CHOLINESTERASE ACTIVITY IN DIFFERENTIATING NEURONS OF THE HUMAN PELVIC PLEXUS

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In the course of differentiation of neurons (from the neuroblast to the mature neuron) of the human pelvic plexus in the periods of prenatal and early postnatal ontogenesis a gradual increase in cholinesterase activity is observed. The enzyme is localized in the cytoplasm of the nerve cells, usually peripherally, and in synaptic structures and nerve endings. Pseudocholinesterase is found in all structures of the neuron and surrounding glia.

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The study of acetylcholinesterase is closely linked with the mediator function of acetylcholine, which, as physiologists consider, plays an important role in the mechanisms of nervous phenomena. The principal function of acetylcholinesterase is to split acetylcholine synthesized in nerve tissue with the participation of choline-acetylase. Acetylcholine and choline-acetylase are constituents of synaptic vesicles, and cholinesterase is localized in the cytoplasm of the neuron and the synaptic membranes. The role of substances of the acetylcholine system in the transmission of the nervous impulse has largely been elucidated by physiological and biochemical investigation, but in certain parts of the nervous system it has been inadequately studied [1, 2, 6-8, 10-12, 15-18].

EXPERIMENTAL METHOD

The cytochemistry of the cholinesterases was studied in the pelvic plexus of human fetuses aged from 15 to 36 weeks and in the newborn. The activity and localization of acetylcholinesterase were determined by the method of Koelle and Friedenwald in Portugalov's modification, using a control in which the sections were incubated in the absence of acetylcholine. The specificity of the reaction for cholinesterase was verified by preliminary treatment of the sections in a solution of inhibitor of this enzyme (1 : 1000 neostigmine solution). The results were assessed with full allowance made for possible diffusion of the reaction products, prevention of which was attempted by using the measures recommended by Portugalov. For convenience of description of the activity of the reaction for cholinesterase in ganglia of the pelvic plexus, a system of notation by plus signs (+) was used. To establish the degree of differentiation of the neurons, some of the material was impregnated by the Gros–Bielschowsky method as modified by Rasskazova. During determination of the stage of maturation of the neurons, the classifications of Knorre [3] and Korochkin [5] were used.

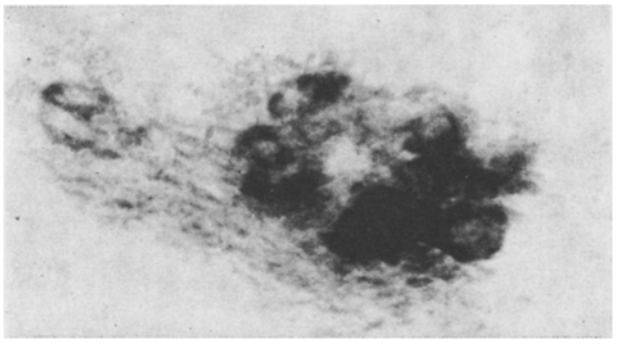

Fig. 1. Cholinesterase activity in neurons at the stage of growth. Pelvic plexus of 15-week human fetus. Koelle–Friedenwald method in Portugalov's modification, 90×.

EXPERIMENTAL RESULTS

Cholinesterase activity in the pelvic plexus of 15-week fetuses varies in intensity in the different groups of nerve cells. In cells of the neuroblastic series the staining reaction is generally pale yellow in character. It can be concluded from the generally accepted descriptions of the results

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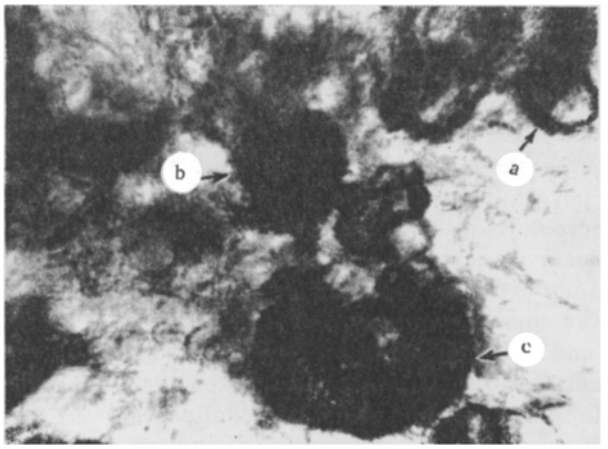

Fig. 2. Cholinesterase activity in neurons at different stages of maturation (a) growing; b) maturing; c) mature). Pelvic plexus of 36-week human fetus. Koelle–Friedenwald method in Potugalov's modification, 90×.

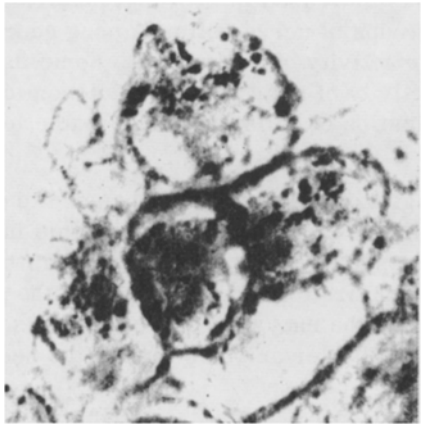

Fig. 3. Group of mature neurons. Cholinesterase activity in synaptic structures. Pelvic plexus of newborn infant. Koelle–Friedenwald method in Portugalov's modification, 90×.

of the reaction for cholinesterase that these cells contain chiefly nonspecific cholinesterase which, as a rule, gives the sections a yellow color. Consequently, the reaction for acetylcholinesterase in all structures of the neuroblasts was negative (–). However, against the yellow background of the ganglia of the pelvic plexus appearing after staining, certain groups of neurons can be distinquished by their large size and brown color. The reaction products were concentrated chiefly at the periphery of the cytoplasm of these neurons, in the form of diffuse granules collectively forming a ring (Fig. 1). The intensity of staining of these rings differed in different groups of growing nerve cells. In most neurons they were dark brown in color (+++), and in some they were paler (++). The bundles of nerve fibers in the pelvic were stained yellow. Sometimes small dark-brown granules were seen along their course. After incubation of the sections in the absence of acetylcholine they remained colorless. Neostigmine solution completely inactivated the acetylcholinesterase in the growing neurons.

In fetuses aged from 21 to 23 weeks an increase in the intensity of the reaction for cholinesterase was observed in the ganglia of the pelvic plexus. Characteristically the neuroblasts were stained yellow as before. They were grouped into discrete nodes or concentrated among the larger neurons. Very rarely tiny brown granules could be observed at the periphery of the cytoplasm of the neuroblasts, indicating the presence of minute traces of acetylcholinesterase (+–). The activity and localization of the enzyme in the remaining neurons of the pelvic plexus varied. Cells of two types were distinquished among the growing neurons also. In some the deposits of copper sulfide were colored brown (++) and were located at the edge of the perikaryon, while in others they were much darker (+++). In contrast to the 15-week fetuses, solitary structures resembling dark-brown rings (+++) with a clear central area were seen on the surface of the growing neurons. Structures of this type on the surface of the neurons are presumably synaptic plates. Besides growing neurons, large nerve cells lying singly or in groups in the ganglia of the pelvic plexus also were frequently encountered. The intensity of the reaction for cholinesterase was much higher in these cells than in the growing neurons. Their neuroplasm was diffusely stained brown. The degree of staining was increased at the periphery of the perikaryon (++++). Cholinesterase activity was reduced (+++) in the other maturing neurons. Synaptic structures, consisting of rings and boutons, were observed more often on the bodies of the maturing cells. The intensity of their staining was increased (++++). In the glia surrounding the growing and maturing neurons a positive reaction for nonspecific cholinesterase was observed, persisting even after treatment with neostigmine solution.

Cholinesterase activity was still further increased in individual groups of neurons in the ganglia of the pelvic plexus of fetuses aged 30-36 weeks and of newborn infants the cholinesterase activity showed a further increase in certain groups of neurons. As before, neuroblasts, forming tiny ganglia or lying alongside more completely differentiated neurons, were stained yellow. Fine brown granules were seen only around individual neuroblasts. The intensity of the reaction for cholinesterase in growing and maturing neurons

was slightly increased compared with that in neurons of the pelvic plexus of 21-23-week fetuses. As before, two groups of cells differing in their intensity of staining (+++++ and +++) could be distinguished. High acetylcholinesterase activity in all groups of neurons was observed in the marginal zone of the neuroplasm, where copper sulfide was deposited in large granules. The remainder of the neuroplasm of the nerve cells was diffusely stained pale brown. Structures described above as synaptic endings were revealed on the surface of the nerve cells (more especially of maturing neurons). As a rule they were stained an intense dark-brown color, often appearing black. In the pelvic plexus of fetuses in the last weeks of embryogenesis and of newborn infants, some very large nerve cells classed as mature neurons from several of their morphological features were found among the neurons just described. The intensity of the reaction for cholinesterase in these nerve cells was similar to that in maturing neurons (++++). However, in some cells a sharp increase in cholinesterase activity was observed (++++++). Their cytoplasm was almost black in color (Fig. 2). Synaptic structures—loops and boutons—of considerable size were seen on the bodies of the mature neurons (Fig. 3). Incubation of sections of the pelvic plexus, taken at the stages of development described above, in neostigmine solution led to total inactivation of acetylcholinesterase. Prolonged washing of the sections with water after incubation in a solution of the inhibitor did not restore enzyme activity. When these sections were treated with a solution of acetylcholine, no brown deposits of copper sulfide could be seen. Areas corresponding to ganglia and nerve fibers were stained pale yellow.

Cholinesterase activity is thus closely linked with the degree of differentiation of the nerve cells of the pelvic plexus. This link can be traced at all stages of development of the fetus studied. The enzyme is localized mainly in the cytoplasm of the nerve cells, more often at its periphery, in synaptic structures and in nerve endings. Pseudocholinesterase is found in all structures of the neuron and of the surrounding glia. The reaction for acetylcholinesterase is more often negative in neuroblasts of the pelvic plexus of 15-36-week fetuses and newborn infants. The intensity of the reaction is increased in growing, maturing, and mature neurons but it is expressed differently in the different groups of nerve cells. Among neurons of the same size two groups can be distinguished: with higher or lower activity of the enzyme. Some investigators [9, 13, 14] hold the opinion that this difference in the enzymic activity of neurons of the autonomic nervous system is connected with differences in the cytochemical organization of cells of parasympathetic and sympathetic nature.

Methods of determination of cholinesterase activity in ganglia of mixed type can be used to differentiate neurons with different functions at various stages of their maturation. The results of this investigation also show that a positive reaction is detected initially in the neuroplasm of the growing neuron; the cholinesterase concentration rises sharply as the cell grows. With maturation of the neurons of the pelvic plexus, interneuronal (synaptic) connections are established [4] and the enzyme then accumulates in the region of the synaptic structures, where it is concentrated in much larger amounts than in the neuroplasm.

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