EFFECT OF THE AGENT OF AMYOTROPHIC LEUKOSPONGIOSIS ON NEUROGLIAL CELLS IN VITRO

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Human slow infections, including amyotrophic leukospongiosis (ALS) and Creutzfeldt—Jakob disease (CJD), combined into the group of subacute transmissive spongioform encephalopathies, are caused by unconventional viruses or prions [1, 10]. In these diseases extensive death of neurons, the development of a status spongiosus, are observed in the CNS, with the development of activation of the astroglia, manifested as its hypertrophy and proliferation [4, 5]. The mechanism of damage to neurons and the role of the astroglia with the development of the degenerative changes in the CNS remain incompletely studied. Some workers consider that the reaction of the astroglia is simply compensatory or substitutive in character [5], whereas others claim involvement of these cells in the pathological process [7]. It has not been established which of the astrocytes are most sensitive to the cytodestructive action of the causative agent.

The aim of this investigation was to identify the ultrastructural changes caused by the agent of ALS in different types of cells in dissociated cultures of the rat embryonic brain and spinal cord.

EXPERIMENTAL METHOD

Dissociated cultures of the spinal cord and brain were obtained from 14-16-day rat embryos and grown on coverslips with collagen substrate [2]. Altogether, 20 cultures of the brain and 23 cultures of the spinal cord of rat embryos were used. In version I of the experiment, a 10% brain suspension from patient D., a man dying from ALS (titer of the causative agent of ALS in the brain tissue 4.0 log ID_{50}/ml) was used to infect these cultures, in series II, culture fluid obtained from a monolayer culture of the brain of this same patient, concentrated 8 times with dry polyethylene-glycol (titer of the agent of ALS in the culture fluid 5.1 log ID_{50}/ml) was used. After introduction of the agent, two infected and two control cultures were taken in version I for ultrastructural investigation on the 3rd, 6th, and 9th days, and five experimental and five control cultures on the 3rd day in version II. The cultures were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and then postfixed with OsO_4. They were then embedded in Araldite and ultrathin sections were cut and studied in the JEM-100 CX-II electron microscope. At each point of the experiment and control 80-100 cells were analyzed.

For the light-optical investigation subcultures were stained with hematoxylin and eosin.

The presence of the agent ALS in the infected cells was determined by the direct immunoperoxidase method, using monoclonal antibodies to PrP_{27-32} ALS, labeled with peroxide by the method in [8].

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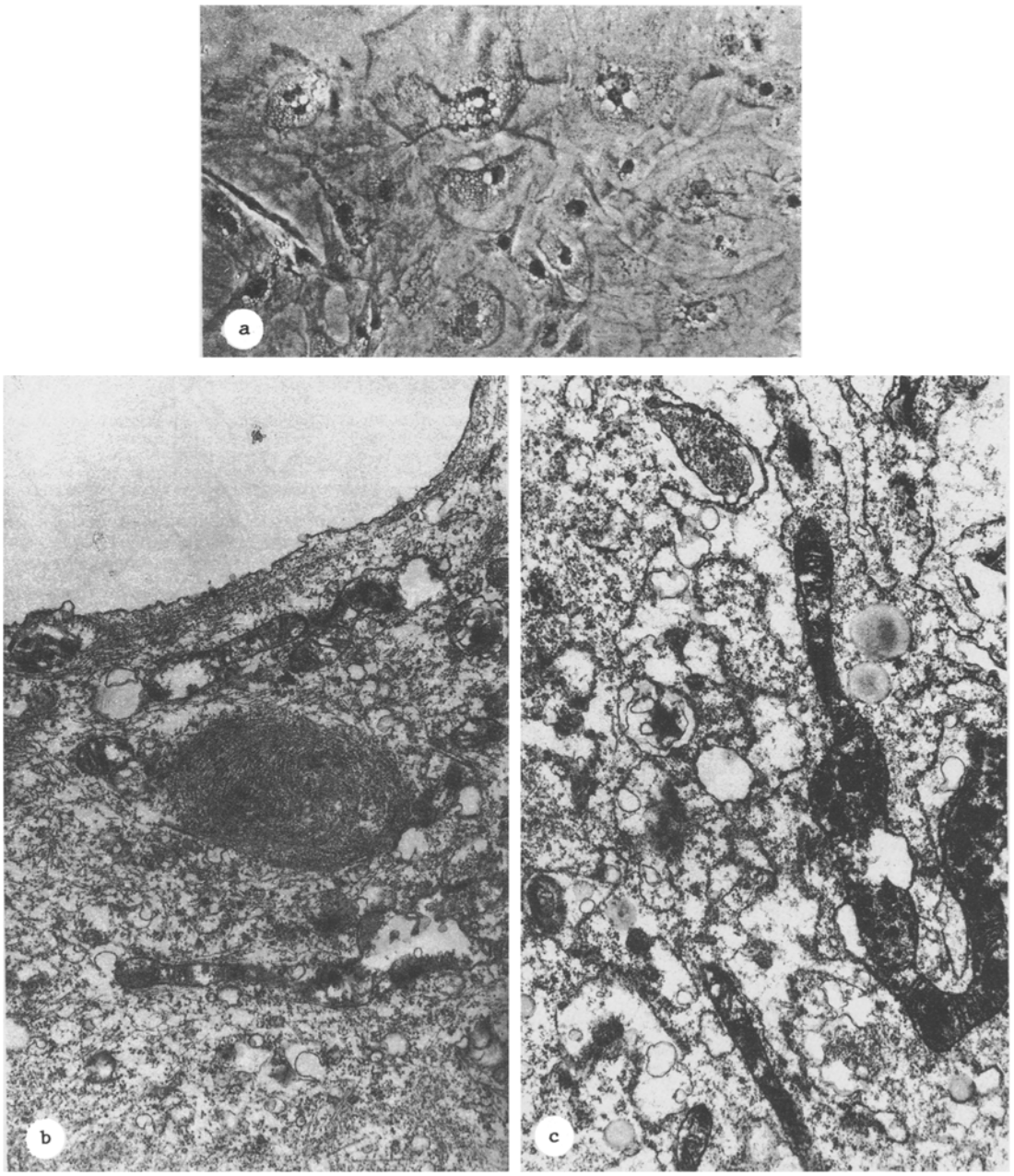

Fig. 1. Development of cytopathic changes in dissociated cultures of rat embryonic spinal cord after introduction of agent of ALS. a) Extensive vacuolation of cytoplasm of degenerating cells. Hematoxylin and eosin, 100×; b) part of ultrathin section of fibrous astrocyte. Among destroyed mitochondria (M) a gliofibrillary coil (GC) can be observed in the cytoplasm; c) the same. Giant mitochondrion with intramitochondrial amorphous inclusions; b, c) 9400×.

EXPERIMENTAL RESULTS

The light-optical studies showed that after introduction of the brain suspension no cytopathic changes were found during the period of observation of the cultures, whereas after introduction of a cultural concentrate of the agent of ALS on the 1st-30th days they were found in 20-30% of cells (Fig. 1a).

When the immunoperoxidase method was used, the agent PrP_{27-32} ALS was found in neurons and glial cells toward the 6th day after infection.

Electron-microscopic investigation showed that the development of the pathological process in neurons and astrocytes in version I of the experiment occurred in 2-4% of cells, not until the 6th-9th day. In version II, most cells were involved in the process after the 3rd day. Ultrastructural injuries, although differing in severity, were uniform in character in all the infected cultures, with destruction of the plasma membranes and the formation of neuron pairs. The extent of the regions with absence of membrane varied considerably: from 50 to 500 nm or more. In large and less frequently, in small neurons foci of lysis of the cytoplasmic matrix were observed with disappearance of subcellular organelles and with the formation of vacuole-like structures. Some polysomes broke up into separate ribosomes. Cisterns of the rough endoplasmic reticulum swelled to form cavities in which myelin-like structures were present in the form of coils. Similar structures were sometimes found in the perinuclear space. The single preserved mitochondria were sharply hypertrophied, with parallel collapse cristae and with increased osmiophilia of the membranes. The nuclei of the modified neurons were pale, with frequent deep invaginations of the nuclear membrane. Little condensed chromatin was present. Sometimes the granular component had completely disappeared in the nucleoli.

Extensive vacuolation of the cytoplasm was observed in degenerating, mainly fibrous, astrocytes, so that they appeared as "reticular cells" with one or more pycnotic nuclei. In the cytoplasm of some astrocytes a concentration of vacuoles, lysosomes, dense bodies, and also gliofibrillary coils and giant mitochondria with partially destroyed cristae and with mitochondrial inclusions could be seen (Fig. 1b). Usually one or two abnormal mitochondria were found in the cell, cylindrical or convoluted in shape, 5-10 μm long, with increased osmiophilia of their membranes and with several intramitochondrial inclusions, formed by the accumulation of amorphous or finely granular material (Fig. 1c). Much less frequently the intramitochondrial inclusions were crystal-like in appearance. The gliofibrillary coils, formed by threads about 10 nm thick, were arranged in the center or around the periphery of the bodies of the astrocytes. Among the fibrils there were some small formations similar to glycogen granules.

Activation of individual astrocytes, characterized by marked hypertrophy, was observed in all the infected cultures. Predominance of cytopathic changes in those neurons which were located next or near to the "reticular astrocytes" was a noteworthy feature.

Most of the oligodendrocytes involved in the formation of myelin sheaths had no disturbances of any kind. The macrophages contained large accumulations of lipids and lysosomes and were actively involved in the ingestion of cellular debris.

The results of these experiments thus showed that the cells most sensitive to the cytodestructive action of the agent of AML in dissociated cultures of rat embryonic spinal cord and brain are large neurons (evidently Betz' cells in brain cultures and anterior horn motoneurons in spinal cord cultures) and fibrous astrocytes. The times of appearance and the degree of severity of the degenerative changes were found to depend on the concentration of the agent used. The formation of paired cells indicates a membranolytic action of the agent of AML. In a model of another slow CNS infection, namely Creutzfeldt-Jakob disease, the formation of conglomerates of several cells also was observed in vitro as a result of destruction of their plasma membranes [10]. The formation of giant mitochondria in vitro under the influence of the agent of AML, together with our observations on changes in these organelles [3, 4], are evidence that the infective agent evidently causes primary injury to the energy-forming apparatus of cells sensitive to it.

It is interesting to contemplate the role of astrocytes in the pathological process. Experiments during recent years have shown that these cells can become involved in immunologic reactions through the production of interleukins 1 and 3, expression of Ia-antigen, and presentation of antigen to T lymphocytes [6, 9]. Probably the astrocytic glia may play not only a passive role in the pathogenesis of AML, as "target" for the agent of the disease, but may also be actively involved in the sequence of cellular reactions preceding the development of degenerative changes.

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USE OF CHEMILUMINESCENCE ANALYSIS TO DETERMINE ACTIVITY OF HEPATIC ANTICHALONE AND CHALONE

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Chalones — tissue-specific inhibitors of DNA synthesis and mitosis — are found in all tissues so far studied [1, 5]. Many investigators have found not only chalones, but also tissue-specific stimulators of DNA synthesis and mitosis, often called antichalones [1, 10, 12]. It has been suggested that these stimulators and inhibitors act together [2]. Methods of obtaining and purifying chalones and antichalones have been developed and the class of chemical compounds to which they belong has been established (glycoproteins [7]). However, the method of determining activity or quantity of antichalone and chalone in an isolated preparation still remains the most difficult problem. Meanwhile, no method of determination of antichalone and chalone activity per unit mass of an organ has yet been developed. We know that DNA synthesis is coupled with the state of many factors. For example, if the level of cell proliferation falls the intensity of free-radical reactions increases, and the quantity of natural inhibitors is reduced. Opposite changes take place in cases of stimulation of cell proliferation and, in particular, after partial hepatectomy [8]. Thus the state of proliferation, including under conditions when factors regulating it are acting, can be judged from changes in the parameters of free-radical reactions.

The aim of this investigation was to determine the antiradical activity of hepatic antichalone and chalone by the use of chemiluminescence analysis for this purpose.

EXPERIMENTAL METHOD

Antichalone and chalone were isolated from bovine liver and from the liver of 27 noninbred male albino rats weighing 130-150 g [13]. The writers' previous investigations showed that preparations obtained from the liver and partially purified stimulate intrahepatic DNA synthesis and cell division (antichalone) and inhibit these processes (chalone), and their optimal doses also were established [1, 4]. Quenching of chemiluminescence by antichalone and chalone was determined in a system generating free radicals (luminol — riboflavin in carbonate buffer, pH 10.0, irradiated by ultraviolet light at 365 nm) [11].

Chemiluminescence was recorded on a KhLMITs-01 chemiluminometer, with continuous flow cuvette, in which the components of the system forming free radicals circulated. No free radicals were generated without irradiation of the luminol-riboflavin system. Luminescence reached a maximum 1-2 min after irradiation. If under these circumstances antichalone or chalone was added, chemiluminescence was quenched. The degree of quenching was expressed as a percentage. Antichalones and chalones are known to be thermolabile. They were inactivated by heating their solutions for 15 min at 65°C. Partial hepatectomy (PHE) was performed on the male albino rats by the method in [9]. The operation was performed on the animals in the morning. The rats were killed by decapitation 24 h (six animals), 72 h (seven animals), and 14 days (five animals) after the operation. Intact animals (nine rats) served as the control. Antichalone and chalone were isolated from the liver by the method in [13]. The alcoholic residue was redissolved and applied to a chromatography column with Sephadex G-50. Eluates from the chromatography column were recorded in the cuvette at 280 nm. Their activity was determined in pooled fractions of antichalone

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