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Response of Smooth Bronchial Musculature in Bronchoconstrictor Substances in Newborn with Lung Atelectasis at the Respiratory Distress Syndrome (RDS)

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ABSTRACT

Objective: Role of the atelectasis (hypoxia) in the respiratory system of the live and exited newborn (250 up to 3000 g. of body weight), which has died due to different causes was studied in this work. **Methods:** Response of tracheal rings to dopamine, serotonine and ethanol in the different molar concentrations (dopamine: 0,05 mg/ml, 0,5 mg/ml, 5 mg/ml; serotonine (5-HT): 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol/dm^3; ethanol: 0,2 ml, 0,5 ml, 1,0 ml; 96%) was followed up. Study of the smooth tracheal musculature tone (STM) was elaborated in 16 tracheal preparations taken following the newborn death due to different causes. **Results:** Based on functional researches of tracheal isolated preparations, it was ascertained as follows: atelectasis (cases born with lung hypoxia) has changed the response of STM to dopamine, serotonine and ethanol in a significant manner ($p<0,01$) in comparison to cases of controlling group, which has died due to lung inflammatory processes (e.g. pneumonia, bronchopneumonia, cerebral hemorrhage), which have also caused significant response ($p<0,05$). **Conclusion:** Results suggest that exited cases from lung atelectasis and cases of controlling group reacts to above mentioned substances by causing significant constrictor action of tracheobronchial system.

Key words: Dopamine, serotonine, ethanol, trachea.

1. INTRODUCTION

Sudden infant death syndrome (SIDS) remains one of most often causes of infant death in the period from birth until the end of first year of life. In despite of long term intensive scientific research and submission of many hypotheses, etiology of this syndrome remains unclear, yet (1, 2, 3).

It is assumed that chronic hypoxia, infections (viruses and different bacterial toxins), inflammatory conditions, biochemical disorders and genetic abnormalities are most important causes in the sudden infant death syndrome. (SIDS) (4, 5, 6, 7, 8).

Respiratory infections are another important factor in the etiology of SIDS. According to some data from the medical evidence, infections of the respiratory tract are cause of the SIDS in 33% of cases (9, 10).

Also, at infants following death from sudden death syndrome, a certain number of viruses and bacteria are isolated in some postmortem cultures that were identified by serological reactions or polymerase chain reaction. Results of these researches shows that respiratory syncytial virus, influenza A virus, adenoviruses, rhino viruses, cytomegalovirus and parainfluenza was most often isolated viruses (11, 12, 13, 14).

It is assumed that caliber of airways is under the control of autonomous nervous system, mainly of parasympathetic (cholinergic) nervous system. In despite of many researches, basic mechanism of hyper-reactivity and bronchoconstriction of the respiratory system remains totally unclear. Release of the acetylthiocholine from the parasympathetic nerve fibers activates muscarinic receptors that are present in the airways smooth musculature, submucosal glands and blood vessels of these airways by causing bronchoconstriction, secretion of mucus and vasodilation (15).

Data from different researches "in vitro" presents that smooth musculature of different organs relaxes by two types of nerve fibers of autonomous nervous system: sympathetic or adrenergic nerve fibers and nonadrenergic noncholinergic nerve fibers (16, 17).

In the smooth bronchial and tracheal musculature, epithelium plays also an important role. Removal of epithelium from the bronchial segments or trachea in researches "in vitro" has shown that it is accompanied with an in-

crease in the sensitivity of airways smooth musculature to acetylthiocholine with subsequent bronchoconstriction (18).

All of the above mentioned mechanisms being involved in the process of breathing are assumed to be altered if exposed to the continuous intrauterine and postpartum hypoxia. Different researches has shown that intrauterine hypoxia of the fetus is usually manifested with a high level of immunoglobulin, increase of muscular mass, about 20%, in pulmonary arteries, increase of airways smooth musculature cells and increase of the level of fetal hemoglobin. Increase of immune stimulation in the mucosa of trachea, duodenal mucosa, and palatine tonsils and increase of the level of interferon in the blood circulation appears as other changes. At the fetus and newborn, we need to add the fact that many mechanisms have not achieved the proper scale of their maturity. All these changes have also subsequent modified response of airways smooth musculature with impact to functional performance of the respiratory system, in general (19, 20).

Hence, studying the role of atelectasis (hypoxia) in the respiratory system at live and dead newborns (250 up to 3000 g. of body weight), which have died due to different reasons, was aim of this work. Impact of hypoxia in the respiratory system was followed up through response of tracheal segments in pharmacological substances such are: dopamine, serotonine and ethanol in different molar concentrations.

2. MATERIAL AND METHODS

Research was conducted in cooperation with the Gynecology Obstetrics Clinic, Pathologic Anatomy Institute and Experimental Unit of Medical Faculty in Prishtina, with permission of the Ethic Commission by respecting principles of Helsinki Declaration.

Classification of tracheal preparation of newborn babies in different weeks of gestations is made on the basis of histopathological examination of tracheal preparation (in blocks of paraffin). The preparations have been stained with standard: hematoylin-eosin (H & E) methods.

Research has been conducted in 16 experimental studies in vitro in isolated trachea of infants died in different weeks of gestations (weight 250 to 3000 g). Trachea were taken immediately after autopsies. Above the bifurcation of trachea, 6 tracheal rings were taken and placed in Krebs solution DIP (pH=7,4).

During the experiment, the water bath temperature was kept at 37 °C, and solution in bath is aerosolized continuously with gas mixture (95% CO_2 and 5% O_2), which has flown continually through the bath solution. Rings were prepared and serially connected to each other. The series consisting of 6 rings was placed in bath for isolated organs (volume 50 ml), so that lower part of the ring is connected to the holder, while upper part is connected to transducer ("Force transducer", Statham UC_2). The smooth musculature response is registered in multi channel recorder (Watanabe HSE 6600).

After 30 minutes, tone of tracheal rings is registered, then preparation was exposed to different molar concentrations (dopamine: 0.05 mg/ml, 0.5 mg/ml, 5 mg/ml; serotonine: 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol/dm^3; ethanol: 0, 2 ml, 0,5 ml, 1,0 ml; 96%).

Doses are changed every 15 minute, while effects of bronchoconstrictor agents are followed 3 minutes, after application. Then, preparation got rinsed several times with Krebs solution, prior application of another substance.

Results are processed with statistic computer program GraphPad InStat III with comparing T test for two working groups.

3. RESULTS

Results of the research in isolated tracheal preparation in newborn shows that dopamine, serotonine and ethanol are applied in different molar concentrations (dopamine: 0,05 mg/ml, 0,5 mg/ml, 5 mg/ml; serotonine: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} mol/dm^3; ethanol: 0,2 ml, 0,5 ml, 1,0 ml; 96%), which acts in different ways, depending on the applied dose.

Based on functional researches of tracheal isolated preparations, it was ascertained as follows: atelectasis (cases born with lung hypoxia) has changed the response of STM to dopamine, serotonine and ethanol in a signif-

	5-HT log-4	5-HT log-3	5-HT log-2	5-HT log-1
Atelectasis	0,25±0,25	2±0,66	1,25±0,84	2,15±1,08
Control	0±0	0±0,56	0,5±0,5	0,5±0,5

Table 1. Serotonine (5-HT) dose response of STM in newborn babies of different age groups with respiratory distress syndrome (RDS). (n= 5; X± SEM).

	Dopamine 0,05 mg/ml	Dopamine 0,5 mg/ml	Dopamine 5,0 mg/ml
Atelectasis	0,37±0,37	1,5±0,98	4,43±1,78
Control	0,5±0,5	0,83±1,25	1,5±2,95

Table 2. Dopamine dose response of STM in newborn babies of different age groups with respiratory distress syndrome (RDS). (n= 5; X± SEM).

	Ethan 0,2/ml (96%)	Ethan 0,5/ml (96%)	Ethan 1,0/ml (96%)
Atelectasis	0,37±1,46	1,5±2,88	4,43±4,47
Control	0,5±0,68	0,83±2,35	1,5±8,68

Table 3. Ethanol dose response of STM in newborn babies of different age groups with respiratory distress syndrome (RDS). (n= 6; X± SEM).

	5-HT log-4	5-HT log-3	5-HT log -2	5-HT log -1	Dopa 0,05	Dopa 0,5	Dopa 5,0	Ethan 0,2	Ethan 0,5	Ethan 1,0
Atelect.	0,25±0,25	2±0,65	1,25±0,83	2,25±0,84	0,37±0,37	1,5±0,98	4,43±1,73	0,37±1,14	1,5±2,88	4,43±4,47
Control	0±0	0±0	0±0,57	0,5±05	0,5±0,5	0,8±1,25	1,5±2,95	0,5±0,68	0,83±2,34	1,5±8,68

Table 4. Cumulative action of 5-HT, dopamine and ethanol of STM in newborn babies of different age groups with respiratory distress syndrome (RDS). (X± SEM).

icant manner (p<0,01) in comparison to cases of controlling group, which has died due to lung inflammatory processes (e.g. pneumonia, bronchopneumonia, cerebral

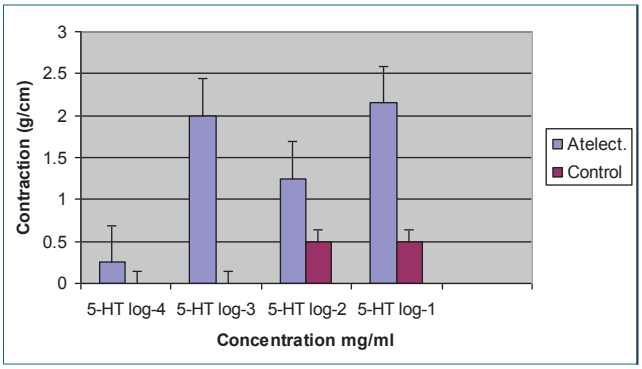

Figure 1. Response of STM to 5-HT at newborns with dominant pulmonary atelectatic changes. (X ± SEM).

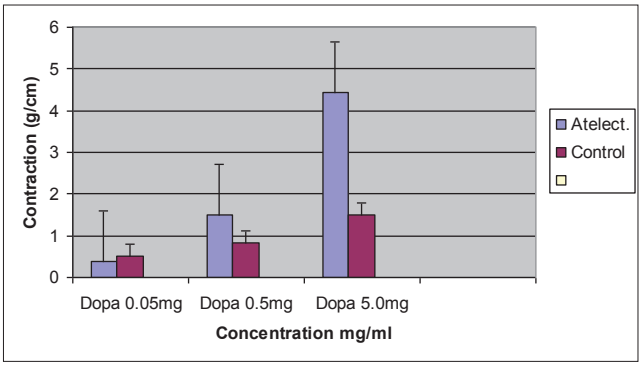

Figure 2. Response of STM to dopamine at newborns with dominant pulmonary atelectatic changes. (X ± SEM).

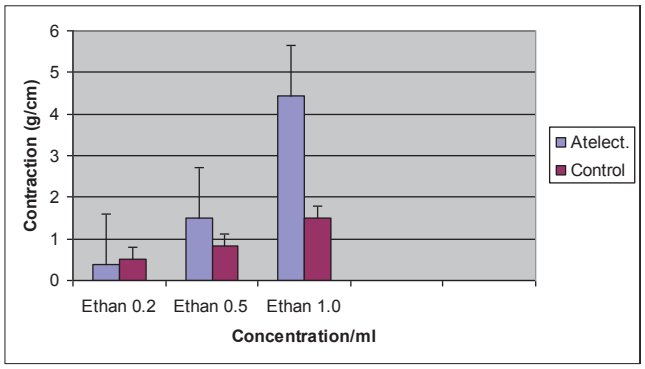

Figure 3. Response of STM to ethanol at newborns with dominant pulmonary atelectatic changes. (X ± SEM).

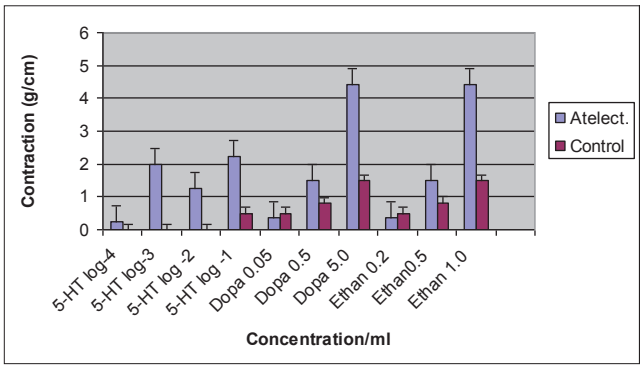

Figure 4. Response of STM to 5-HT, dopamine, and ethanol at newborns with dominant pulmonary atelectatic changes. (X ± SEM).

hemorrhage), which have also caused significant response (p<0,05).

In the Tables 1, 2, 3, 4 and Figures 1, 2, 3, 4 action of 5-HT, dopamine and ethanol in STM at newborns died due to different reasons appears. (X ± SEM).

4. DISCUSSION

Chronic hypoxia at the fetus and infants, besides organic changes in tissues and different organs, is accompanied also with changes in the level of cell metabolism. These changes in the level of cell metabolism are affected in particular to metabolism of some endogenous lipophilic substances such are: steroids, liposoluble vitamins, prostaglandin, troboxan and some exogenous substances (21, 22).

In children with sudden death syndrome, as a consequence of chronic hypoxia, TNF-[alpha] and other inflammatory cytokines, aradonic acid and polystaurated fatty acids stimulates production of the superoxide from polymorphonuclear leukocytes that affects in damaging of different tissues with different mechanisms, including airways also (23, 24, 25, 26, 27).

These damages of airways manifest with ultrastructural changes in cells of smooth musculature, tissue matrix and damage of epithelial integrity (28, 29).

Results of the research in isolated segment present that during the stimulation with serotonine, contraction of smooth musculature is significantly more emphasized in the group with atelectasis comparing to the controlling group in all concentrations of serotonine. It is difficult to find any other neurotransmitter that is more widely spread in the organism than serotonine. Author Kinney emphasizes that 5-HT innervate almost "all nervous system" (30).

Due to wide inclusion in the function of vital organs, depletion of serotonine depot in terminal phase prior death of infants is evident. Data from a research in rats shows that average hypoxia has not deranged connection of serotonine to effectors receptors, whilst heavy forms of hypoxia derange the connection of serotonine to effectors receptors including also airways receptors (31).

Therefore, based on the facts of respective authors, it is observed that response of tracheal musculature to serotonine in our research in the group with atelectasis was maintained, whereas in the controlling group is weaker.

During the stimulation of tracheal segments with dopamine in small concentrations, contractile response in the group with atelectasis is lower in comparison to controlling group, without any significant distinction, but contractile response increases significantly along with the increase of the dopamine dose in the group with atelectasis in comparison to controlling group, this distinction is especially emphasized in the concentrations of dopamine 5 mg/ml.

Author Kanairo, with collaborators, presents that dopamine has not changed the tone of smooth musculature in rats (32).

Analyses of gained results during the stimulation of the tracheal segments has showed that ethanol causes constriction of smooth musculature at both groups in concentration of 0.2 ml (96%) with difference in fact that this response is more emphasized in the group with atelectasis comparing to controlling group. Whereas, during the stimulation with ethanol in concentration of 0,5 ml (96%) we have gained constrictor response of smooth tracheal musculature as more emphasized in the group with atel-

ectasis rather than in the controlling group. Meanwhile, ethanol in concentration of 1.0 ml (96%) has caused significant constriction of smooth musculature of groups with more emphasized effect in the group with atelectasis.

Author Trevisani, with et al., during his research with pigs has ascertained that ethanol stimulates sensory nerve fibers of respiratory system by transient receptor potential vanilloid-1-dependent mechanism TRPV1 by causing the release of some sensory neuropeptides and initiation of the inflammatory process by causing bronchoconstriction and pulmonary edema (swelling) (33).

Some other authors has ascertained that bronchoconstrictor effect of ethanol derives from release of local acetylthiocholine produced by the ethanol metabolism even though this hypotheses remains contradictory and yet not fully verified (34).

Results of our research go in favor of this fact that in despite of increase of ethanol concentration we have increase of the constriction of smooth tracheal musculature. Therefore, similar to author Trevis, we propose more detailed research of mechanisms by which mediators of inflammation regulates the intracellular response in sensory organs including also respiratory system.

From what was ascertained, we can conclude that different etiologic factors that have caused the death of the fetus or newborns, have crucial impact in the response of smooth tracheal musculature in applied substances (in different concentrations) with significant differences in between groups.

5. CONCLUSION

Based on functional researches of tracheal isolated preparations, it was concluded that: atelectasis (cases born with pulmonary hypoxia) have changed the response of STM to dopamine, serotonine and ethanol ($p<0,01$), whereas cases of controlling group, which has died due to pulmonary inflammatory processes (e.g. pneumonia, bronchopneumonia, cerebral hemorrhage), has caused significant response ($p<0,05$). Results suggest that cases died from lung atelectasis and cases of the controlling group reacts on above mentioned substances by causing a significant constrictor action.

List of Abbreviations

RDS	-	Respiratory distress syndrome
STM	-	Smooth tracheal musculature
5-HT	-	Serotonine
SIDS	-	Sudden infant death syndrome
Dopa	-	Dopamine
Ethan	-	Ethanol

CONFLICT OF INTEREST: NONE DECLARED

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