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DISTRIBUTION OF NMDA RECEPTORS IN HUMAN BRAINSTEM DURING DEVELOPMENT AND ASSESSMENT OF POSSIBLE IMPLICATION WITH SIDS

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ABSTRACT - Background: Many neuroactive molecules play an important role in the CNS during development. Among them glutamate, acting through NMDA and non-NMDA receptors, has been indicated as crucial in neurotransmission, neuronal maturation, plasticity but also in excitotoxic injury.

NMDA receptors in particular seem to be involved, during fetal life and early infancy, in neuronal rearrangement also by means of apoptosis. Particularly we were interested in clarifying the role of these receptors in brainstem nuclei regulating respiratory and autonomic responses to hypoxic-ischemic insults. Indeed a developmental disorder in these nuclei, and in particular in the arcuate nucleus of the ventral medullary surface, seems to be involved in sudden infant death syndrome (SIDS), the leading cause of postnatal death in rich countries.

Methods: on the basis of these considerations we decided to apply, despite the difficulties of operating on human brain postmortem tissues, the immunohistochemical technique on formalin fixed and paraffin embedded slices using, in the first set of experiments, a monoclonal antibody against the NR1 subunit of the NMDA receptors. The study was carried out in 29 fetuses at different gestational ages, 23 SIDS cases and 10 age-matched control cases consisting of infants who died for identified pathologies and 26 adult cases.

Results:

1- The NR1 subunit is expressed with different degrees of intensities in all the nuclei analyzed (Hypoglossal N., Dorsal Motor Nucleus of the Vagus, N. Solitary Tract, Ambiguous N., Principal Inferior Olive N., Arcuate N.).

2- The NR1 subunit is expressed in glial cells at very different rate. Preliminary results give evidence in favour of an astrocytic expression. Anyhow, further characterization with a double staining procedure using antibodies specific for glial fibrillary acidic protein (GFAP), microglia and brain macrophages is on going.

3- Statistical analysis of the different group of age expression levels in the nuclei analyzed showed a significative increase between midgestation, early infancy and maturity ($P=61603;0.0001$ in all Nuclei except the Dorsal Motor Nucleus of the Vagus $P=0.027$).

4- Statistical analysis of SIDS and control data distributions revealed no significative differences of NR1 expression between the two groups.

5- NR1 expression didn't correlate ($p=0.519$) with the hypoplasia of the Arcuate Nucleus, a condition occurring in the 30% of SIDS cases (*Acta Neuropathol.* 99:371-75, 2000).

In conclusions the developmental expression of the NR1 subunit of the NMDA receptors may be relevant to the maturational changes occurring during late pregnancy and early infancy. Even more significant could be the results obtained studying the expression of the other subunits of the receptor. Molecular subtypes composition differences in fact, give rise to changes in ligand affinity and kinetics properties of the receptors, this indeed could be relevant to the maturational changes in the physiologic response to hypoxia. Analysis of the other NMDA receptor subunits expression following the same protocols is one of our next steps.

Differences in glial expression of the NR1 subunit is also intriguing because it has been suggested that astrocytic receptors activity can monitor glutamate release by neighboring axon terminals.

ANATOMO-PATHOLOGICAL TECHNIQUES FOR THE STUDY OF BRAINSTEM IN SUDDEN INFANT DEATH SYNDROME (SIDS) AND UNEXPECTED LATE FETAL STILLBIRTH

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ABSTRACT - Recent observations have identified, both in SIDS victims and stillborn fetuses, frequent developmental abnormalities in the brainstem, particularly in the arcuate nucleus (ARCn), an important cardio-respiratory center of the ventral medullary surface. ARCn hypoplasia, sometimes associated with alterations in other brainstem structures, has been detected in 30-35% of SIDS and stillbirth cases. Aim of the present work is to define our anatomo-pathological techniques for the study of the brainstem. The procedure for the complete examination of the brainstem involves trasversal serial 5-6µm sections made throughout the entire brainstem. The number of serial sections varies in relation to the age of the analyzed victim, from 360 in the stillborn to 600 in the term fetus to over 1,400 sections in the SIDS victims. Such procedure, although having the merit of the completeness and accuracy, it is not routinary applicable in all histopathological laboratories, for the obvious necessities of additional technical personnel. The procedure for the simplified examination of the brainstem allows a remarkable reduction of the sections number. It requires a careful and precise sampling. The brainstem is divided in three blocks: the first cranial one is extended by the border between medulla oblongata and pons up to the upper pole of the olivary nucleus; the second, intermediary one, correspondent to the sub-median area of the inferior olivary nucleus, has as point of repere the obex and it is extended 2-3 mms above and under the obex itself; the third, caudal one, includes the lower pole of the inferior olivary nucleus and the lower adjacent area of the medulla oblongata. The examination of brainstem in 106 SIDS victims, in 30 control infants died within the first year of life, and of 51 stillborn after the 25th week of gestation, has underlined a remarkable variability, particularly of the ARCn, both in dimension and the neuronal density. Therefore, the complete evaluation the brainstem's structures and their possible abnormalities and/or alterations, requires its full examination on serial sections or, in the simplified procedure, the individualization of defined and constant section levels, identifiable through anatomical repere points. In the first cranial block, the ARCn is well recognizable, either medially and in the initial lateral portions, the hypoglossus nucleus, the dorsal nucleus of the vagus, the tractus solitarius nucleus and the ambiguous nucleus are just recognizable, while the reticular formation and the inferior olivary nucleus are well evident. In the second block, the ARCn appears clearly in the lateral portions, while is reduced in the medial portion; the hypoglossus, dorsal of the vagus, ambiguous, inferior olivary nuclea and the reticular formation are clearly detectable. In the third block the ARCn is detectable, particularly in the fetuses, in the lateral portions, while medially it is absent; all the other nuclei are well recognizable. From the comparative examination of our observations on the ARCn with those reported in the main anatomical textbooks and precisely the texts by Testut, by

Martin, and the Olszewski and Baxter atlas, agreements and discordances have emerged. On the basis of these considerations, we conclude that the autopsy protocol for the SIDS and stillborn victims, must include the examination of the autonomous nervous system according to the herein described guidelines, available at our web site: http://users.unimi.it/~patol/sids/tecnica_e.html.

POSTNATAL APOPTOSIS OF THE CARDIAC CONDUCTION SYSTEM IN CRIB DEATH: PRELIMINARY RESULTS

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ABSTRACT - The cardiac theory that crib death may be related to lethal arrhythmias or heart block due to abnormalities of the conduction system is gathering a renewed interest. The aim of this study is to determine the presence and significance of apoptosis in the cardiac conduction system in the crib death. Postnatal morphogenesis of the human heart is an important part of its normal development. However, the nature of the postnatal changes in the cardiac conduction system and its relation to crib death is not yet fully explained. The cells protruding from the atrio-ventricular node and His bundle are undergoing cardiac molding, named resorptive degeneration. Such molding consists of degeneration, cell death and replacing in an orderly programmed way. Apoptosis, with its unpredictable but rapid occurrence, could play a role in the pathogenesis of crib death. Such process, if defective, could leave in place some accessory communication between the atrio-ventricular pathway and the adjacent ordinary myocardium. We analyze several hearts from autopsied crib death cases and from age-matched controls. The cardiac conduction system was removed in two blocks: the first included the sino-atrial node and the crista terminalis, the second contained the atrio-ventricular node, His bundle, and bundle branches. Histological examination of the cardiac conduction system was performed on serial sections, using in situ endolabeling of fragmented DNA (TUNEL) method. Apoptosis of the cardiac conduction system is discussed as a process favoring lethal electrical instability.

STUDY OF THE CYTOARCHITECTURE OF THE PARABRACHIAL/KÖLLIKER-FUSE COMPLEX IN SIDS AND FETAL LATE STILLBIRTH

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ABSTRACT - The parabrachial / Kölliker-Fuse (PB/KF) complex has been defined in different animal species as lying in the dorsolateral part of the pontine tegmentum and subdivided into three well defined regions: the medial parabrachial nucleus (mPBn), the lateral parabrachial nucleus (lPBn), and the Kölliker-Fuse nucleus (KFn). Experimental studies have demonstrated that the PB/KF complex is involved in a variety of functional activities and plays an important role in the respiratory activity.

In man, the impossibility of using experimental approaches, makes it difficult to characterize the cytoarchitecture and the physiology of these structures. Only a few studies have provided morphological data regarding the human PB complex, but these observations are imprecise and conflicting.

The aim of the present study was to examine a large sample of human brainstems in order to define the precise morphology of the mPB, lPB and KF nuclei and to determine whether these centers show morphologic alterations in SIDS and fetal late stillbirth, whose etiopathogenesis is still unknown.

In serial sections of 24 human brainstems of subjects aged from 32 gestational weeks and one year (from 6 stillbirths, 7 SIDS cases, and 11 infants with defined cause of death) we studied by morphologic and morphometric analyses the cytoarchitecture and the extension of the PB/KF complex.

We observed that its morphology is homogeneous in all cases; therefore we defined the precise structure of the three nuclei. The features of the lPBn and of the mPBn are largely consistent with those reported in experimental studies.

The lPBn in transverse sections is located between the lateral surface of the pcs and the lateral lemniscus. It extends vertically from the level of the pons-mesencephalon junction (cranial pole) to the level where the lateral lemniscus nucleus is clearly visible (caudal pole). The size of the lPBn decreases from cranial to caudal pole. The neurons are round or tapering, with a light, often central nucleus, prominent nucleolus and scarce cytoplasm.

The mPBn lies medially to the pcs in transverse sections, between the motor nucleus nervi trigemini and the locus ceruleus up to the ventral termination of the pcs. Longitudinally its size does not change from the rostral pole (pons-mesencephalon junction) to the caudal pole (where the lateral lemniscus nucleus is clearly visible). It contains numerous oval and polygonal neurons, which are usually larger than the lPB neurons and have darker and more evident cytoplasm.

Instead, we found some morphologic differences between the KFn in man and those of other animal species, to wit: 1) It has a lesser degree of definition and circumscription; 2) as appreciated in transverse sections, it is made up of an area of clustered neurons (subnucleus compactus) and an adjacent area with dispersed neurons (subnucleus dissipatus); 3) It is not restricted to the cranial portion of the pons, but also detectable in the caudal tract of the mesencephalon. On the basis of these observations, it can be concluded that the KFn appears to be more developed in man than in other animal species, and that it shows a more extended and complex structure, with connections to the mesencephalon.

We plan to examine an even wider sample of cases in order to confirm our observations and to determine whether there are no morphologic differences in the parabrachial area of stillbirths and SIDS victims. We are also studying another nervous center (the gigantocellular nucleus) located in the caudal tract of the pons, which seems to be closely connected to the PB/KF complex in the control of respiratory activity, by inhibiting the interruption of inspiration.