positivity was found in this localization in the 13th week In older studied material (13–14th week of the intrauterine life) apoptotic nuclei failed to be proved in nervous system, eye and nasal cavity Single apoptotic cells were found in the ectodermal and mesodermal parts of the 9-week-old tooth germ and in the lingual anlage Later (in the 14th week) apoptosis disappeared in this localization The other parts of the orofacial region were negative

Expression of PCNA and Ki-67 increases with the age In correlation with expression of some other proteins it gives the information about the regulation of the cell cycle during embryonic and fetal differentiation of the studied organs Results of our study can be of some value for explanation of the proliferative activity of cells and at the same time for elucidation of the origin of anomalies in the studied regions

Detection of apoptosis was performed in the same material All studied regions were areas with high cell proliferation , appearance of apoptotic cell nuclei was rare in comparison with other organs which undergo the complicated development in early developmental period (e g kidneys, limbs) Our findings testify for hypothesis that the normal human embryogenesis is under multiple level control (Le Brun *et al* 1993)

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Detection of Ischemic Changes in the Cytoplasm of Neurocytes from Rat Brain and Spinal Cord by Densitometric Measurement of Methylene Blue Binding

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Abstract. Ischemic changes in neurocytes from brain and spinal cord of rats were studied by densitometric measurement of bound basic stain – methylene blue Statistically significant differences in integrated optical density (I O D) of cytoplasm near to cell nucleus in brain and spinal cord neurocytes were detected after ischemia After 10 minutes of

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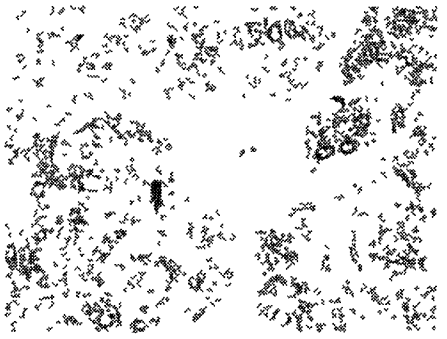

Figure 1. Neurocyte from the spinal cord - digitalized image, electronicaly filtered Mag 640 ×

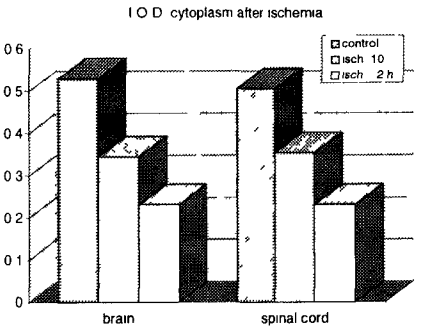

Figure 2. Optical density of the cytoplasm of neurocytes after ischemia

ischemia, the average values of I O D decreased to 65% and to 69,9% of I O D values of controls After 2 hours of ischemia, the average values of I O D in brain cell cytoplasm reached only 43,6% and in the spinal cord cells they fell to 54,5% of control values

Introduction

Disorders of the central nervous system blood supply following e g cardiovascular diseases have often serious consequences We studied ischemic changes of neurocytes of the brain and spinal cord in rats In one group of experiments, the optical density of cytoplasm was followed by densitometric measurement of methylene blue binding

The method of image analysis was used also for detection of ischemic changes in CNS (Gerhardt *et al* 1991, Tanaka *et al* 1994, Ginsberg *et al* 1996) We used this method to exactly define the optical density of cytoplasm after ischemia

Materials and Methods

Changes of optical density of the cytoplasm of neurocytes near cell nucleus from grey matter of the frontal lobe of the brain and from anterior horns of grey matter of the spinal cord were measured after 10 minutes and 2 hours of ischemia, respectively The ischemia was achieved by bilateral ligation of common carotid arteries The setup of the whole experiment and specimen preparation were described in details in one of our previous papers (1)

Semithin sections (1mm) from resin embedded tissue blocks were stained for 3 min by methylene blue and scanned by CCD camera equipped Olympus IMT 4 light microscope on line with a suitable computer Digitalized images (Fig 1) were analyzed using the transmission densitometric mode of RMS CUE-2 software (Galai Production Ltd, Israel)

20–40 subareas $1\mu m^2$ large in a distance of $1\mu m$ from nuclear membrane were analyzed Optical density of area unit was expressed in the form of integrated optical density - I O D Data obtained from optical density measurements are shown in Fig 2

Results

Some differences between neurocytes after ischemia and from controls can be recognized by light microscopic examinations After digitalization of images and consequent image analysis these differences can be more precisely determined

Statistically significant difference in integrated optical density after ischemia against controls was detected both in the neurocyte perinuclear cytoplasm from the brain and from the spinal cord In controls, the average values of I O D were 0,5305 " 0,0984 and 0,5063 " 0,0873, respectively (Fig 2) After 10 minutes of ischemia, the average values of I O D decreased to 65% and to 69,9% of I O D control values, respectively (0,3450 " 0,0506, $t_{4445} = 1{,}68$, $P < 0{,}5$, 0,3542 " 0,0746, $t_{3691} = 1{,}33$, $P < 0{,}5$) The differences were not statistically significant due to larger data scattering There was a statistically significant difference between I O D values from controls against those after 2 hrs of ischemia Average I O D values decreased to 0,2315 " 0,0445 ($t_{3825} = 2{,}77$, $P > 0{,}01$) and 0,2762 " 0,0554 ($t_{3825} = 2{,}23$, $P > 0{,}5$) This means, that in the analyzed region of neurocyte cytoplasm, the average values of I O D in brain cells reached only 43,6% and in the spinal cord cells 54,5% of control values

Discussion

Our results indicate that biochemical and structural changes in cell cytoplasm can clearly be recognized by densitometric method Numerous papers described the effects of ischemia in various types of cells – e g changes in brain lipids (Chavko and Nemoto 1992), in protein synthesis (Raley-Susman and Murata 1995), in cytochrome oxidase activity (Dimlich *et al* 1990, LaManna *et al* 1996), in alpha-tubulin and beta-actin mRNA accumulation (Kondo et al 1994), a s o Our results correspond with those reported in papers mentioned above, with our earlier electron microscopic observations (Beňuška *et al* 1986, Remiš *et al* 1989) as well as with our observations of I O D after hypoxia (Beňuška et al 1994, 1996) In conclusion, we can confirm, that measurement of methylene blue binding by densitometric mode of RMS software is useful for exact definition of changes in the optical density of the cell cytoplasm from CNS The method can contribute to speeding up and setting a correct diagnosis in cell pathology

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The Role of the Apoptosis and the Genes Regulating Apoptosis in the Early Differentiation of Human Embryo

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Abstract. Apoptosis (programmed cell death) is an important process participating in the formation of organs and tissues during embryogenesis Our aim of the work is studying the role of the apoptosis during the human embryonic differentiation We tend to give acquired findings into the correlation with expression of proteins Bcl-2 and Bax (products of genes regulating apoptosis) Detection of the apoptosis was carried out on 25 routinely processed human embryos by means of TUNEL technique The level of expression of Bcl-2 and Bax was determined using standard three-step immunohistochemical procedure Results were achieved by the comparison of apoptoic index and the level expression of Bcl-2 and Bax was semiquantitatively evaluated The low value of apoptic index was mostly accompanied by the high expression of Bcl-2 and the Bax expression was not proportionally related to the value of apoptic index

Key words: Apoptosis — Bax — Bcl-2 — Embryo — TUNEL

For every cell, there is a time to live and a time to die There is one mode of cell death that occurs under the normal physiological condition so orderly that it is often called programmed cell death (Kerr *et al* 1972) This process (also called apoptosis) plays an important role in the maintenance of homeostasis (e g hormone-dependent involution in the adult, death of immune cells after cytokine depletion or deletion of autoreactive T-cells in the developing thymus) as much as is needed for proper development of organs and tissues during embryogenesis (e g resorption of the tadpole tail during its metamorphosis into a frog, removal of the cells in interdigital parts of primitive autopodia, extinction of

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