An increase of activity was found in the oligodendrocytes after exposure for 3 h to the immune serum and a decrease in activity of these enzymes in the same cells after exposure of the cultures to the serum for 12 h. By contrast to the oligodendrocytes, high activity of all these enzymes was found in the astrocytes after exposure for 12 h to the serum.

KEY WORDS: culture of cerebellar cells; enzyme activity; newborn rats; allergic whooping-cough encephalomyelitis.

The study of the histoenzymology of nerve tissue in the so-called demyelinating diseases of man and their experimental model – allergic encephalomyelitis (EAE) – has recently achieved widespread popularity [2, 9, 11]. Enzymes hydrolyze the basic protein of myelin to form encephalitogenic peptides; these, by acting on the lymphoid system, form an autoallergic response [7, 11]. The suggestion has been made that, in addition to sensitized lymphocytes, monocytes, and polymorphs rich in lysosomes, an important role in this response is also played by the oligodendrocytes. The study of enzyme activity during the development of autoimmune responses in nerve tissue is accordingly of great interest.

The writers have studied the activity of some oxido-reductases concerned with the Krebs cycle (succinate dehydrogenase – SD) and with electron transport (NAD- and NADP-diaphorases), using as the model for this purpose a culture of nerve tissue, for in that way it is possible to study the response of individual cell groups to the altering factor.

The activity of the above-mentioned enzymes in various cells of an intact culture has previously been studied during the cultivation of cells from the central and peripheral nervous system [1, 10, 12]. The present investigation, a continuation of these previous studies, was aimed at detecting enzymic changes by means of histochemical reactions in nerve tissue culture during immunological transformation.

EXPERIMENTAL METHOD

Dissociated cultures of neonatal rat cerebellum were used [3]. Blood was taken from guinea pigs with EAE, induced by injection of a homogenate of homologous brain in an oily suspension of Bordetalla pertussis cells (2 mg per animal), at the height of development of clinical manifestations of the disease (14th-16th day). The serum of the experimental and control (normal) animals was added in 25% concentration to the nutrient medium of 12-day cultures of rat cerebellum.

After exposure to the serum for 3 and 12 h the cultures were washed in physiological saline and transferred into incubating solutions, which differed for each enzyme. The activity of the enzymes was determined by the methods described in Pearse's textbook, with certain modifications as regards the

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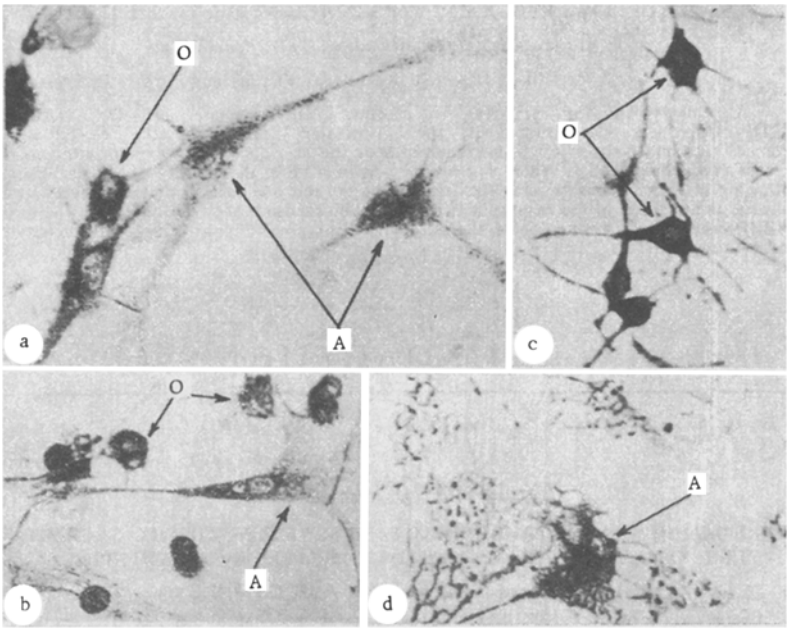

Fig. 1. Changes in SD activity in a culture of newborn rat cerebellum (age of culture 12 days) depending on duration of incubation with serum of animals with EAE: a) intact culture of cerebellum: SD activity in astrocytes (A) weaker than in oligodendrocytes (O), 150 ×; b) high SD activity in oligodendrocytes after exposure for 3 h to serum of animals with EAE, 100 ×; c) marked decrease in SD activity in oligodendrocytes after exposure of culture for 12 h to serum of animals with EAE, 100 ×; d) increase in SD activity in astrocytes of culture of rat cerebellum incubated for 12 h with serum of animals with EAE, 100 ×.

concentration of some reagents and the incubation time [6]. For instance, after incubation at 37°C for 40 min, the substrate was washed off with physiological saline and the cultures were fixed with 10% neutral formalin; after treatment for 10 min in 10% alcohol, the preparations were mounted in glycerol–gelatin. As survey methods, the cultures were stained by Nissl's method and with hematoxylin–eosin.

EXPERIMENTAL RESULTS

Considerable changes in both size and shape were found in the dissociated intact 12-day cultures of neonatal rat cerebellar cells, and this made strict application of the criteria of identification used for histological preparations of nerve tissue to them difficult. Microglial cells were found in large numbers, as a rule in layers; they formed a delicate network in the loops of which astrocytes and modified giant multinucleated forms of astrocytes were present. Among them it was possible to distinguish also large granular, frequently branching cells, with a vesicular nucleus and a single nucleolus, which were classed as Purkinje neurons. Oligodendrocytes with a large nucleus, occupying a large part of the cell body, and with short, delicate processes, were observed diffusely throughout the culture. Granule cells were also well represented in some cultures.

In preparations of the same cultures stained by Nissl's method, many lumps of tigroid material could be seen in certain of the Purkinje cells; their nuclear membrane and nucleus stained dark blue against the background of pale cytoplasm, whereas the cell processes were completely unstained.

In normal cultures of the cerebellum and in cultures incubated with normal guinea pig serum, a positive histochemical reaction for all the enzymes studied, as shown by an irregular deposition of diformazan granules in the cytoplasm of the cells, was found chiefly in the neurons and macroglial cells: astrocytes and oligodendrocytes. Small diformazan granules completely filled the zone of the perikaryon of the Purkinje cells, and often because of their large number they joined together to give the cytoplasm and processes of the cell a diffuse staining. The activity of the granule cells was low and only solitary granules were present in the perinuclear zone. So far as the astro- and oligoglia are concerned, differences in the

level of activity of the various enzymes were discovered. Whereas in the astrocytes and spongioblasts, diffusely scattered, weakly stained granules could be seen in the zone of the perikaryon and processes, high enzyme activity was observed in the oligodendrocytes in the perinuclear and juxtanuclear zones and in the cell processes also (Fig. 1a).

High activity of all the enzymes studied was found in cultures with no visible morphological changes in their cells after exposure for 3 h to the serum of animals with EAE. Particularly high activity was found in the oligodendrocytes (Fig. 1b).

After exposure for 12 h to the serum of the experimental animals, among the numerous swollen, vacuolated, and necrotic cells in the cerebellar cultures a few neurons still retaining fairly high activity of the oxido-reductases were found. So far as the neuroglial cells are concerned, exceptionally strong staining for enzymes was found in the hypertrophied astrocytes compared with the oligodendrocytes (Fig. 1d). The number of the latter in the cultures was appreciably reduced and their enzyme activity was very low (Fig. 1c). No appreciable changes in enzyme activity were found in the remaining cells of the culture.

On the 7th day of sensitization a decrease in the intensity of energy metabolism expressed as reduced activity of SD and NAD- and NADP-dehydrogenases has been observed in the brain homogenates of guinea pigs with EAE [4, 5]. The work of Bickis et al. [8] has shown that respiration and glycolysis are inhibited in the cells of tissue cultures during immunological transformation. A decrease in the activity of oxido-reductases in the oligodendrocytes under the influence of immune serum from animals with EAE confirms these findings and demonstrates once more that it is the oligodendrocytes that are the target of the immunologic reaction. Activation of hydrolytic enzymes of the lysosomal apparatus probably takes place under these circumstances, as is confirmed by the further lysis of these cells in nerve tissue culture. The functional significance of the adaptive changes in enzyme activity discovered by the writers in the present experiments may be connected with the reaction of these cells to breakdown products.

The discovery of early histochemical changes in nerve tissue during allergic reactions thus reveals how a vicious circle arises, causing the transformation of the natural process of metabolism into autoaggressive reactions.

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