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REACTION OF MOUSE CNS CELLS TO THE SCRAPIE AGENT IN EARLY STAGES OF EXPERIMENTAL INFECTION

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Summary. — Accumulation of the scrapie agent, histological lesions and cytometric parameters in layers III and V of the cerebral cortex and caudate nucleus were investigated in BALB/c mice during the incubation period of scrapie after intracerebral (i.c.) inoculation of the agent. The following cell parameters were determined: areas of the body, nucleus, and cytoplasm of neurons, concentration and amount of protein within the nucleus and cytoplasm. One month after i.c. inoculation, titre of the agent in the brain was 6.3 log LD_{50}/g. Histological examinations of the infected brains revealed no lesions. Cytointerferometric studies showed a statistically significant increase in the size of the body, nucleus and cytoplasm of neurons, namely in the layer III of brain cortex. Increased amounts of protein were observed only in the nuclei of these cells. No such changes were found following i.c. inoculation of normal brain tissue used as a substrate for the agent.

Key words: subacute transmissive spongiform encephalopathy; scrapie agent; mouse neuron size; protein content; cytometry

Introduction

The scrapie agent belongs to the peculiar group of the agents of subacute transmissive spongiform encephalopathies (STSE) causing slow infections in man and animals (Gajdusek, 1978). The growing interest in the study of these CNS diseases is due to both the features of the clinical course (incubation period of many months combined with 100% lethality) and peculiar properties of the agents distinguishing them from conventional viruses. Investigations of the nature of STSE agents and on the pathogenesis of slow virus diseases induced by them are considerably impeded by virtual inability of the STSE agent to replicate in tissue cultures. At the same time, the study of interactions of STSE agents with susceptible cells is extremely important for elucidation of the features of infectious process. This paper describes the interaction of the scrapie agent with neurons of CNS in BALB/c mice at early stages of infection.

Materials and Methods

Animals. Two to three-week-old BALB/c mice were used.

The agent and inoculation conditions. The agent of scrapie, strain C-506, isolated in the laboratory of Dr. D. C. Gajdusek (U.S.A.) from the brain of a sheep with scrapie and passaged 4 times in mice was received from Dr. N. V. Loginova (D. I. Ivanovsky Institute of Virology, Moscow). The strain was maintained and titrated by i.c. inoculations of BALB/c mice. The results of inoculation were assessed by the number of animals developing a typical clinical picture and histological lesions in the CNS within 11 months observation period. The titre of the agent was calculated by the method of Reed and Muench. Altogether 60 mice used in the study were obtained simultaneously from one source and divided into 3 groups: a) group 1, animals inoculated i.c. with the scrapie agent (10% mouse brain suspension with initial titre of 5.7 log LD_{50}/0.03 ml); b) group 2, animals inoculated i.c. with 10% suspension of normal mouse brain; c) the animals of group 3 were left intact. Up to 5 months post inoculation (p.i.), 5 mice from each group were sacrificed at one-month intervals to determine the degree of accumulation of the agent in their brains along with histological and cytometric examination. The experiments were carried out in the winter-spring period.

Histological and cytometric studies. For histological examinations, mouse brains were fixed in a 10% formalin solution. Paraffin blocks were prepared and sections stained with hematoxylin-eosin and according to Nissl. For cytometric studies, the brains were fixed according to Carnoy's technique followed by dehydration and embedding in paraffin-celloidin. Serial unstained paraffin sections were examined in interference microscope MBIN-4 to determine the amount and concentration of total protein in the nucleus and cytoplasm as well as the area of the body, nucleus and cytoplasm of neurons (Brodsky, 1966; Mats *et al.*, 1979). Pyramidal neurons of layers III and V of sensomotor cortex as well as the caudate nuclei neurons were examined. Brains of the experimental and control mice were embedded into one block, and identical areas limited by fields 4—6 of the cortex and rostral parts of the caudate nucleus were taken for examinations. At least 50 cells were counted in each layer of the cortex and caudate nucleus. The results obtained were treated statistically using Student's t-test.

Results

One month p.i. no differences in the appearance or behaviour of scrapie-inoculated and control animals were observed. At this time the titre of the agent in the brain of scrapie-infected mice was 6.3 log LD_{50}/g. A general histological examination of infected mouse brains revealed no marked lesions typical of histopathological picture of advanced scrapie infection (spongiosis, loss of neurons, gliosis). Individual neurons and groups of neurons showed pycnosis of the nucleus and cytopasm, sometimes vacuolization and swelling of the cytoplasm. Comparison with brain preparations of control animals (both intact and inoculated with normal mouse brain), however, revealed no significant differences either in the intensity or in the extent of dissemination of these changes. Cytointerferometric examinations demonstrated certain changes caused by the scrapie agent, which are presented in Table 1 and Fig. 1. For more complete analysis, the changes occuring after inoculation of normal mouse brain alone (Fig. 1-II) were differentiated from those developing after inoculation with the scrapie agent (Fig. 1-I). When only statistically significant changes in neurons of brain cortex layers III and V and of caudate nucleus cells are considered, neurons were not found remaining indifferent to inoculation of normal mouse brain; they responded by decrease in the area of the nucleus and cytoplasm and an increase in the cellular protein concentration. When taking into account not only the stat-

Table 1. Changes of cerebral cortex and caudate nucleus neurons in mice inoculated with a normal and scrapie-infected mouse brain suspensions

Cell parameter	IIIrd cortical layer			Vth cortical layer			Caudate nucleus		
	1	2	3	1	2	3	1	2	3
Cell body area ($\mu^2 \pm m$)	100.71 $\pm$1.17	96.82 $\pm$0.96	107.33 $\pm$1.25	200.79 $\pm$2.68	188.56 $\pm$2.43	194.37 $\pm$2.66	57.18 $\pm$0.71	55.71 $\pm$0.62	60.52 $\pm$0.74
Nucleus area ($\mu^2 \pm m$)	48.39 $\pm$0.62	45.70 $\pm$0.54	50.99 $\pm$0.57	81.98 $\pm$1.20	80.59 $\pm$1.08	82.46 $\pm$1.21	34.64 $\pm$0.44	33.40 $\pm$0.45	36.10 $\pm$0.45
Cytoplasm area ($\mu^2 \pm m$)	52.40 $\pm$0.83	51.05 $\pm$0.69	56.12 $\pm$0.96	117.46 $\pm$1.98	106.58 $\pm$1.82	111.32 $\pm$1.98	22.60 $\pm$0.47	22.49 $\pm$0.42	23.20 $\pm$0.53
Amount of protein in the nucleus (pg $\pm$ m)	30.56 $\pm$0.54	31.54 $\pm$0.59	33.29 $\pm$0.66	40.12 $\pm$0.95	43.64 $\pm$0.97	42.84 $\pm$1.05	30.82 $\pm$0.52	31.70 $\pm$0.56	31.60 $\pm$0.59
Amount of protein in the cytoplasm (pg $\pm$ m)	53.14 $\pm$0.96	55.04 $\pm$1.19	59.03 $\pm$1.19	108.14 $\pm$2.12	108.37 $\pm$2.26	106.48 $\pm$2.44	41.30 $\pm$0.88	43.13 $\pm$0.79	43.34 $\pm$0.97
Concentration of protein in the nucleus ($pg/\mu^3 \pm m$)	0.1252 $\pm$0.0018	0.1279 $\pm$0.0021	0.1212 $\pm$0.0033	0.1021 $\pm$0.0044	0.1070 $\pm$0.0021	0.1030 $\pm$0.0017	0.1770 $\pm$0.0024	0.1888 $\pm$0.0021	0.1763 $\pm$0.0021
Concentration of protein in the cytoplasm ($pg/\mu^3 \pm m$)	0.2028 $\pm$0.0023	0.2143 $\pm$0.0028	0.2104 $\pm$0.0025	0.1851 $\pm$0.0024	0.2039 $\pm$0.0025	0.1925 $\pm$0.0065	0.3697 $\pm$0.0025	0.3813 $\pm$0.0025	0.3759 $\pm$0.0022

1 — intact mice; 2 — normal mouse brain suspension; 3 — scrapie-infected mouse brain suspension.

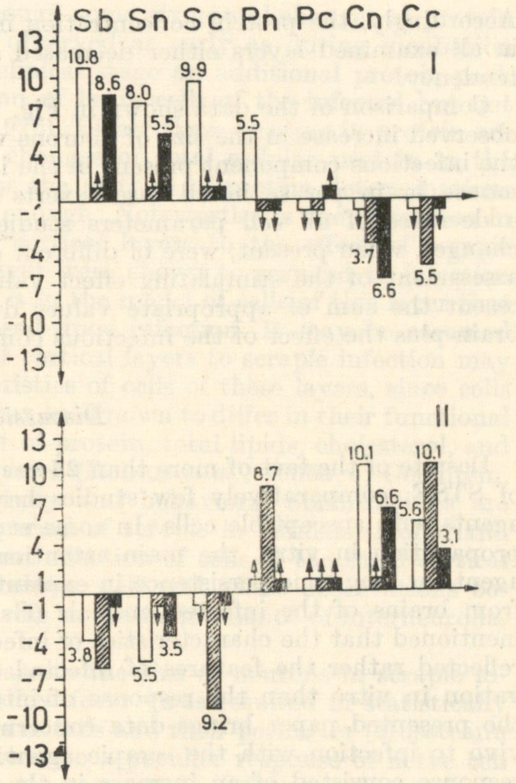

Fig. 1.
Changes in CNS of mice experimentally infected with the scrapie agent
Abscissa: S_b — cell body area, S_n — nucleus area, S_c — cytoplasm area, P_n — protein amount in the nucleus, P_c — protein amount in the cytoplasm, C_n — protein concentration in the nucleus, C_c — protein concentration in the cytoplasm.
Ordinates: I — per cent ratio of the parameters of brain cells of mice infected with the scrapie agent to those of mice inoculated with normal mouse brain suspension; II — per cent ratio of parameters of brain cells of mice inoculated with normal mouse brain suspension to those of intact mice.
empty columns — statistically significant changes in parameters of layer III cells in cerebral cortex
dashed columns — statistically significant changes in parameters of layer V cells in cerebral cortex
dashed columns — statistically significant changes of parameters of the caudate nucleus cells
↑↓ — direction of statistically insignificant changes

istically significant changes but also their direction (Fig. 1-II), it could be seen that the observed changes in the parameters were more or less manifested in all neuron layers of the brain. From the lack of quantitative changes of protein in the nucleus and cytoplasm of neurons, with an exception of the cells of layer V, the observed increase in the cellular protein concentration after inoculation of normal mouse brain may be considered to be due to cell compression.

Inoculation of mice with the scrapie agent resulted in definite and significant changes in some of the parameters under study, first of all, in an increase in the area of the body, nucleus, and cytoplasm of neurons. This effect, however, was not observed in all the layers examined but was most marked in cells of cortex layer III. In these cells, both nuclear and cytoplasmic areas increased proportionally. At the same time in the caudate nucleus the cell area increased only at the expense of increasing nucleus area, and cells of layer V showed only a trend for increase in these parameters (Fig. 1-I). As a rule, the increase in the cell size was not accompanied by significant changes in the protein amount in the nucleus and cytoplasm but only by an increase in the amount of protein in the nuclei of layer III neurons.

Accordingly, the protein concentration in nuclei and cytoplasm of neurons in all examined layers either decreased significantly or showed decreasing tendency.

Comparison of the data shown in Fig. 1-I and 1-II demonstrates that the observed increase in the size of neurons was completely due to the effect of the infectious component present in the inoculum. It is evident that normal mouse brain per se in all cases exerts a directly opposite effect causing a decrease of all cell parameters studied. Even statistically insignificant changes, when present, were of different directions. Thus, final quantitative assessment of the stimulating effect values after inoculation actually represent the sum of appropriate values due to the effect of normal mouse brain plus the effect of the infectious component alone.

Discussion

Despite of the fact of more than 25 years research on the causative agents of STSE, comparatively few studies have dealt with relationships of the agents with susceptible cells. In some studies attempting the scrapie agent propagation in vitro, the main attention was paid to the capacity of the agent for long-time persistence in explants and trypsinized cultures derived from brains of the infected animals (Gajdusek *et al.*, 1972). It should be mentioned that the characteristics of infected cells described in these papers reflected rather the features of infected animal cells upon long-term cultivation in vitro than the response of intact cells to infection. In contrast, the presented paper brings data concerning the response of intact cells in vivo to infection with the scrapie agent. As follows from our results, this response consisted of an increase in the size of neurons at the expense of increasing areas of both cytoplasm and nucleus. Since there was no marked increase in the amount of protein in the nucleus and cytoplasm, and protein concentration decreased slightly, these results are difficult to interpret in one way since they may be early stages of cell hypertrophy or phenomena associated with disorders in the watersalt balance. It is known that scrapie--infected animal brain cells cultivated in vitro showed an increased growth activity as compared to controls, an enlargement of nuclei, an increase in the number of multinuclear cells and a proliferation of neuroglia (Field and Windsor, 1965; Gustafson and Kanitz, 1965; Heig and Pattison, 1967; Webb, 1967; Clarke and Heig, 1970; Buenig and Gustafson, 1971; Caspary and Bell, 1971).

When evaluating the possible effect of the scrapie agent on nerve cells the capacity of STSE agents to induce cell fusion should be considered (Kidson *et al.*, 1978). A final evaluation of the nature of neuronal changes in the early stages of infection can be apparently obtained by further studies in the time course of infection. At the stage of infection studied in this work we failed to reveal quantitative changes in the amount of protein with exception of increased amounts of protein in nuclei of cells of the third cortical layer. In scrapie infection, the total protein synthesis in the brain is known not to change (Hunter, 1974), although some structural changes of protein in synaptosomal membranes were observed as early as during incubation period (Viret *et al.*, 1981). In the clinical stage an additional protein peak was found in the membrane fraction of brain cells of the infected but not control animals (Somerville *et al.*, 1976). Either these changes in protein are not accompanied by quantitative changes of total cellular protein in the early stages of incubation period or the method of protein determinations used in this study is not sensitive enough. Noteworthy is the difference in sensitivity of neurons of different cortical layers to the effect of scrapie agent. Cells of the third cortical layer were shown to respond to infection by most marked changes in size. It is in the nuclei of cells of this layer that the total amount of protein increased upon infection. It may be assumed that different sensitivity of different cortical layers to scrapie infection may be associated with diverse characteristics of cells of these layers, since cells of layers III and V of the motor cortex are known to differ in their functional importance as well as in the content of protein, total lipids, cholesterol, and in the level of activity of some enzymes (Robins *et al.*, 1956*a*, *b*; Gershtein, 1975). Disorders of the motor activity and behavioral abnormalities are known to occur in the incubation period of scrapie in animals (McFarland and Hotchin, 1980). The predominant affection of cells of the third cortical layer at this stage of infection correlates well with these observations because these cells play an important role in the maintenance of interneuronal contacts (Polyakov, 1965).

It is of interest that unlike increase in the size of neurons in scrapie infection, inoculation of mice with yellow fever virus resulted in statistically significant decrease of the area of nerve cells and their perimeter (Museteanu *et al.*, 1980). This may one again attest to a peculiar response of nerve cell to infection with STSE in vivo. At the same time, infection of monolayer cell cultures with conventional viruses is known to induce an increase in the size of nuclei (Khesin, 1967) and latent infection with influenza virus in L cells leads to an increase in the area of the infected cells (Timakov *et al.*, 1971).

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