IN VITRO EXAMINATION OF DEGENERATIVE EVOLUTION OF ADRENERGIC NERVE ENDINGS IN PULMONARY INFLAMATORY PROCESSES IN NEWBORNS

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Abstract

Morphological aspect of tracheal preparations and pulmonary tissue was studied in vitro. The material was obtained from autopsy of newborns that died from different causes. Examinations were made in different gestational periods (immature 23-29 weeks; premature 30-37 weeks; mature >38 weeks). Material for examination was obtained up to 6 hours after death. Pulmonary and tracheal tissue was incubated for fixation in buffered formalin (10%). Special histochemical and histoenzymatic methods were used for coloring of pulmonary and tracheal tissue and the activity of ATP-ase and dopaoxidase was monitored. Cut out models were made in series of 7μ, 10 μ and 20 μ. In peripheral axons of tracheobronchial pathways, degenerative alterations of adrenergic nerve endings in lung inflammatory processes were documented. These morphologic neuronal changes were described: Walerians degeneration, neuro-axonal degeneration and segment demyelinisation. These changes are well seen with argentafine coloring (Sevier-Munger modification for nerve endings) and with dopaoxidase reaction. In mature newborns that died from respiratory distress syndrome, we found different forms of metabolic and toxic degenerative damage in peripheral axons, such as: segment demyelinisation, neurotubular fragmentation, Schwan cell prolifera-

tion, fragmentation and bulging out of axonal neurotubules and neurofilaments. In tracheo-bronchial tissue, chromafine granules are homogenously distributed on Lamina propria layer and through glandular structures. This gives as a contradiction, according to some authors, that adrenergic nerve fibers for muscle tissue are absent and that adrenaline and noradrenalin diffuse in muscle tissue from interstice.

KEY WORDS: adrenergic receptors, human trachea, bronchus and pulmonary tissue

Introduction

The main characteristic of tracheal nerve architecture is the positioning of the nerve trunk alongside smooth tracheal muscles and tracheal rings. The nerve trunk in the rostral part is smaller than in the caudal part. Branches of the nerve trunk anastomise and form a superficial net, which spreads on the surface of tracheal muscles from larynx to tracheal bifurcation. On the surface of tracheal plexus, the drawn in fibers branch deep in the muscle thus creating a neuronal coil. In the frontal part of trachea, the superficial coil and the lower neuronal coil intercommunicate and create a net of nerve fibers near sub mucosal glands, a net that is in between tracheal cartilage rings (1). Studies using electronic microscope and histochemical methods do not give clues about the way in which the direct innervation of aerogenic pathways is created but only determine the presence of adrenergic fibers in ganglions, blood vessels and sub mucosal glands. Lack of possibility to distinguish direct adrenergic innervation of bronchial muscles from adrenergic fibers does not mean that muscle membrane has no adrenergic receptors without neurotransmitters (2). It is proved that these receptors are not uniformly distributed in tracheal smooth muscle. The density of beta-adrenergic receptors is greater in lower parts of bronchi, while the density of alfa1-adrenergic receptors increases in upper parts of respiratory pathways. Axons derive from sympathetic ganglia as sympathetic postganglionic non-myeline nerve fibers. The greater part of nerve ending varicoses is distributed in axons, which allows economizing on axonal branches. Fast conduction of impulses is also possible. Adrenergic nerve endings are found in ganglion synapses or near effector organs (mainly near muscle tissue). Adrenergic nerve endings have a diameter of 3μ and contain granulated vesicles. The majority of vesicles is 40-60μ and contains smaller granules, which makes a greater electric density possible. Some vesicles have only electrodensitive membrane without the presence of vesicle membrane granules. We still have no sufficient data about the morphology and function role of vesicles in their normal and pathological state *in vivo* and *in vitro* condition in human material. Characteristics of adrenergic vesicles are diameter of 30-60μ and electrodensitive granules. There are also some vesicles with a diameter of approximately 50μ, which have great electric density. Larger granulated vesicles are also found in nonadrenergic nerve endings that do not contain catecholamines. It is proved that non-terminal part contains more granulated vesicles. With systematic studies of adrenergic nerves, a conclusion is reached that there are same groups of small and large vesicles in dense nerve endings. Genesis of small and large granulated vesicles during inflammatory processes is not explained. It is suggested that big granulated vesicles are formed in perikarion and then they transform in granulated vesicles with a smaller diameter after the release of contents and neurotransmitters. We still have no sufficient data on the morphology and pathological physiological aspect of the vesicle (3). Intramural nerve plexus is created from axons of different nature and origin. It is characteristic that big axonal parts face each other and their size is 15-20μ. Based on experimental, morphologic and pharmacologic studies an interaction between cholinergic and adrenergic nerve endings is documented (4). The aim of this study is to examine, *in vitro*, adrenergic nervous system in the airways, its morphogenesis and pathogenesis, degeneration of adrenergic nerve fibers in newborns, which are the cause of high mortality rate in different weeks of gestation in respiratory distress syndrome.

Material and Methods

Examination was conducted on 26 experimental studies *in vitro* on isolated tracheas of newborns that died in different gestational weeks. Samples were taken immediately after autopsy.

Group		Weight (g)	Gestational week
I	Immature	500-1100	23-29
II	Premature	1100-2500	30-37
III	Mature	>2500	>38

TABLE 1. Classification of examined cases according to gestation period of the newborns (n=26)

Special histochemical and histoenzymatic methods were used for coloring of pulmonary and tracheal tissue and the activity of ATP-ase and dopaoxidase was monitored. Cut out models were made in series of 7 μ, 10 μ and 20 μ.

Selected material for examination was
taken from these organ parts:

1. trachea above the bifurcation,
2. main intrapulmonary bronchus on both lungs,
3. pulmonary tissue from all lobes.

a) Material from main intrapulmonary bronchus was divided in two parts: one part was used fresh for histochemical methods, while the other part was fixed in 10% buffered formalin solution for histochemical methods.

Weight (g)	Age	HP. DG.	C.K.	A.D.	R.A.	NFD.
250	-	IP	-	-	-	-
500	-	IP	-	-	-	-
550	-	IP	-	-	-	-
700	5 hours	IP	-	-	-	-
850	1 day	IP	-	-	-	-
1000	-	IP	-	-	-	-
1050	1 day	MHP	-	+	-	-
1200	-	ALAM	-	-	-	-
1300	-	ALAM	-	-	-	-
1550	6 hours	MHP	+	+	+	-
1600	3 days	MHP	-	-	-	-
1940	1 day	MHP	-	+	-	-
2000	1 day	MHP	-	+	-	-
2050	-	ALAM	-	-	-	-
2250	8 days	BPPB		+	+	-
2300	1 day	MHP	-	+	-	-
2380	2 days	MHP	-	+	-	-
2650	-	ALAM	-	-	-	-
3000	1 day	MHP	-	+	-	-
3200	3 months	BPPB	+	+	+	+
3300	2 days	ALAM	-	+	-	-
3350	3 days	MHP	+	+	+	-
3450	4 days	BPPB	+	+	+	-
	4 months	BPPB	+	+	+	+
	4 months	BPPB	+	+	+	+
	4.5months	BPPB	+	+	+	+

Table 2. Degenerative ganglion and axon changes in neuronal populations in MNDV and NTS nucleus in medulla oblongata

Legend: HP.DG.- histopathologic diagnose; IP-immaturatio pulmonum; MHP-mebranae hyalinea pulmonum; ALAM-aspiratio liquoris amnii et meconialis; BPPB-bronchopneumonia purulenta pulmonum bilateralis; CK-central chromatolysis; AD-axonal degeneration; RA-reactive astrocitosis; NFD-neurofibrilar degeneration.

These methods were used: histochemical and histoenzymatic methods-hematoxilin and eosin coloring, Cresyl-echt-violet coloring for nerve and glial cells, coloring of argirophylle granules (Grimelius), Servier-Munger modification for coloring of nerve endings. Preparations were made with microtone and criotone in 7 μ and 10 μ.

Results

In our study, we used material obtained from dead and alive newborns, in different gestation periods. We used histochemical and histoenzymatic methods for morphologic examination of trachea and pulmonary tissue in different fetal development. On Table 2. ganglion and axonal degenerative changes of neuronal population of nerve cells in DMNV (dorsal motoric nucleus vagal) and NTS (nucleus tractus solitarius) in medulla oblongata are shown. Morphologic changes of adrenergic nerve endings in certain lung pathologic processes are shown in the pictures below (see Figure 1, 2, 3, 4, 5, 6, 7).

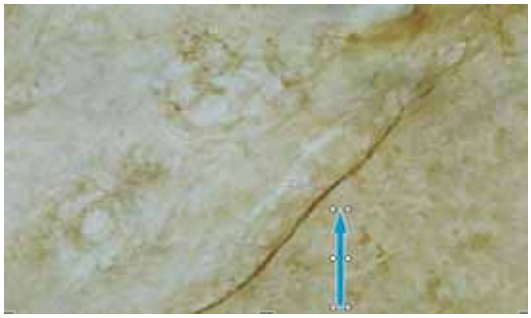

FIGURE 1. Peribronchial sensor nerve endings. Peribronchial sensor nerve endings do not contain vesicles. (dopaoxidase; 400x)

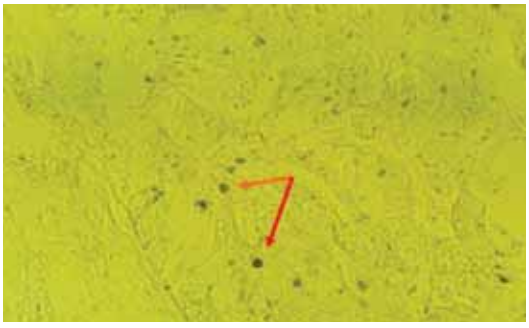

FIGURE 2. Light-positive ATP-ase in tracheal mucous glands. (ATP-ase; 400x).

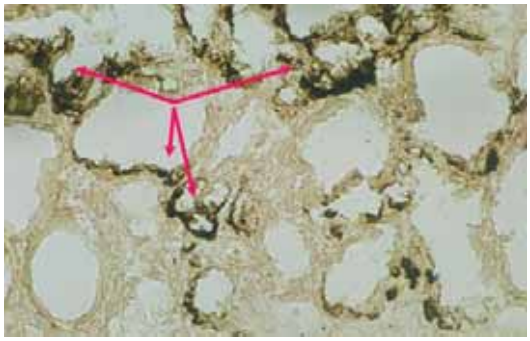

FIGURE 3. Clear positive argenophylle reaction in pulmonary tissue in mature newborn. (Sevier-Mnger modification; 400x)

FIGURE 4. Tracheal nerve fibers with axonal degenerative changes, segment demyelinisation. (dopa-oxidase; 400x).

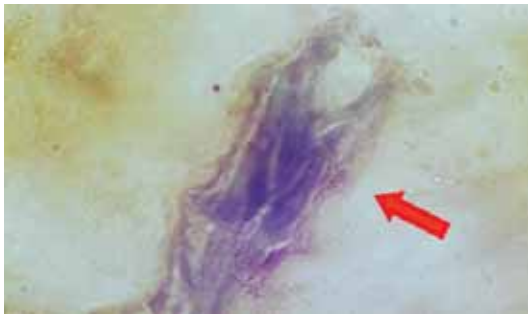

FIGURE 5. Axonal segment demyelinisation, axonal neurotubular fragmentation of Schwan cells in motoric tracheal nerve ending that contains vesicles. (dopa - oxidase; 400x).

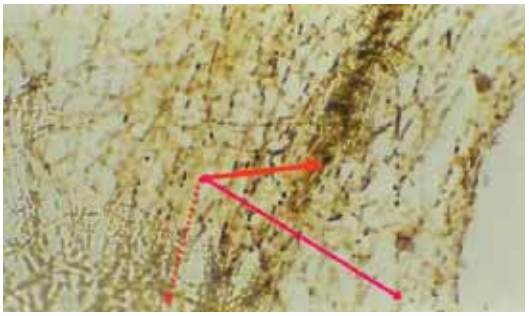

FIGURE 6. Axonal degeneration, fragmentation – bulging out of tracheal axons. (Sevier-Muniger modification; 1000x).

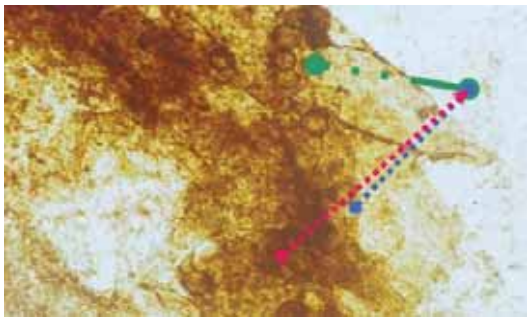

FIGURE 7. Axonal Waleron degeneration, disintegration of myeline wraping in form of globoid formulations, morphologic characteristic of this degeneration. (dopa - oxidaze, 400x).

Discussion

Noradrenaline is the main neurotransmitter of sympathetic nervous system, which can be found in preganglion synapses. Noradrenaline, adrenaline and dopamine were found later in central nervous system due to the application of fluorescence method of tissue monoamines induced by formaldehyde vapour. With use of imunocytochemical methods and production of specific antibodies against enzymes responsible for synthesis of catecholamines (tyrosine hydroxilase, dopamine beta-hydroxilase and fentolamine N-methyltransferase) new ways for anatomic investigation of catecholaminergic nervous system were found. It is thought that neurons, which contain dopa-oxidase on their surface, are noradrenergic; respectively use noradrenaline as neurotransmitter (5, 6). Adrenergic nerve fibers through postganglionic nerve fibers penetrate the lung and innervate the airways. A characteristic of nervous system is that neurons from different parts migrate in higher zones of nervous system. Neurons migrate in two directions: radial and tangent. It is thought that one of the mechanisms for migration of young neurons is through radial fibers of glial cells. In the beginning of neuronal cell development, they migrate as much as 1 mm per 24 hours, while in later stages of neurogenesis two weeks are needed for migration of cortical neurons (7). In our material, we note perivascular neuronal migration of respiratory neuron population. As a possible mechanism for migration, other authors emphasize it too (8). Pulmonary pathology in different stages of maturation

in newborns shows significant morphologic changes in population of neuronal cells in dorsal vagal motoric nucleus (MNDV) as well as in sensor dorsal nucleus (NTS) in shape of central chromatolysis "axonal retrograde degeneration", reactive astrocytosis, neurofibrilar degeneration and axonal degeneration (9,10,11). These changes are significant in newborns that lived more than 6 hours. In more mature newborns, which have more advanced pathologic substrate, because of chronic hypoxia, toxic metabolic impairments, as well as vascular circulation insufficiency, neurofibrilar degeneration is also seen. Three types of granules can be seen that correspond with axo-dendritic, axo-somatic and axo-axonal synaptic interconnections in tracheal glands, smooth muscle and in the layer of Lamina propria: small-granulated vesicles (SGV), big granulated vesicles (BGV) and vesicles with amino acids, with gamma-aminobutyric acid and glycine (GAG) (10, 11). Cholinergic system is filogenetically dominant, while other systems develop depending on the age. Adrenergic and histaminergic system are developed in mature age (12). Enzymatic activity is more expressed in serous glands, in muscles and in epithelia (neuroepithelial bodies). In terminal bronchioles, small and big granules (SGV, BGV) can be noted in epithelia and sub epithelia in direct contact with nerve endings (12). Sensor nerve fibers are less represented in tracheobronchial tract than motor nerve fibers. Main criteria for distinguishing sensor fibers from motor fibers are the presence of vesicles in motor nerve endings. Sensor nerve endings do not contain vesicles. Perichondrial sensor nerve fibers get mechanically irritated during the pathologic process of tracheal cartilage shrinkage (11,12,13). Pathological prolonged asphyxia (hypoxic states) cause depletion of adenosine triphosphate energy (ATP) (13). Adaptive reactions of neuronal cells are induced by hypoxia and degree of neuron cell maturation. In peripheral axons of tracheobronchial ways, these neuronal morphologic changes were seen: Walerian degeneration, neuro-axonal degeneration, segment demyelinisation, Schwan cell proliferation in tracheal motor nerve ending, which contains vesicles (dopa-oxidase reaction; 1000x). These changes are well seen with argentaphyle coloring (Sevier-Munger modification for nerve endings). In more mature age, we found different forms of degenerative, metabolic and toxic damage in peripheral axons, such as segment demyelinisation, neurotubular fragmentation, Schwan cell proliferation, fragmentation and bulging out of neurotubules and axonal neurofilaments. Observations made above suggest the direct influence of cardiorespiratory insufficiency in adrenergic nerve ending damage, as well as in other nervous systems. As a result, a "neurogenic inflammation" is created, which in human material still has no adequate response.

Conclusion

Based on data gained from morphologic investigation of trachea and pulmonary tissue in different gestational phases, we conclude the following:

- Enzymatic activity is more expressed in serous glands, muscle and epithelia (neuroepithelial bodies). In terminal bronchioles small and big granules can be noted in epithelia and sub epithelia in direct contact with nerve endings (which corresponds to adrenergic nervous system).
- In tracheobronchial tissue chromafine granules are homogenously distributed on Lamina propria layer and through glandular structures. This gives as a contradiction, according to some authors, that adrenergic nerve fibers for muscle tissue are absent and that adrenaline and noradrenalin diffuse in muscle tissue from interstice.
- In superficial axons of tracheobronchial ways of newborns that died from different factors, these neuronal morphologic changes were seen: Walerian degeneration, neuro-axonal degeneration, segment demyelinisation. These changes are well seen with argentaphyle coloring (Sevier-Munger modification for nerve endings) and with dopa-oxidase reaction.
- In more mature newborns that died from respiratory distress syndrome, we found different forms of degenerative, metabolic and toxic damage in peripheral axons, such as segment demyelinisation, neurotubular fragmentation, Schwan cell proliferation, fragmentation and bulging out of neurotubules and axonal neurofilaments.

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