ULTRASTRUCTURAL STUDY OF BRAIN SAMPLES OF THE LEFT TEMPORAL LOBE OF FETUSES FROM SCHIZOPHRENIC MOTHERS

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The neurodevelopmental hypothesis in the etiology and physiopathology of schizophrenia is considered one of the most consistent at present. It is based on a series of evidences that guide toward an affection in the critical period of the human being development due to pregnancy and delivery complications, particularly those with known or presumed impact on fetal neurological development, that result in increased risk for schizophrenia psychosis. Among the possible etiological candidates are viral infections. The minor physical and functional anomalies, manifesting as soft neurological sings, slight anatomical defects of the head, hair, eyes, mouth, hands and feet, as dermatoglyphic asymmetries, are due to some injury occurring during the first and more probable second trimester of fetal life, and are more common among patients with schizophrenia and in their unaffected siblings than in the general population. A virus acting in this important and critical stage of the development interacting or not with genetic factors can be responsible for the cascade of biological events that appear later on and could explain the period of relative stillness that exists between the birth and the appearance of the symptoms in the puberty that could be related to the reactivation of a latent viral infection. In the present work additional results are presented in an ultrastructural study carried out in samples of the left temporal lobe of two monozygotic and monochorionic fetuses aborted for medical reasons from a paranoid schizophrenic mother with strong familial antecedents of schizophrenia. The findings obtained are compatible with an active infection of the central nervous system by herpes simplex hominis type I [HSV1] virus during the second trimester of pregnancy in one of the studied fetuses. Until our recent report evidences supporting the concept of virus-cell interaction in the neurodevelopmental hypothesis of schizophrenia had been indirect. Virus particles had never been demonstrated. The present paper is the first direct evidence that demonstrate the presence of virus particles in the central nervous system of monozygotic fetuses in the critical period of the second trimester of foetal development. The importance of this finding can have practical applications in the prevention of the illness keeping in mind its direct relationship to the aetiology and physiopathology of schizophrenia.

Previous informed consent a brain sample is obtained [tip of the left temporal lobe] of two males 16 weeks gestational age aborted foetuses [monozygotic, monochorionic and biamniotic twins] of a paranoid schizophrenic mother who her pregnancy was interrupted for medical indications. One of the twins presented cryptorchidism. The samples were fixed in glutaraldehyde-paraformaldehyde and transported to the electron microscopy laboratory for electron microscopic techniques: general technique for transmission and immuno-electron microscopy. The obtained samples were being proceeded to their immediate fixation in 1% glutaraldehyde-paraformaldehyde during one hour. Washed in buffer phosphate of five minutes each one. Proceeded later on to the blockade of the endogenous peroxide with methanol more hydrogen peroxide during thirty minutes to ambient temperature. Later washed with several volumes of PBS during 15 minutes. Washed with TRIS, three volumes for five minutes every time. Normal goat serum was used diluted at the 1:5. It was added the antisera [HSV1] later during 24 hours, being proceeded later on to washing with TRIS (three volumes five minutes each one). DAB, eight minutes, to ambient temperature and washed with TRIS, post-fixation with osmium tetroxide to one percent during thirty minutes, washed with buffer phosphate (3 X 15 minutes), ethanol to thirty percent during five minutes, to fifty percent during five minutes, to seventy percent during ten minutes and absolute ethanol (3 X 20 minutes). propylene oxide (3 X 15 minutes): being proceeded to the inclusion in Epon I during sixty minutes, Epon II during five minutes each one. DAB, eight minutes, to ambient temperature and washed with TRIS, three volumes for five minutes every time. Normal goat serum was used diluted at the 1:5. It was added the antisera [HSV1] later during 24 hours, being proceeded later on to washing with TRIS (three volumes five minutes each one). DAB, eight minutes, to ambient temperature and washed with TRIS, post-fixation with osmium tetroxide to one percent during thirty minutes, washed with buffer phosphate (3 X 15 minutes), ethanol to thirty percent during five minutes, to fifty percent during five minutes, to seventy percent during ten minutes and absolute ethanol (3 X 20 minutes). propylene oxide (3 X 15 minutes): being proceeded to the inclusion in Epon I during sixty minutes, Epon II during the whole night, ending the definitive inclusion inclusions was made for blocks with dilutions from the antisera to 1/10 and 1/20, blocks controls of each dilution, blocks of the controls more control of the blocks controls in each dilution and general technique. In one of the studied foetuses [the one that presented cryptorchidism] it was observed within the nucleus of neurons the presence of spherical empty particles of 100 nm occupying the centre of an electron-lucid area [Fig.1]. The inclusions with particles appeared in number from 2 to 8 per nucleus, with great incidence in their appearance, practically in all the neurons. The size and form of the particles coincides with the observations made of similar particles in the brain of adult schizophrenics, in other studied foetuses and in animals experimentally inoculated with cerebrospinal fluid from schizophrenic patients using the same electron-microscopic techniques. The rest of the cells of the nervous system didn't present these particles. Presumptive evidence for a viral aetiology of schizophrenia requires the demonstration of a virus, antigen or viral antibody. In previous works we have obtained results that constitute a direct evidence in two of three of these requirements, virus and antigen [21]. Until our recent report evidence supporting the concept of virus-cell interaction in the neurodevelopmental hypothesis of schizophrenia had been indirect. Virus particles had never been demonstrated. The present paper is the first direct evidence that demonstrate the presence of virus particles in the central nervous system of monozygotic foetuses in the critical period of the second trimester of foetal development. The importance of this finding can have practical applications among other important aspects in the prevention of the illness keeping in mind its direct relation to the aetiology and physiopathology of schizophrenia. It should be considered the use of antiviral drugs in a preventive form in those susceptible women of having an offspring with high risk to suffer schizophrenia. Future studies directed to clarify the moment that is the first stage of subsequent biological events that lead to the illness would be necessary in order to contribute to a better knowledge of its cause and physiopathology.
This finding is a direct evidence obtained with a high resolution power technique at cellular level in a stage of the foetal development not explored previously and theoretically signal as vulnerable and explanatory of the later biological events in schizophrenia related to an aggression of the cell by environmental factors. An active viral infection of the central nervous system in this stage of the human development is demonstrated by the presence of immature viral particles similar in size and form to those observed in the brain of adult schizophrenic patients, other foetuses of schizophrenic mothers and animals inoculated with cerebrospinal fluid from schizophrenic patients. In this electron microscopic technique an anti-herpes HSV1 antibody without DAB was used. Observe the presence of several vacuoles in the nucleus of neurons [arrows Fig. 1A] of the left foetal temporal lobe of a monozygotic twin. Within these vacuoles appear virus empty capsids [red arrow Fig. 1B]. Bar: 100 nm Herpes virus has the characteristic of in novo membrane formation within the nucleus of neurons. In chicken embryos inoculated with cerebrospinal fluid from schizophrenic patients HSV1 antigen is present within the nuclei of neurons. [arrows] Fig. 1C. A mature viral particle is observed [inset] labelled by anti-herpes peroxidase conjugated antibody. The reactivation of latent virus in culture media is a characteristic of herpes virus. Although alternative explanations are possible, a most attractive possibility is that the infected neuron by HSV1 virus in foetal brain will serve as a source of latent virus and its later reactivation in limbic structures by environmental stressors would explain the recurrent character of schizophrenia. A vertical type transmission to offspring would restart the cycle of the illness in new generations.