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Arousal deficiency theory in sudden infant death syndrome with reference to neuronal plasticity

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Abstract

Objective: Among 27,000 infants studied prospectively to characterize their sleep–wake behavior, 38 infants died under 6 months of age (including 26 infant victims of sudden infant death syndrome (SIDS), five with congenital cardiac abnormalities, two from infected pulmonary dysplasia, two from septic shock with multi-organ failure, one with a prolonged seizure, one from prolonged neonatal hypoxemia and one from meningitis and brain infarction).

Method: The frequency and duration of sleep apnea events recorded some 3–12 weeks before the infants' deaths were analyzed. Brainstem material from these 38 infants was studied in an attempt to elucidate the relationship between sleep apnea and neuronal pathological changes in the arousal pathway. The histochemical analyses included Bielschowsky staining and the immunohistochemical analyses included the evaluation of growth-associated phosphoprotein 43 (GAP43) and of synaptophysin as markers for synaptic plasticity. Neurofibrae with positive pathological reactions were quantitatively analyzed. Pathological and physiological data were linked for each infant.

Results: The correlation between sleep apnea and neuronal plasticity in the arousal pathway of the SIDS victims was not seen in the control infants and the correlation between sleep apnea and neuronal plasticity in the arousal pathway found in the control infants was not seen in the SIDS victims.

Conclusion: These findings suggest that neuronal plasticity in the brainstem arousal pathway is related with SIDS.

Keywords: Sudden infant death syndrome; Sleep apnea; Neuronal plasticity; Gap43; Synaptophysin; Bielschowsky staining; Arousal

1. Introduction

Several etiological hypotheses have been proposed for sudden infant death syndrome (SIDS), including the role of apnea [1,2] and sleep arousal [3–5].

Neuropathological studies suggest that the brainstems of infants who succumbed to SIDS were characterized by minute changes resulting from hypoxia. These changes include gliosis and apoptosis [6]. Gliosis was evaluated by the immunohistochemical detection of glial fibrillary acidic protein (GFAP) in the brainstem of SIDS victims [7–9].

Few reports have substantiated the presence of depressed arousal from a neuropathological aspect. A recent report suggests that abnormality of neuronal plasticity was detected in the arousal pathway by growth-associated phosphoprotein 43 (GAP43) [10].

This study tested additional pathological stainings that could show changes of neuronal plasticity. The presence of sleep apnea was correlated with the histological findings for each infant.

2. Methods

2.1. Physiological analyses

2.1.1. Subjects studied

The sleep characteristics of 38 apparently healthy infants were prospectively recorded some 3–12 weeks before they

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died. They had been selected from over 27,000 infants studied prospectively over 20 years to determine their sleep–wake characteristics in several Belgian pediatric sleep laboratories. The families were invited to join the study when leaving the maternity ward and gave their written informed consent. The infants were included in the study if they were born at term after a normal gestation, and if there was no family or personal history of apnea, apparent life threatening event (ALTE) or SIDS. At the time of recording, the infants were aged between 2 and 27 weeks, were healthy, and were receiving no medication.

Some weeks after the sleep recordings, 38 infants died suddenly and unexpectedly (Tables 1 and 2). Following postmortem examinations and death scene investigations, 26 of the 38 infants were considered to be SIDS victims [11]; five died from congenital cardiac abnormalities; two from infected pulmonary dysplasia; two from septic shock; one from a prolonged seizure; one from prolonged neonatal hypoxemia, and one from meningitis and brain infarction. The maximum delay between the estimated time of death and the postmortem examination was 24 h.

2.1.2. Polygraphic monitoring

The 8-h sleep studies were conducted overnight in a sleep laboratory, following standard recording techniques [12–15]. The recordings were performed in a quiet and darkened room, at an ambient temperature ranging between 20 and 23°C. All infants slept supine, without restraints. Recording started around 21:00 hours. The infants were observed continuously during recording and were fed on demand. Their behavior and any nursing intervention were charted. No infant had a pacifier during the recording sessions. The following variables were recorded simultaneously: two scalp electroencephalograms with central and occipital leads, two electrooculograms and an electrocardiogram. Thoracic respiratory movements were measured by impedance and airflow with thermistors taped under both nostrils and on the side of the mouth. Oxygen saturation was recorded continuously by a transcutaneous sensor (Nellcor, USA). Gross body movements were measured using an actigram placed on one arm. The data was collected on a computerized infant sleep recorder (Alice recording system III, Healthdyne, USA).

2.1.3. Ethical issues

This study was approved by the Ethical Committee of the University Children's Hospital and was performed in accordance with the ethical standards prescribed by the 1964 Declaration of Helsinki.

2.1.4. Data analysis

Based on the polygraphic recordings, sleep stages and sleep apnea events were scored according to standard definitions [12–16]. Apnea events were scored when they lasted 3 s or longer. They were classified as central apnea when flat tracings were obtained simultaneously from the strain gauges and thermistors. Obstructive apnea was defined as continuous deflections from the strain gauges, with a flat tracing recorded from the thermistors. Mixed apnea was defined as a central apnea directly followed by an obstructive apnea and was scored together with the obstructive episodes. The frequency of obstructive apnea events was measured by dividing the total number of apnea events by the total sleep time in minutes and multiplying by 60. The type, frequency (number per hour of sleep) and duration (in seconds) of sleep apnea events were computed. The recordings were analyzed visually by two independent scorers without knowledge of the subject's age or sex to ensure reliability. Discrepancies were discussed and the agreed score was computed for analysis.

Table 1
Significant correlations between physiological data and pathological data only in SIDS victims[a]

Physiological data	Pathological data	Correlation coefficients	*P*-value
Frequency of obstructive apnea	Number of GAP43-positive neurons in MBDR	−0.8124	0.026*
Frequency of obstructive apnea	Number of GAP43-positive neurons in PPTN	0.9010	0.006**
Frequency of obstructive apnea	Amount of Bielshowsky-positive neurofibrae in MBDR	−0.9739	0.026*
Frequency of central apnea	Amount of Bielschowsky-positive neurofibrae in MBDR	−0.9597	0.010*
Frequency of central apnea	Amount of Bielschowsky-positive neurofibrae in MBPG	−0.9597	0.010*
Frequency of central apnea	Amount of Bielschowsky-positive neurofibrae in PPTN	−0.9010	0.006**

[a] GAP43, growth-associated phosphoprotein 43; MBDR, dorsal raphe in midbrain; PPTN, pedunculopontine tegmentum nucleus; MBPG, periaqueductal

Table 2
Significant correlations between physiological data and pathlogical data only in control cases[a]

Physiological data	Pathological data	Correlation coefficinents	*P*-value
Frequency of obstructive apnea	Number of spines of GAP43-positive neurons in PPTN	0.8289	0.021
Frequency of central apnea	Number of spines of GAP43-positive neurons in PPTN	−0.8999	0.006

[a] GAP43: growth-associated phosphoprotein 43, PPTN: pedunculopontine tegmentum nucleus.

Statistical analysis was conducted using SAS statistic analysis system, release 6.12.

2.2. Pathological findings

2.2.1. Subjects analyzed

A total of 48 paraffin blocks were collected from the brain stems of the 38 infants who died unexpectedly: seven blocks from the midbrain, 22 from the pons, and 19 from the medulla oblongata. The delay between the death and the postmortem examination was within 24 h.

2.2.2. Histological and histochemical brain examination

Hematoxylin–eosin (HE) staining and Bielschowsky staining [17] were carried out as standard histological brain staining.

2.2.3. Immunohistochemical examination

The blocks were subjected to immunohistochemical analysis, using an anti-GAP43 monoclonal antibody (YLEM, Italy; 1:20) and an antihuman synaptophysin polyclonal antibody (A0010, DAKO Japan, Japan, 1:100). The 4-micron thick sections made from each block were preincubated with 1 mM of EDTA solution (pH, 8.0) using a microwave oven (Panasonic) at 800 W for 10 min to stain GAP43 and preincubated with target retrieval solution (DAKO S1699) using an autoclave at 120°C for 10 min to stain synaptophysin. After the blocking of intrinsic peroxidase by 3% hydrogen peroxide for 5 min and washing, the sections were incubated with antibody overnight for GAP43 and for synaptophysin at 4°C. Finally, GAP43 and synaptophysin were immunohistochemically visualized with the aid of an LSAB2 kit (Dako) and DAB reaction.

2.2.4. Quantitization of histochemistiy and immunohistochemistry

Measurements were made in the periaqueductal gray matter in the midbrain (MBPG) and the dorsal raphe nucleus in the midbrain (MBDR) as well as the pedunculopontine tegmental nucleus (PPTN).

The number of spines per one GAP43-positive neuron and the number of GAP43-positive neurons were counted manually. Counting in an area $625 \times 102\ \mu m^2$ was repeated five times in different overlapped sites and the average was recorded. The density was measured as a percentage of the number of reaction positive glias against the number of total glias. The average number of GAP43-positive dendritic spines per neuron was calculated and recorded.

As for the staining for synaptophysin and Bielschowsky staining, the neurofibrae with positive staining were quantitatively computerized by image analytical software, MacScope version 2.3 (Mitani Shoji, Japan).

The pathological measurements were performed twice by the same pathologist; quantitized data with large standard deviation were recounted or rejected.

2.2.5. Data analyses

The scorers of the sleep recordings and the pathologist were not informed of the causes of death of the infants.

Correlation analysis was conducted between each infant's physiological data on sleep apnea and pathological data on neuronal plasticity, using SPSS version 8.0.

2.2.6. Ethical issues

This study was approved by the Ethical Committee of the University Children's Hospital and was performed in accordance with the ethical standards prescribed by the Declaration of Helsinki.

3. Results

3.1. Characteristics of the physiological data

Compared to the control infants, the SIDS victims were characterized by a greater frequency of obstructive apnea ($P = 0.001$) and a greater duration of both obstructive and central apnea ($P = 0.026$, 0.049).

3.2. Correlation analyses between the physiological and the pathological data

3.2.1. Correlations found in the SIDS victims only

The following six correlations were found in the SIDS victims but not in the control infants:

1. a negative correlation between the frequency of obstructive apnea and the number of GAP43-positive neurons in the MBDR ($P = 0.026$);
2. a positive correlation between the frequency of obstructive apnea and the number of GAP43-positive neurons in the PPTN ($P = 0.006$);
3. a negative correlation between the frequency of central apnea and the amount of Bielschowsky-positive neurofibrae in the MBDR ($P = 0.0l0$).
4. a negative correlation between the frequency of central apnea and the amount of Bielschowsky-positive neurofibrae in the MBPG ($P = 0.010$);
5. a negative correlation between the frequency of obstructive apnea and the amount of Bielschowsky-positive neurofibrae in the MBDR ($P = 0.026$);
6. a negative correlation between the frequency of central apnea and the amount of Synaptophysin-positive neurofibrae in the PPTN ($P = 0.006$).

3.2.2. Correlations found in the control infants only

The following two correlations were found in the control infants but not in the SIDS infants:

1. a positive correlation between the frequency of obstructive apnea and the number of spines of GAP43-positive neurons in the PPTN($P = 0.02\ 1$);

2. a negative correlation between the frequency of central apnea and the number of spines of GAP43-positive neurons in the PPTN ($P = 0.006$).

4. Discussion

Some correlations were found in the brainstem arousal pathway of SIDS victims, while other correlations were found in the control subjects only. These findings suggest a relation between changes of neuronal plasticity in the brainstem arousal pathway and SIDS.

In conclusion, these findings suggest an inverse relation between hypoxic load induced by sleep apnea and the importance of neuronal plasticity in SIDS victims.

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