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Developmental alterations of the auditory brainstem centers — Pathogenetic implications in Sudden Infant Death Syndrome

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ABSTRACT

Sudden Infant Death Syndrome (SIDS), despite the success of campaigns to reduce its risks, is the leading cause of infant death in the Western world. Even though the pathogenesis remains unexplained, brainstem abnormalities of the neuronal network that mediates breathing and protective responses to asphyxia, particularly in the arousal phase from sleep, are believed to play a fundamental role. This is the first study to identify, in SIDS, developmental defects of specific brainstem centers involved in hearing pathways, particularly in the cochlear and vestibular nuclei, in the superior olivary complex and in the inferior colliculus, suggesting a possible influence of the acoustic system on respiratory activity. In 49 SIDS cases and 20 controls an in-depth anatomopathological examination of the autonomic nervous system was performed, with the main aim of detecting developmental alterations of brainstem structures controlling both the respiratory and auditory activities. Overall, a significantly higher incidence of cytoarchitectural alterations of both the auditory and respiratory network components were observed in SIDS victims compared with matched controls. Even if there is not sufficient evidence to presume that developmental defects of brainstem auditory structures can affect breathing, our findings, showing that developmental deficit in the control respiratory areas are frequently accompanied by alterations of auditory structures, highlight an additional important element for the understanding the pathogenetic mechanism of SIDS.

1. Introduction

The current definition of SIDS (acronym of Sudden Infant Death Syndrome) is, as well known, "the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history" [1]. Despite the success of campaigns to reduce the risks introduced worldwide, the rate of SIDS has remained relatively stable since 2001, with around 2300 infants dying each year in the USA [2]. Even if the pathogenesis of this syndrome remains unexplained, a disturbance in respiratory activity, due to developmental alterations of a specific central neural network, is believed to play a prominent role [3,4]. Indeed, breathing is the result of connections and interactions among several neuronal populations within the brainstem, including the preBötzinger nucleus (preBötN) in the medulla oblongata, and the Retrotrapezoid/Parafacial complex (RT/PFC) and the Kölliker–Fuse nucleus (KFN) in the pons [5–10]. This network is crucial in generating respiratory rhythm and in controlling the breathing, particularly in the responsiveness to arousal from sleep, that demands an increased ventilatory activity.

Acoustic stimulation has been shown to interfere with this central respiratory pattern [11]. Animal and human research has demonstrated that specific brainstem centers are involved in the hearing pathways, namely: the dorsal and ventral cochlear nuclei (DCN and VCN), the medial and inferior vestibular nuclei (MVN and IVN) in the rostral medulla oblongata, the superior and lateral vestibular nuclei (SVN and LVN), the superior olivary complex (SOC) in the caudal pons, and the inferior colliculus (IC) in the caudal midbrain [12–14]. The anatomical proximity of the auditory centers to the respiratory network components in the brainstem allowed us to presume, given obviously the impossibility to perform experiments in humans, that there are synaptic connections between them, thus explaining the influence of acoustic inputs on respiratory activity [15]. We therefore hypothesized that an intact

Abbreviations: ABS, auditory brainstem system; ANS, autonomic nervous system; CN, central nucleus; DC, dorsal cortex; DCN, dorsal cochlear nucleus; EC, external cortex; IC, inferior colliculus; IVN, inferior vestibular nucleus; KFN, Kölliker–Fuse nucleus; LSO, lateral superior olivary nucleus; LVN, lateral vestibular nucleus; MSO, medial superior olivary nucleus; MVN, medial vestibular nucleus; PO, periolivary nuclei; preBötN, preBötzinger nucleus; RT/PFC, Retrotrapezoid/Parafacial complex; SIDS, Sudden Infant Death Syndrome; SOC, superior olivary complex; SVN, superior vestibular nucleus; TEOAE, transient evoked otoacoustic emission; VCN, ventral cochlear nucleus.

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development and function of the auditory brainstem system (ABS) might be necessary to maintain a regular ventilatory activity, above all during the vulnerable sleep phase in infancy, and that a disruption or defective development of auditory nuclei might play a critical role in inducing a predisposition to SIDS. Previously, we have already reported cytoarchitectural and functional alterations of the SOC and of the IC in victims of sudden perinatal death, suggesting a potential association between hearing centers and ventilatory activity [16,17].

Our interest in the present study was focused on the developmental state of all the brainstem centers involved in hearing control, in a cohort of infants who died in the first year of life of known and unknown causes. The main aim was to validate the supposed intersection between auditory function and arousal and, in particular, to evaluate whether alterations of the ABS development may contribute in triggering a sudden infant death, thereby taking a further step toward a fuller understanding of the pathogenesis of SIDS.

2. Materials and methods

2.1. Study subjects

The study included 69 infants, sent to our Research Center and diagnosed according to the application of the guidelines stipulated by Italian law n.31/2006 "Regulations for diagnostic post mortem investigation in victims of Sudden Infant Death Syndrome (SIDS) and sudden fetal death" [18]. This law decrees in particular that all infants who died suddenly within the first year of age, of suspected "SIDS", must undergo an in-depth anatomo-pathological examination, with a specific in-depth study of the autonomic nervous system (ANS). Parents of all subjects provided written informed consent to the autopsy examination, under protocols approved by the institutional review board (IRB) of the "Lino Rossi" Research Center, University of Milan.

After the routine autopsy and clinical history analysis, the death remained unexplained and hence a diagnosis of "SIDS" was made for 49 infants, 21 females and 28 males, who died within the first 8 months of life (mean age ± SEM, 3.64 + 0.47 months). In the remaining 20 cases (10 females and 10 males, mean age ± SEM, 3.57 + 0.42 months), a precise cause of death was formulated at autopsy. Specific diagnoses were congenital heart disease ($n = 10$), severe bronchopneumonia ($n = 4$), myocarditis ($n = 1$), pulmonary dysplasia ($n = 3$), pneumonia with acute respiratory distress ($n = 1$), and mucopolysaccharidosis type I ($n = 1$). These cases were regarded as "controls", also being similar with regard to gender, ethnicity and age at the time of death to SIDS cases.

2.2. Neuropathological examination

In all cases an in-depth histological examination of the ANS was performed, with the principal aim of detecting even fine developmental alterations of the brainstem structures controlling the vital functions. The study methodology is available at the website: http://users.unimi.it/centrolinorossi/en/guidelines.html of the "Lino Rossi" Research Center, University of Milan.

Briefly, after fixation in 10% phosphate-buffered formalin, the brainstem was processed and embedded in paraffin. Then three specimens were taken. The first specimen included the upper third of the pons and the adjacent caudal portion of midbrain, below the superior colliculus and including the inferior colliculus; the second specimen extended from the upper third of the medulla oblongata to the adjacent caudal portion of the pons; and the third specimen included the obex. Transverse serial sections from the midbrain, pons and medulla oblongata samples were made at intervals of 60 μm. For each level, six 4 μm sections were obtained, three of which were routinely stained for histological examination using hematoxylin–eosin, Klüver–Barrera and Bielschowsky's silver impregnation technique. The remaining sections were saved for further investigations and stained as deemed necessary.

2.2.1. Histological examination of the brainstem

The routine histological evaluation of the brainstem was focused on the hypoglossus, the dorsal motor vagal, the tractus solitarius, the ambiguus, the inferior olivary, the pre-Bötzinger, the arcuate, the dorsal and ventral cochlear nuclei, the medial and inferior vestibular nuclei, and the obscurus and pallidus raphé nuclei in the medulla oblongata; on the locus coeruleus, the retrotrapezoid/parafacial complex, the superior olivary complex, the superior and lateral vestibular nuclei, the Kölliker–Fuse, and the median and magnus raphé nuclei in the pons; and on the inferior colliculus, the substantia nigra, and the dorsal and caudal linear raphé nuclei in the caudal mesencephalon. Histological and histochemical observations were carried out blindly by two independent pathologists. Comparison of results was performed employing K statistics (K Index — KI) to evaluate the inter-observer reproducibility. The Landis and Koch system [19] of K interpretation was used, where 0 to 0.2 is slight agreement, 0.21 to 0.40 indicates fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 strong or substantial agreement, and 0.81 to 1.00 indicates very strong or almost perfect agreement (a value of 1.0 being perfect agreement). The application of this method revealed a very satisfactory KI (0.87).

2.3. Risk factor information

For each case, all available information about pregnancy, delivery and about the environmental and familial situation where the death occurred, besides information related to both "preventable" and "unpreventable" risk factors [20], was collected and categorized during post-mortem family interviews. All the information sheets were recorded in the registry of a dedicated data bank, as stipulated by Italian law n. 31. Attention has been given to the preventable factors well known in literature as associated with SIDS, and that could be avoided. In particular, mothers were asked to complete a questionnaire probing smoking habit and, if they were smokers, detailing the number of cigarettes smoked before, during and after pregnancy. Smoking habit was assigned to two categories (smokers vs. nonsmokers). Overall, 32 of the 69 mothers (46%) were smokers of more than 3 cigarettes/day already before the onset of pregnancy, and 37 were nonsmokers. Since the retrospective assessment of the smoking habit of a mother, mainly after the death of her son, is sometimes unavoidable, the negative self-reports were validated by the urinary measuring of cotinine, the main metabolite of nicotine.

Twenty-six women in the smoker group were mothers of a victim of SIDS and 6 of control cases.

2.4. Statistical analysis

Quantitative data were expressed as means ± SEM. The significance of differences between parameters of the groups of victims was evaluated by the Student-t test, the χ^2 test or Fisher's test. In case of skewed distribution, a nonparametric Whitney rank sum test was used. One-way ANOVA was used for quantifying and partitioning variance between groups. Statistics were compiled and plotted using SigmaPlot® (version 13, Systat Software Inc., Chicago, IL). The threshold level set for statistical significance was $p < 0.05$.

3. Results

In accordance with the aim of this study, an in-depth histological examination of the ANS was mainly focused on the brainstem centers presiding over the sense of hearing.

3.1. Examination of the brainstem auditory network

3.1.1. Cochlear nuclei

The cochlear structure is the first synaptic relay station of the auditory pathway and the termination site of all auditory nerve fibers [21,22]. It is best analyzed at the rostral pole of the medulla oblongata at the

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