

POSTNATAL - PHYSIOLOGICAL RESEARCH OF THE BRONCHIAL RECEPTOR SYSTEM DEVELOPMENT ON THE ISOLATED PREPARATION OF THE HUMAN TRACHEA IN VITRO

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ABSTRACT

Research was done on pharmacological-physiological development of the bronchial receptor system on the smooth muscles of trachea in the newborn children, alive-born and stillborn children. Monitored was the response on: acetylcholine, dopamine, histamine and serotonin in different molar concentrations 10^{-4} , 10^{-3} , 10^{-2} , 10 mol/dm^3 , $\mu\text{mol/dm}^3$). Research was done on tonus of tracheal smooth muscles of 23 tracheal preparations taken by autopsy after death from different factors. Based on pharmacological-physiological research on the preparations of human isolated trachea it was find out that: acetylcholine stimulation effect is significant ($p > 0,01$) in 38-41 weeks of pregnancy comparing with that in 30-37 weeks of pregnancy ($p > 0,01$), while dopamine stimulation effect is significant ($p > 0,05$) in 30-37 pregnancy weeks comparing with the effect of acetylcholine and dopamine on the still-born infants of the same pregnancy period ($p < 0,01$). Histaminic receptors were developed during intrauterine life after 38 weeks of pregnancy ($p > 0,025$). Serotonin has caused contraction of the bronchial smooth muscles after 30 pregnancy weeks, but response was not significant ($p < 0,01$). This suggests that cholinergic and adrenergic system of the airways in alive newborn infants develops in parallel intrauterine, contrary to other systems which develop in certain extrauterine life phases.

KEY WORDS: cholinergic, adrenergic, histaminergic and serotonergic receptors,
human trachea

INTRODUCTION

In the fourth month of intrauterine life starts the trachea differentiation from the gastrointestinal tract. Ganglia vascularization originates from the bronchial artery. One-way trachea and gastrointestinal systems innervation development shows to interaction of the two systems. Current knowledge and histo-chemical methods demonstrate the existence of a free plexus of intra-arterial nerves on the carotid artery, and also on arteries and veins of smaller diameter in newborn children, but nerves are absent. Lymphatic plexus has an abundant innervation on both branches of vagus. The nerve endings were not found on alveolar walls, but the encapsulated nerve endings were found on visceral plexus, diaphragmal part and upper pleural part. Intrabronchial part of wall has a good innervation of muscles, epithelium and glands, particularly above the tracheal bifurcation. Ganglions are mainly situated in peribronchial plexus, but some fibers similar to acinous glands were also found (1,2,3). In the beginning of fifth month of fetal life many nervous fibers start assuming the white appearance, a myelin deposit sheath, formed after the repeated circular movements of Schwann's cells membrane around axon. Myelin membrane covering the nerve fibers along the spinal chord has quite different origin, as it is formed from oligodendroglial cells. Nevertheless, myelin furnishing of nerve fibers in the bone marrow starts around the fourth month of intrauterine life, while the nerve fibers descending from upper brain cells to the spinal chord are not myelinated before expiry of one year from the birth. Tracheas with the nervous system are myelinated approximately when they start functioning (4,5). In certain pulmonary pathological states causing the respiratory insufficiency with newborns, the varying susceptibility to chlonergic, adrenergic, histaminergic and serotonergic substances was found (6,7). Dysfunction of respiratory epithelium may lead to the bronchial hyperactivity and affect the function of respiratory airways (8,9). Viruses tend to attack the epithelium and to lessen the action of relaxing substances (RS), thus increasing the bronchoconstricting effect. Maturing of contractile response in postnatal period is gradual and maturation of the system is concomitantly followed by neuropeptides activity (10,11). For the purpose of verifying the pharmaco-physiological development of bronchial receptor system in dependence of its developmental phase, examined in this study were the effects of acetylcholine, dopamine, histamine and serotonin on isolated human trachea preparations of newborn infants in different gestation weeks, from pharmaco-pathophysiological aspect.

MATERIALS AND METHODS

Examination was made in 23 experimental studies in vitro with isolated tracheas of alive-born and still-born children in different gestation weeks. Tracheas were taken immediately after autopsy; six rings were taken above the tracheal bifurcation and immersed in Krebs' solution pH =7,4. While carrying out the experiment, solution temperature in the bath was maintained on 37oC and aerosolized with mixture of gasses, i.e.95% O₂ and 5% CO₂; the mixture was streaming uninterruptedly through the solution in the bath. The rings were prepared and interconnected in a string. The interconnected tracheas in string of 6 rings were placed in the bath for isolated organs (volume 50 ml), so that the lower part of rings was connected to the holder, while their upper part was thread tied to the "force transducer" (Statham UC2). The response of smooth muscles of tracheal rings was registered on the monochannel recorder (Watanabe HSE 6600). After 30 minutes in rest, tonus of tracheal rings was recorded and then the preparation was exposed to different molar concentrations 10⁻⁴, 10⁻³, 10⁻², 10 mol/dm³, μmol/dm³) of acetylcholine, dopamine, histamine and serotonin. Doses were changed every 15 minutes, while the effect of relevant bronchoconstrictor agent was monitored in 3 minute time after application. The preparation was then rinsed several times with Krebs' solution, before adding the other substance. The zero hypothesis was used, based on the assumption that cholinergic and adrenergic systems in smooth muscles of tracheal rings are developed equally in different gestation weeks, both in alive-born and still-born children, as well as in certain pathological lung conditions, and that they do not influence the response of bronchial muscles after use of acetylcholine, dopamine, histamine and serotonin. Significant changes were identified by t-test (Student-Fisher t-test).

RESULTS

Test results of isolated tracheal preparations function in newborn and still-born infants are showing that acetylcholine, dopamine, histamine and serotonin applied in different concentrations (10⁻⁴, 10⁻³, 10⁻², 10 mol/dm³, μmol/dm³) have different effect

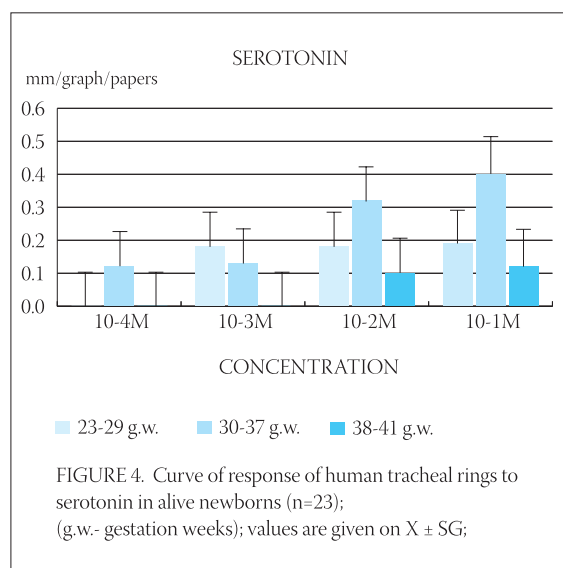
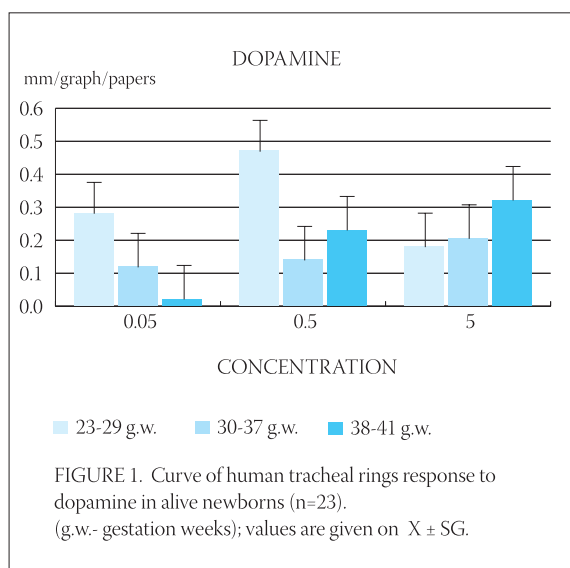
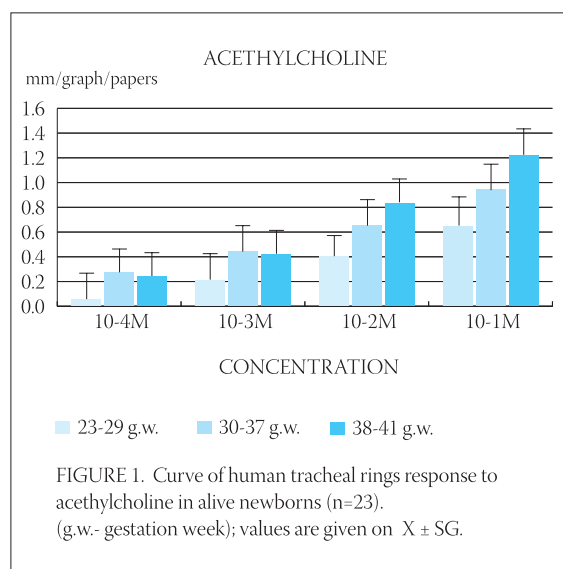
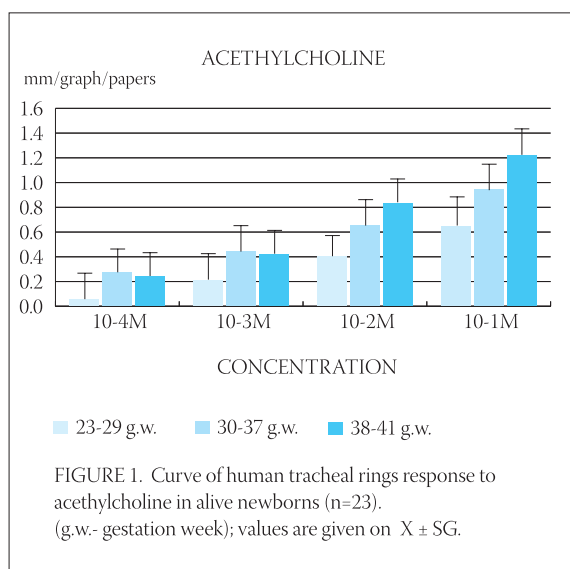
Group	Infant	Mass (g)	Gestation weeks
I	Immature infant	500-1100 g	23-29
II	Premature infant	1100-2500 g	30-37
III	Mature infants	>2500	>38

TABLE 1. Sequence of tested cases based on gestation phase of the newborn infants.

on tracheal smooth muscles. Table 1 shows the infants selected by body mass and gestation weeks.

- a. Acetylcholine brought to the smooth muscles response in alive newborns of 30-37 gestation weeks. This indicates that the cholinergic system in airways of alive newborns is completely developed ($p < 0,025$) in comparison with influence of acetylcholine in still-born children of the same gestation age (see Figure 1.)
- b. Dopamine effect on the smooth muscles of tracheal rings in alive newborns after 38-41 gestation weeks in comparison with that experienced in 23-29 and 30-37 gestation weeks, is significantly higher ($p < 0,025$). This suggests that the adrenergic system, similar to the cholinergic system, is getting developed after 38 gestation weeks, what confirms that such developed system is giving response to the concerned substance ($p < 0,05$), Figure 2.

- c. Histamine effect on the smooth muscles of human trachea in alive newborns after 38-41 gestation weeks was significant ($p < 0,025$) in comparison with the effect recorded after 23-29 gestation weeks, what shows that histaminic receptors are developed during the extrauterine life (Figure 3.)
- d. Serotonin brings to the contraction of bronchial smooth muscles of human trachea after 30 gestation weeks, but the response is insignificant ($p > 0,1$). This shows that serotonergic receptor system in bronchial smooth muscles is not yet fully developed to be able to respond to the above bronchoconstricting substance (Figure 4.). This shows that the receptors specific for this substance are developed later in life.



DISCUSSION

Based on literature data, the sensory bronchial system is insufficiently developed in the first weeks of extrauterine life. This was proven by testing the smooth muscles of isolated tracheal rings in pigs (12). The function of most receptors is considerably influenced by environmental conditions, neuronal connections and age (13,14). Functions of calcium ions and calcium channels are also age dependent. Although the role of acetylcholine and noradrenaline neurotransmitters is known for a long time, it is relatively little known about ontogenesis, morphology, distribution and connections between cholinergic and adrenergic neurons. Reasons for that are the unavailability of specific methods to enable the safe identification of such cells. Major information on cholinergic and adrenergic structures derive from histo-chemical determination of acetylcholine esterase and dopa-oxidase (15), as well as from pharmaco-histochemical procedures improving the visualisation of positive neurons (16). In pathological cases the reaction of smooth muscles in airways is contrary to normal reactions (17). Cholinergic excitation of smooth muscles tonus is increased in pathological cases. Ganglia damage does not show to the adequate muscles functioning, what results in spasm and uncontrolled movements. Similar changes are seen in air-ways alterations. Increased excitation of vagus is the result of hyperactivity of smooth muscles airways potentiating the cholinergic activity, as well as result of alteration in structure of plasmatic membranes of smooth muscles (18). Increased excitation of vagus increases the inhibitory system activation, where we face with damage of neurotransmitter of nonadrenergic system (8,19,20). The obtained data of tracheal smooth muscles response are evidencing the susceptibility of bronchial muscles to acetylcholine, histamine, serotonin and dopamine, when going from younger to the maturing age in newborn infants. Acetylcholine has a more pronounced excitatory effect in immature and mature infants. Histamine has a significant effect in mature infants. Dopamine is also showing a significant effect in mature infants. The above data indicate that the bronchial susceptibility in newborn infants to cholinergic and adrenergic substances is evident in immature age. Cholinergic system is phylogenically predominant, while the other systems are showing the susceptibility depending on the age: Dopamine and histamine are showing the excitatory effect in mature age (mature infants). Based

on pharmaco-physiological responses, it seems that cholinergic and adrenergic systems in airways begin to develop at the same time of intrauterine life, they show the same maturity of these systems, what is manifested in significant response ($p < 0,025$) of bronchial smooth muscles to the relevant substances. Histaminic receptors are developing during the extrauterine life after 38 gestation weeks, with significant response ($p < 0,025$). Serotonergic system in bronchial smooth muscles during extrauterine life is not completely developed even after 41 gestation weeks to be able to give a significant response to bronchoconstricting substance. This indicates that specific serotonin receptors are developed later in life. With lower and mean doses, beta1 and beta2-adrenergic receptors are stimulated, while with high doses the stimulation of alpha1-adrenergic receptors is predominant. The response of smooth muscles of tracheal rings to dopamine suggests that this substance can exhibit its constricting effect through alpha₁-adrenergic receptors. However, the presence and role of these receptors in healthy and diseased persons with increased bronchial reactivity are disputable. Stability and balance of internal body medium in newborns are to a large extent depending on regular functioning of the autonomous nervous system. The cholinergic system is important for bronchomotor tonus regulation and it plays an important role in bronchial reactivity. Tracheal-bronchial constriction is depending on distribution of muscarine receptors. Adrenergic receptors are not equally distributed in smooth muscles of tracheo-bronchial stem. Density of beta₂-adrenergic receptors is higher in lower airways, and their stimulation brings to bronchodilatation. According to Barnes (21), the density of alpha1-adrenergic receptors is also low in trachea, but it is getting higher (and in other peripheral parts it reaches the density of beta₂-adrenergic receptors), and their stimulation causes the bronchoconstriction. Response of smooth muscles of tracheal rings to dopamine suggests that this substance may express its constricting effect through alpha₁-adrenergic receptors. Disturbed balance between the two systems and their interaction with histaminic-serotonergic receptor system are responsible for maintaining of bronchial tonus in physiological conditions. Disturbance of relation between these systems brings to domination of one system over the other, what is manifested in increased bronchial reactivity. There is the necessity for further investigations that will encourage the explanation of change in balance of these systems, manifested in air-ways obstruction.

CONCLUSION

Results of the study (except in case with serotonin) are showing that the response of isolated tracheal preparation during 38-41 gestation weeks is more pronounced in comparison with other gestation weeks; it means that cholinergic, adrenergic and histaminic receptor systems during gestation weeks of maturing are more pronounced, while the serotonergic receptors are not sufficiently developed to be able to react in significant degree to the bronchoconstricting substances.

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