Development Ontogenesis of Receptor Tracheobronchial System in Isolated Preparations of Pigs Trachea in Vitro

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1. INTRODUCTION
In most of the mammals’ types, airways smooth musculature has cholinergic and adrenergic innervations and bronchi-motor tonus depends from the effect of the both systems. Vagus nerve contains prangaglionic nerve fibers that emerge from the vagal nucleus in medulla oblongata up to the ganglions in airways. These parasympathetic ganglions are spread in an irregular manner throughout posterior wall of the trachea and big bronchi (1). From these ganglions, emerge postganglionic fibers, which innervate SM of the airways, bronchial circulation glandular acinuses (2). Histological studies do not show any efferent postganglionic fibers under the level of terminal bronchi, and studies of the function do not show the effect of vagal stimulation in bronchiole and in alveoli (3).

Ach acts on both receptors, muscarinic and nicotinic. Five types of muscarinic receptors (M1-up to M5), are genetically ranked. Muscarinic receptors M1-M3 also can be identified based on the different affinity to connect with different antagonists. Muscarinic receptors M2 are blocked in a selective manner from pirenzipine; muscarinic receptors M4 are blocked by AF-DX 116 and galamina; receptors M5 are blocked by 4-DAMP; whereas receptors M1 are antagonized by hombocina; it is hard to identify the selective agonist for receptors M1. Auto-radiographic studies have demonstrated receptors M1 and M2 muscarinic over nerve plexuses and within cholinergic ganglions (4.5). Primary cultures of postganglionic cholinergic neurons from trachea show that these nerve provides information for ARN as an informative messenger only for receptors M1 (6,7,8). Smooth musculature of the airways has muscarinic receptor M1 and M2 that causes contraction of SM.

Based on the pharmacologic and electrophysiological experiments, there are data showing that in tracheal musculature of the guinea pigs and in human’s airways a non-adrenergic – non-cholinergic nervous system is present (NANC) (9,10). It seems that neurotransmitter of these nerve is a vaso-active intestinal polypeptide (VIP) even though purines can also be incorporated here. Role of this system in a physiologic and pathologic condition is not proved. Local-reflexive contractile axon in afferent nerve routes, participates actively in keeping of the smooth musculature tonus of trachea-bronchial system. They provide response to mucosal irritation, and cause local constriction of the smooth musculature through release of substance P. Importance of this system is not assessed in whole, yet (10). Retention of the airways tonus is done by the influence of mediators, which do manifest their action through specific receptors.

Bronchial receptor system is not quiet enough developed in first weeks of extra-uterine life (11, 12, 13). In order to prove the development of bronchial receptor system in dependence from life age, in this work we have researched effects of Ach, propranolol + Ach, histamine, without presence and in presence...
of propranolol, and PGF$_2$-alfa in isolated preparation of pigs trachea.

2. MATERIAL AND METHODS

Research was conducted on 24 series of experiments. Experiments were done in vitro in piggy’s tracheal isolated rings of ages from 1 day to 4 weeks (1 day, 1 week, 2 weeks and 4 weeks). Previously, animals were sacrificed and then isolated tracheal preparation was placed in Krebs solution. Research of the airways isolated tracheal preparation response was done within 3 hours, since the moment of taking of the sample (cattle) from piggy’s. During the experiment, preparation was placed in a bathroom with Krebs solution with pH = 7.4 whilst bathroom temperature during the development of the experiment was held as constant one, in 37°C. Solution in the bathroom was aerosolized continuously with gas mixture (95% O$_2$ and 5% O$_2$), that has flown continuously in the bathroom solution. Rings were prepared and serially connected in between themselves. Serial composed of 2 up to 6 rings (depending from the age of experimental animal) was placed in bathroom for isolated organs (volume 50 ml), in such a way that lower part of the rings is connected for retainer, whilst upper part of the ring is connected with elastic thread for transducer („Force transducer“, Statham UC$_2$). Response of TSM was registered in a multi-channel registration (Watanabe HSE 6600). (See scheme 1. of the experimental model in vitro).

After 30 minutes, first tonus of tracheal rings was registered (silence potential), afterwards preparation was exposed to different molar concentrations (10$^{-4}$, 10$^{-3}$, 10$^{-2}$, 10$^{-1}$, mol/dm$^3$) of Ach, histamine, and PGF$_2$-alfa with propranolol and without propranolol. Doses have changed every 15 minutes, whereas effects of bronchoconstrictor agents were monitored for 3 minutes, after the application. Afterwards, preparation got rinsed couple of times with Krebs solution, prior applied solutions.

Ach has caused response of smooth musculature since the first day of extra-uterine life (p<0.01), suggesting that cholinergic and adrenergic system in airways at piggy’s is developed during intrauterine life. See fig. 1, 2, 3, 4, and 5. Within work, effect of propranolol in emphasizing of the bronchi-constriction in Ach was studied, also. This shows that propranolol emphasizes constriction of the bronchial smooth musculature caused by Ach, but the action is not significant (p>0.1).

There was no registration of constriction caused by histamine and PGF$_2$-alfa in tracheas of pigs since the
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Figure 4. Cumulative reaction of Ach, propranolol+Ach in TSM of pigs, age 4 weeks (n=6; X±SEM).

Figure 5. Cumulative reaction of Histamine in TSM of pigs, age 1 day, 1, 2 and 4 weeks (n=6; X±SEM).

Figure 6. Cumulative reaction of PGF2-alfa in TSM of pigs, age 1 day, 1, 2 and 4 weeks (n=6; X±SEM).

first day up to the 4th week of extra-uterine life, which shows that these systems develop later during lifetime. Tracheas of guinea pigs of different ages (reaction to: Ach, Ach + propranolol; histamine, histamine + propranolol; PGF2-alpha, PGF2-alpha + propranolol) were used as a control.  

4. DISCUSSION 

Research of tracheal ganglions at guinea pig, with electronic microscope, shows similarity of ganglion cells with other nerve spinal and parasympathetic cells (13). Whole surface of the ganglions is covered with satellite coverage. Within ganglions, blood vessels, mielinized and non-mielinized nerve fibers can be found. These hard bronchi-constrictor mechanisms should be controlled; this can be done best by controlling the release of the Ach, in fact control of Ach release from cholinergic postganglionic nerve is achieved by Ach itself. By acting in the muscarinic M3-inhibitor autoreceptors localized in borders of postganglionic nerve limits the further release of the Ach. Thus, these receptors act as autoreceptor (14). Function of neuronal autoreceptors M2 is demonstrated in vivo with antagonists of receptors M2 such as galamina that causes bronchi-constriction depending on vagus. In addition, receptors M3, described at guinea pig are described in all of the studies including humans (15).

Sensitive supply of neurons in trachea and in bronchi was found in nodal ganglion and in vagal jugular ganglion as well as in the ganglions of tracheal posterior ways. Morphology of neurocytes and tracheal ganglions is at most studied in guinea pig and mice’s. Most of the neurocytes are multi-polar, with short extensions. Number of tracheal ganglions in examined species is different. In guinea pig 166 – 327 (average of 222) and mice’s 63 -78 (average of 70). In our studies, 95-210 ganglions were found in cat (19). These results suggest that there is a correlation in between number of nerve cells and size of the respiratory system; this can be proved by a huge number of data. Two plexuses in dorsal wall of trachea are found in mice’s. First one with more delicate structure is in cervical part and second one as 80% of all ganglions with AChE positive, and nerve fibers in tracheal are denser than those in cervical part, but the difference is not as high as in the mice’s (19).

Recently, it has been concluded that different tachykinin are localized in the sensory fibers of the airways including substance CGRP (Calcitonin Gene Related Peptide) (20). Also, in motor parasympathetic nerve endings acetylcholine is produced, which is released in the neuromuscular connection by causing the constriction of the smooth muscle. Ach stimulated also epithelial cells and leads towards production of nitric oxide (NO). It is presumed that tachykinin also release endogen NO. Thus, e.g. NO is released by itself in airways and tachykinin that induces release of the NO do this through stimulation of receptors NK1 (21). Ach through activation of enzyme syntaase of nitric oxide (NOS) of epithelial cells releases NO, a molecule that relaxes airways smooth muscle (22). It is proved that NO has a role in the flogistic action of the substance P and other inflammatory mediators (23, 24).

Results of this research of piggy’s trachea isolated preparation function since the first day of extra-uterine life up to the fourth week of the age, shows that Ach, propranolol+Ach as applied in different concentrations affects the smooth tracheal musculature depending on age of the piggy’s and on concentrations of applied solutions. This is a specific process for every species. Possible explanation of this mecha-
nism of constriction of tracheal rings smooth musculature, dependence-age, is increase of the Calcium ions influx throughout “receptor-activating” and “voltage operating” channels in cells of the piggy’s tracheal smooth musculature in the first days of extra-uterine life.

5. CONCLUSION
Based on the experimental data, conclusions are as follows:

• Acetylcholine has caused response of the smooth musculature since the first day of extra-uterine life (p<0.01), which indicates that constrictor mechanism in the airways of piggy’s is developed during intrauterine life.

• Propranolol emphasizes easily constriction caused by acetylcholine of the smooth bronchial musculature at the first day of extra-uterine life, but this emphasis is not significant (p>0.1). This suggests that even adrenergic system is developed parallel to cholinergic system during intrauterine life.

• Reaction in local mediators such: histamine and PGF₂-alfa (that are released by mastocytes) do not show up until the fourth week of extra-uterine life.

REFERENCES