Influence of Hypoxia in Bronchial Response to Bronchoconstrictor Substances at Newborn with Distress Respiratory Syndrome (DRS)

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Objective: In this work, role of hypoxia in respiratory system on live and exited newborns (weight 250-3000 g.) which have died due to different reasons is studied. Methods: Response of tracheal rings in acetylcholine and histamine in different molar concentrations (10⁻⁴, 10⁻³, 10⁻², 10⁻¹ mol/dm³) was monitored. Study of the tracheal smooth musculature (TSM) tonus was performed in 12 tracheal preparations obtained from the newborns exited due to different reasons. Results: Based on the functional analysis of the isolated tracheal preparations, we have concluded that the cases born with lung atelectasis have caused significant response of TSM to Acetylcholine and Histamine (p > 0.01). In cases of control group, which has exited due to different reasons from lung inflammatory processes (e.g.: pneumonia, bronchopneumonia, cerebral hemorrhage), Acetylcholine and Histamine have also caused significant constriction of tracheal smooth musculature. Conclusion: Results suggest that exited cases with lung atelectasis (hypoxemia) and cases of lung inflammatory processes have significant constrictor action to Acetylcholine and Histamine in tracheobronchial system. Key words: Trachea, Acetylcholine, Histamine.

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1. INTRODUCTION

Hypoxia as pathologic state, in the beginning, usually manifest changes in the respiratory system through activation of peripheral chemoreceptors in the carotid sinus. This response towards hypoxia is composed of stimulatory and inhibitory component. Stimulatory component of the response towards hypoxia is fast one whilst inhibitory (depressive) component is slower being associated with changes in the centers of central nervous system which are in charge for regulating of breathing. Mechanisms of respiratory depression, as response to hypoxia, are localized in nervous structures of the central nervous system being associated with changes in the synaptic transmission (stimulation of neuroinhibitor neurotransmission) and changes in the features of cell membrane of respiratory neurons (modulation of ionic channels of receptors and neuromediators) (1).

Many studies “in vivo” suggest that inhibitory non-adrenergic nerve fibers are in charge for the airways smooth musculature tonus (2, 3).

Role of the muscarinic receptors in the regulating of the airways smooth musculature tonus was also well defined from these scientific studies. It is known that activation of the muscarinic preganglionic receptors of subtype M₂ affects the contraction of airways smooth musculature, whilst role of the receptors of type M₁ is not yet entirely known. M₁ muscarinic receptors are identified mainly at parasympathetic ganglions and it is deemed that they do stimulate neurotransmission, respectively activation of these receptors potentiate the induced bronchoconstriction of nervus vagus fibers, whilst their antagonism will reduce the release of acetylcholine (4, 5).

Though different authors, in their studies, have presented that airway smooth musculature of many beings including human contains mainly M₂ and M₁ receptors (6, 7, 8, 9).

Lately, scientific evidence strengthens regarding the role of the muscarinic receptors of subtype M₂ in the regulation of the smooth musculature tonus through negative response in the release of the acetylcholine from the parasympathetic nerve endings (10, 11, 12, 13).

Active bronchogenic substances such are histamine and acetylcholine may cause different pulmonary re-
sponse in studies “in vivo” depending on experimental conditions in which study is carried out. In a research conducted in cats under anesthesia, in the vagotomy condition, it was seen that histamine has manifested stronger bronchoconstrictor effect of peripheral ways in comparison to acetylcholine, whilst bronchoconstrictor effect of acetylcholine was stronger in central respiratory ways in comparison to histamine. Difference of the effect of these bronchogenic substances in the central and peripheral level of airways may result due to differences of these substances in connection, distribution and metabolism of these substances or from the difference in the sensitivity of receptors localized in different levels of airways. (14).

In different pathologic conditions, structure and interrelation of muscarinic receptors may change also in airways which may be responsible for the airways smooth musculature tonus. Pathologic conditions impacting these changes are chronic hypoxia, pulmonary infections, cerebral hemorrhages characterized with a series of pathologic and metabolic changes which have as a consequence the alteration of structures in the level of muscarinic receptors and also in response of airways smooth musculature (15, 16, 17).

Therefore, aim of this work was study of the role of hypoxia in respiratory system on live and exited newborns which have died due to different reasons. Influence of hypoxia in the respiratory system was monitored through eventual changes of the response of tracheal segments of smooth musculature in pharmacologic substances such as histamine and acetylcholine at different molar concentrations.

2. MATERIAL AND METHODS

Research was conducted in cooperation with the 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 gestation is made on the basis of histopathological examination of tracheal preparation (in blocks of paraffin). The preparations have been stained with standard: hematoxylin–eosin (H & E) methods. Research has been conducted in 12 experimental studies in vitro in isolated tracheas of exited children in different weeks of gestation (250 to 3000 g, of body weight). Tracheas were taken immediately after autopsies. Above the bifurcation of trachea, 6 tracheal rings were taken and placed in Krebs solution (pH=7.4).

During the experiment, the water bath temperature was kept at 37 °C, and solution in bath was aerosolized continuously with gas mixture (95% O₂ and 5% CO₂), which has flown in continual mode 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), with lower part of the ring being connected to the holder, and upper part connected to transducer (“Force transducer” Statham UC). The response of TSM was registered in a multi channel recorder (Watanabe HSE 6600). (See scheme 1. of the experimental model in vitro).

After 30 minutes, tonus of tracheal rings is registered, and afterwards preparation was exposed to different molar concentrations (10⁻⁴, 10⁻³, 10⁻², 10⁻¹ mol/dm³) of Acetylcholine and Histamine (figure 1).

Doses are changed every 15 minute, while effects of bronchiconstric- tor agents are monitored for 3 minutes, after application. Afterwards, preparation got rinsed several times with Krebs solution, prior application of another substance.

Results were processed with statistic computer program GraphPad InStat III with T test of comparing two working groups.
3. RESULTS

Results of the study in isolated tracheal preparations in newborn shows that acetylcholine and histamine applied in different molar concentrations (10⁻¹, 10⁻², 10⁻³, 10⁻⁴ mol/dm³) act on a different manner, depending on the applied dose. It is ascertained that: cases born with lung atelectasis has caused a significant response of TSM in acetylcholine and histamine (p < 0.01). In cases of control group, which has exited due to different reasons from pulmonary inflammatory processes (e.g.: pneumonia, bronchopneumonia, cerebral hemorrhage), acetylcholine and histamine have also caused significant constriction of tracheal smooth musculature (p < 0.01). See fig.1,2,3.

4. DISCUSSION

Manifestation of hypoxia endangers the function of brain centers during entire cycle of intrauterine development of fetus up to the birth and it may be one of the most important pathologic factors for intrauterine death of the fetus including after the birth of children, also. Supply of the brain with oxygen is crucial in terms of regulating of other organs functions in general. Chronic hypoxia induces the damage of neuronal cells, neurodegeneration and death of neuronal cells. Hypoxia manifest these changes mainly through activation of anaerobic metabolism which results in metabolic acidosis with development of degenerative changes in respiratory nerve nuclei and central nervous ways as its consequence and also with changes in the level of airways and lungs. Respectively, disorder of the aerobic metabolism is a consequence of severe anoxia, by resulting in the decrease of ATP, which is needed for the work of the ion pump for Na⁺/K⁺ and Ca⁺². This state results with loss of K+ from the neuron by causing the depolarization of the membranous potential (18).

These changes manifest with changes in cell neurotransmission of central nervous system centers, lung immaturity, insufficient secretion of the surfactant and degenerative changes of the airways alveolar epithelium. These changes may reflect with changes also in the mechanisms for maintaining of the airways tonus, respectively in the structure of different receptors and mediators (19, 20).

Influence of hypoxia and of other pathologic factors also in dead and live newborn was analyzed through analyses of tracheal segments, respectively through research of the bronchogenic substances such are acetylcholine and histamine in the tonus of the smooth musculatures of these segments.

Results of our research have shown that in the high concentrations of acetylcholine and histamine, contraction of the smooth musculatures of these tracheal segments is in lower values, whilst lowering of these concentrations in a linear manner increases also the muscular contraction and this contraction has higher values in concentration of Ach log-1. Significant changes regarding the contraction of the tracheal smooth musculature were ascertained in between the testing group and the control group and this change is more evident in lower molar concentrations of acetylcholine and histamine. Effect of muscular contraction is emphasized more at histamine rather than in acetylcholine but without any significant change.

Similar results in the contraction of the smooth musculature in bronchogenic substances were also presented by author Habre with et al. In the experiment with rabbit, they have studied the role of the muscarinic and histaminergic receptors in the bronchoconstriction induced by myorelaxant substances. These authors have concluded that manifestation of muscarinic and histaminergic effect as a result of affinity of connecting of myorelaxant substances to M₁, M₂ and M₃ muscarinic receptors is responsible for this bronchoconstriction. Nonetheless, these authors have concluded that these ascertains during the experiment with rabbit are to be proved in human due to changes in the topography and in the structure of muscarinic receptors and possible changes within the action mechanisms (21).

Meanwhile, author Drazen with et al. has analyzed the contractile response of the parenchyma and trachea in pigs to action of acetylcholine and histamine. These authors have concluded that segments of parenchyma has more emphasized contractile response on histamine action rather than acetylcholine whilst at tracheal segments have similar contractile response on two substances. Results of these authors regarding contractile response in tracheal segments towards acetylcholine and histamine are in accordance with the results of our research by proving for a similarity in structure and the mechanism through which the airways tonus of smooth musculature is adjusted (22).

Influence of hypoxia in the mediator response and the neurotransmission of the airways smooth musculature was studied also by author Fujiwara with et al. Effect of hypoxia were studied in the isolated segments “in vitro” of pig’s trachea and they have ascertained that hypoxia has no influence in the basal tonus of the trachea musculature contractility, but yet response to action of histamine changes evidently whilst response to acetylcholine remains unchanged (23, 24).

As a conclusion, we can present that hypoxia may be a potential factor for modification of the smooth musculature response to acetylcholine and histamine. Modification of the smooth musculature response is supposed to be as consequence of changes in nervous stimulation or from changes in the volume of bronchogenic substance transmission and require additional systematic studies.

5. CONCLUSION

Based on the experimental research of the tracheal isolated preparations, ascertains are as follows: In cases born with lung hypoxia, Acetylcholine and Histamine have caused significant response of TSM (p > 0.01). In cases of control group, which has died due to lung hypoxic processes (e.g.: atelectasis, cerebral hemorrhage), have also caused significant response of TSM. Results suggest that deaths of newborn from lung hypoxia, cholinergic and histaminergic nervous system are developed in different gestation weeks of intrauterine life.

REFERENCES

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“AMNuBiH Days 2013” and EASE Conference

Academy of Medical Sciences of Bosnia and Herzegovina (AMNuBiH) organized on June 21, 2013 in Sarajevo, its 6th Annual Scientific Meeting, which is dedicated to the importance of publishing papers in biomedical and other journals. An integral part of the meeting were the promotion of three journals published under the auspices of AMNuBiH - Med Archives, Acta Informatica Medica and Materia Sociomedica, which was promoted by academician Mirko Grusic (June issues), and promotion of the book by academician Izet Hozo “Clinical propedeutics”, which has promoted academic Muhammed Zildjic.

AMNuBiH celebrates four years of its existence and has 29 members, respected scientists from B&H and other countries. Unfortunately, as the President of AMNuBiH academic Izet Masic pointed out, in our setting the attitude of the state towards the science is very poor, while investment in scientific research is extremely small, but regardless of the attitude to science and scientists, local academics work, have success and receive significant awards at home and abroad. AMNuBiH plans to organize another similar meeting before the end of this calendar year and receive several new members, among which are respected persons that presented their papers at this meeting. Admission conditions in this professional body are PhD, rank of associate professor and at least ten papers published in world biomedical databases.

Izet Masic