DB 169

5-AZACYTIDINE INHIBITS CLOSURE OF THE CEPHALIC NEURAL TUBE IN EAT EMBRYOS.

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The head-fold stage rat embryos (9.5 days of gestation) were cultured for 48 h in rat serum supplemented with or without 0.2 ug/ml 5-azacytidine. Control embryos cultured for 33 h (16-somite stage) completed the fusion of cephalic neural folds. In contrast, the cephalic neural plate remaind open in 5-azacytidine-treated embryos after 48 h of culture. However, the extent of their development, which was scored according to the method of Brown and Fabro (1981), was not different between control and 5-azacytidine-treated embryos except the head region. There was no significant difference in DNA and protein contents between control and treated embryos cultured for 36 h. Embryos were sensitive to 5-azacytidine during 6-12 h in culture (3-5-somite stages). Immunocytochemical observations using 5-methylcytosine-specific antibody indicated that the methylation of DNA was depressed by 5-azacytidine in the median part and apices of the neural plate and in the area around the apices. These results suggest that the methylation of cells in these areas play an important role in closure of the cephalic neural tube.

DB 170

A GOLGI STUDY ON THE HISTOGENESIS OF THE NEOSTRIATAL NEUORNS IN THE CAT.

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The histogenesis of the neostriatal neurons was studied in the cat using the rapid Golgi method. The neostriatal neurons were derived from the striatal elevation protruding into the ventrolateral region of the lateral ventricle. Cells seen in the striatal elevation had irregularly shaped somata which generated several processes, and migrated toward the pial surface to differentiate into primordial neostriatal neurons. Primordial neostriatal neurons had irregularly shaped cell bodies which extended one thin long process and two to four short ones. The precursor form of the neostriatal medium-sized spiny neurons was first identified as spindle- or irregularly shaped cells. As the development proceeded, immature spiny neurons increased in somatic size as well as in the number and length of processes. In later developmental stages, the processes became thick and possessed numerous spines. The primordium of neostriatal medium-sized aspiny neurons had oval or triangular cell bodies, from which three to six thin processes extended. In older specimens, the number and length of processes increased considerably. However, many of the processes remained thin and had few spines. Aspiny neurons differentiated later than spiny neurons, and some showed immature features even in postnatal stages.

DB 171

GAP JUNCTION mRNA EXPRESSION IN THE NEONATAL RAT BRAIN.

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Gap junctions are considered to play an important role in intercellular communication. Possible participation of gap junctions is presumed in the neuronal organization of developing brain. We studied the cellular distribution of the mRNA for gap junction protein in the brain of rats at postnatal day 2 by using in situ hybridization. A complementary DNA specific for the mRNA for rat liver gap junction protein (connexin 32)(a gift from Dr. D.A. Goodenough) was applied to in situ hybridization histochemistry on cryostat and paraffin sections of the middle level of the brain. Autoradiographic signals for connexin 32 mRNA were found to distribute in various regions of the brain such as frontal cortex, hippocampus, thalamus, striatum and anterior hypothalamus. These signals were localized on neurons, glial cells and ependymal cells. The level of expression of connexin 32 mRNA on cryostat sections was much higher than that on paraffin sections. These results indicate that connexin 32 mRNA may be expressed in neural substrates in the neonatal rat brain, and that the process of paraffin embedding may result in degradation of connexin 32 mRNA in tissues.

DB 172

EFFECTS OF HORSE SERUM ON THE DEVELOPMENT OF ORGANOTYPIC CULTURE OF NEWBORN MOUSE CEREBELLUM.

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Cerebellar culture has been used as a useful model for studying the development of central nervous system. Previously, we reported that under standard conditions (feeding medium contains 23% horse serum) biochemical changes are closely related to morphological development in nearly same manner as in vivo. Without serum, myelin formation does not occur. To clarify the role of serum, we examined several biochemical changes in the early developmental stages in the absence of horse serum. In explants incubated in serum-free medium (SFM) from first day in vitro(1 DIV), protein contents decreased rapidly during first week of incubation, but, the amounts of γ-aminobutyric acid, an inhibitory neurotransmitter, increased according to the same pattern observed in serum-containing medium(SCM). In explants transferred to SFM from SCM at 8DIV, cerebrosides, the major glycolipids of myelin, did not increse during the incubation period. In explants transferred to SFM at 15 DIV, myelin could be observed even at 22 DIV, indicating that serum is not necessary for the maintenance of myelin.

From these results, it was supposed that horse serum contains the factor(s) which induce oligodendrocyte differentiation.