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The influence of aminophylline on the contractility of urinary bladder smooth muscle in rabbits
Michal Hudec, Mária Jakubesová, Jozef Urdzik, Juraj Mokrý, Ján Švihra

Manometric profile of esophagus in children with bronchial asthma and recurrent respiratory diseases.
Vladimír Zošák, Peter Bánovčin jr., Milan Dragula, Daniel Bočíneck

Development of heart rate variability during the first three days of life
Lumír Kantor, Václava Curtisová, Lubomír Dubrava

Multiple neoplasms in patient with hairy cell leukemia – a case report
Lubomír Straka, Katarína Poliaková, Peter Szépe, Katarína Adamicová

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The acute exposure to high concentration of sulfur dioxide did not affect nasal reactivity in anaesthetized guinea pigs

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Abstract
This study was designed to test the hypothesis that sulfur dioxide, as a very important air pollutant, could affect reactivity of the nasal cavity to histamine at the period of 24 hours after the exposure in guinea pigs.

16 male TRK strain guinea pigs were exposed to SO2 (400ppm for 3 hours) in special plastic box. Controls (n = 8) were in the same conditions exposed to room air. 1 hour after the end of exposure animals of the control group and 8 animals of the group exposed to SO2 were anaesthetized, tracheotomized and a tygon nasopharyngeal cannula was introduced into their nasopharynx via proximal tracheal opening. The same procedure was performed in 8 animals of the group exposed to SO2 24 hours after the end of exposure to SO2. Histamine (1.25; 5.0 and 20.0 mmol.l⁻¹) was instilled into the nasal cavity and nasal airway resistance (Rn) was measured to evaluate nasal reactivity to histamine.

Nasal airway resistance (Rn) was determined as a ratio of transnasal pressure and known airflow (1.0; 1.5; and 1.0 l.min⁻¹) applied through the nasopharyngeal cannula and nasal cavity in expiratory direction.

Nasal reactivity to histamine was not significantly changed 1 hour and 24 hours after the end of exposure to high concentration of sulfur dioxide in comparison to control values.

We can conclude that acute exposure to high concentration of sulfur dioxide did not affect nonspecific nasal reactivity in anaesthetized guinea pigs.

Key words: sulfur dioxide – nasal reactivity – guinea pigs

Introduction
Air pollution is an inevitable consequence of industrialization. A number of epidemiological studies have shown acute effects of increased amounts of ambient air pollution on the prevalence of respiratory symptoms in population (1). Not only acute, but also continuous exposure to air pollution may cause or trigger respiratory diseases, as well (2). This connection is very important in persons with existing airway disease or airway hyperresponsiveness (3).

Sulfur dioxide of its high water solubility is primarily an upper respiratory tract irritant. This substance is extremely irritating to the nasopharynx and whole respiratory tract. Bronchoconstriction, thoracic pain, coughing, sneezing, wheezing, dyspnea and upper airway or pulmonary oedema with cyanosis may occur. Reactive airway disease, obstructive and restrictive lung disease or chronic bronchitis may develop in victims that survive exposure to high concentrations (4). So the target organ for the acute effects of sulfur dioxide appears to be confined to the upper respiratory tract and the lung (5).

We have found in our previous investigations that repeated inhalation of high concentration of sulfur dioxide did not affect bronchial reactivity in guinea pigs determined 24 hours after exposure to this gas (6). But it is known, that responsiveness to SO₂ is not uniform throughout the respiratory tract (7).

Absorption of SO₂ is believed to be complete in the conducting airways only a small percentage of inspired SO₂ appears to reach the trachea (8). Miller et al. (9) described the upper respiratory tract and the large bronchi as the major site of absorption and toxicity of SO₂. It is well known that sulfur dioxide dissolves easily in the layer of fluid on the surface of the epithelium of the nasal passages and upper airways with formation of reactive sulfite species. Major percentage of inspired SO₂ acts in the nasal passages.

Very high concentrations of inhaled SO₂ and aerosols of sulfuric acid are surprisingly well tol-
erated by many animal species and morphological damage of epithelial lining cells is detected in the upper respiratory tract after high or prolonged exposures. Chronic exposure to urban levels of air pollution induces secretory cells hypertrophy, combined with a shift toward mucus secretion and ciliary damage (10) in rats – changes that causes mucociliary clearance impairment (11). This finding is consistent with the idea that prolonged exposure to low levels of air pollution deteriorates respiratory defenses against infectious agents and may cause an increase in respiratory morbidity and perhaps mortality. Takenaka et al. (12) also reported morphological changes observed in nasal cavities of beagle dog after long term exposure to SO2, that were characterized by thickening of epithelial layer resulting from epithelial proliferation, by a loss of secretory material and by a moderate mononuclear cell infiltration.

Brief exposure to SO2 at a concentration of 4ppm or less is unlikely to cause significant nasal dysfunction in nonhyperreactive animals, e.g. guinea pigs. Sulfur dioxide does not acutely increase nasal symptoms or nasal resistance in subjects with rhinitis or in subjects with bronchial responsiveness to sulfur dioxide (7) Short-term exposure to low concentrations of sulfur dioxide did not reveal any significant morphological differences in human nasal epithelium (13) on the other hand Carson et al. (14) reported appearance of compound cilia in the nasal mucosa of normal human subjects following acute exposure to sulfur dioxide at concentration 0.75 ppm.

**AIM**

This study was designed to ascertain whether acute short-term exposure to high concentration of sulfur dioxide could affect nasal reactivity to histamine during 24 hours after the end of exposure to sulfur dioxide in anaesthetized guinea pigs.

**METHODS**

**Animals**

24 male TRIK strain guinea pigs weighing 400g - 510g were recruited for the study. Animals were housed in central animal house of Jessenius Faculty of Medicine and were kept in standard living conditions. All of these animals underwent veterinary examination to exclude possible health disorders.

**Exposure to sulfur dioxide**

16 animals were placed into the plastic box and exposed to sulfur dioxide with concentration 400 ppm (parts per million) during 3 hours. 8 controls were exposed to room air in the same conditions.

**Determination of nasal airflow resistance (Rn)**

Animals were anaesthetized with intraperitoneal administration of urethane 1.1g, kg\(^{-1}\) (Riedel de Haen, AG Germany) and they were placed in the supine position on the heated pad. Rectal temperature of the animals was continuously measured and maintained in the range 37-38 \(^\circ\)C. The trachea was explored and widely opened to allow spontaneous breathing. A thin tygon cannula (external diameter 3.5 mm) was introduced into the nasopharynx through the proximal tracheal opening and fixed by a ligature. Cannula was connected to electromanometer HSE (Hugo Sachs Electronic).

The principle of recording of the nasal resistance is the measurement of transnasal pressure (difference between the pressure in the nasopharynx and atmospheric pressure) during the periodization of the known airflow through the nasal cavities. The resistance is given by the ratio of transnasal pressure and the accompanying airflow. Nasal passages were blowing with the constant airflow of humidified and tempered air that was delivered by the airflow generator. The airflows of 1.0; 1.5 and 1.0 l/min were bubbled through the water of the thermostat 57°C. The air moistened with water vapors and warmed up to approximately 37°C was introduced to the nasopharyngeal cannula. At the same time nasopharyngeal pressure was measured and recorded. Rn was measured during gradually increasing airflows (1.0 to 1.5 l/min) - the first phase of measurement, and back to 1.0 l/min – the second phase of measurement.

Nasal airways resistance was determined from the formula:

\[ R_n = \frac{P_{tn}}{airflow - Rc} \]

\[ P_{tn} \] - transnasal pressure

\[ Rc \] - airflow resistance of nasopharyngeal cannula

\[ R_n \] - nasal airway resistance (kPa.min\(^{-1}\))

**RESULTS**

Intranasal histamine challenge was used as nonspecific provoking stimulus to induce a response of nasal mucosa. Histamine is one of the most frequently used stimuli for nasal reaction (15). Nasal passages were gently filled with histamine (Spofa) at concentrations 1.25; 5.0 and 20.0 mmol/l in increasing order. Intranasal stimulation with histamine lasted for 3 minutes and then residual histamine solution was gently expressed from the nasal cavities. Just after the end of this maneuver nasal airflow resistance was measured.

**Design of experiment**

Nasal airflow resistance (Rn) was determined in control animals (1\(^{st}\) group, n = 8) that were exposed to room air. These values were compared to these obtained for Rn in animals exposed to SO2 – 1 hour after the end of exposure (2\(^{nd}\) group, n = 8) and 24 hours after the end of SO2 exposure (3\(^{rd}\) group, n = 8). Rn was determined in these above groups after intranasal saline and histamine (from 1.25, 5 to 20 mmol/l), during airflows 1.0; 1.5 and again 1.0 l/min.

**Statistics**

Data obtained for nasal airflow resistance are expressed as an arithmetic mean and standard error of mean. Values were evaluated using Friedman test and Dunn’s test for multiplicative comparisons, p < 0.05 was considered to be significant.

**Table 1. Changes of nasal airflow resistance caused by intranasal histamine challenge in guinea pigs exposed to sulfur dioxide**

<table>
<thead>
<tr>
<th>group</th>
<th>Rn (kPa.min(^{-1})) after saline</th>
<th>Rn (kPa.min(^{-1})) after histamine 1.25 mmol/l</th>
<th>Rn (kPa.min(^{-1})) after histamine 5 mmol/l</th>
<th>Rn (kPa.min(^{-1})) after histamine 20 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) mean</td>
<td>S.E.M.</td>
<td>19.33</td>
<td>19.89</td>
<td>15.36</td>
</tr>
<tr>
<td>2(^{nd}) mean</td>
<td>S.E.M.</td>
<td>24.09</td>
<td>21.52</td>
<td>18.91</td>
</tr>
<tr>
<td>3(^{rd}) mean</td>
<td>S.E.M.</td>
<td>20.53</td>
<td>18.01</td>
<td>16.2</td>
</tr>
</tbody>
</table>

\(*) p<0.05 – the effect of intranasal histamine challenge in comparison to saline challenge

1 \(^{st}\) group – Rn in animals exposed to room air

2 \(^{nd}\) group – Rn in animals exposed to sulfur dioxide 1 hour after the exposure

3 \(^{rd}\) group – Rn in animals exposed to sulfur dioxide 24 hours after the exposure
upper respiratory ways is experimentally rarely studied (17). of the total airflow resistance of the respiratory passages, the limitation of airflow through the mostly in the region of lower airways. Despite the fact that nasal cavities represent about a half

Nasal hyperreactivity play a crucial role in the pathogenetic process of diseases of nasal cavities. Nasal reactivity is characterized by appearance of nasal symptoms – sneezing, nasal discharge and decreased nasal patency as a response of nasal mucosa to appropriate stimuli. Inappropriate reactivity of the structures of nasal mucosa is manifested as a nasal hyperreactivity (hyperresponsiveness).

Nasal hyperreactivity play a crucial role in the pathogenetic process of diseases of nasal cavity and sinuses (16). The questions of hyperreactivity of respiratory tract are intensively studied, mostly in the region of lower airways. Despite the fact that nasal cavities represent about a half of the total airflow resistance of the respiratory passages, the limitation of airflow through the upper respiratory ways is experimentally rarely studied (17).

Tatár et al. (18) developed a method for observing the nasal reactivity in anaesthetized guinea pigs. Volume changes of the nasal mucosa (nasal patency) were estimated from the changes of airflow through the nasal cavity and transported by a mucociliary system up to the nasopharynx, where due to nasopharyngeal cannula its swallowing was blocked. The accumulation of mucus could contribute to the increase of nasal resistance in the 1st phase of measurement. In the second phase of the measurement of nasal resistance it was decreased in comparison to the values of 1st stage of measurement. This difference could be ascribed to removal of accumulated mucus from the nasal cavity during airflow 1.5 l/min applied in expiratory direction, which allowed to clean up the nasal cavities from accumulated secretions.

In conclusion: Acute exposition to high concentration of sulfur dioxide did not affect nasal reactivity measured 1 and 24 hours after the exposure to sulfur dioxide.

REFERENCES

THE INFLUENCE OF AMINOPHYLLINE ON THE CONTRACTILITY OF URINARY BLADDER SMOOTH MUSCLE IN RABBITS

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ABSTRACT

Introduction: Urinary bladder smooth muscle is under the influence of autonomic nervous system. Its contractions are evoked by parasympathetic nervous system, with its main mediator acetylcholine. Acetylcholine acts through the Gq-protein (receptor M1, M3, M5) and activation of enzyme phospholipase C or through the inhibition of adenylate cyclase on Gs protein (receptor M2, M4). The contractions can be inhibited by two basic mechanisms - inhibition of contraction (anticholinergic drugs) or induction of relaxation (sympathomimetics, calcium channel blockers). In this study, we investigated the effect of aminophylline - inhibitor of phosphodiesterase – on urinary bladder smooth muscle contractions evoked by acetylcholine.

Methods: Samples of smooth muscle from rabbit urinary bladders (11 rabbits, weight 1500-2500g) were analyzed in vitro in organ baths. From the samples thin slices were made, which were adapted in Krebs-Henseleit’s solution for one hour (30 minutes under tension of 4 g and 30 minutes under tension of 2 g). The first recordings (control) were made under the influence of acetylcholine. Aminophylline - inhibitor of phosphodiesterase - was then added to the organ chamber in order to reach the concentration of 10^3 or 10^4 mol/l and after 15 minutes incubation, the second recordings were made.

Results: The contractile responses to cumulative doses of acetylcholine were significantly decreased after adding of aminophylline in concentration of 10^-4 mol/l. Aminophylline in concentration of 10^-4 mol/l caused only a non-significant inhibition of contractile responses.

Conclusion: We found that aminophylline is an effective inhibitor of urinary bladder smooth muscle contractions in rabbits evoked by acetylcholine in vitro in concentration of 10^-4 mol/l.

Key words: aminophylline, contraction, acetylcholine, urinary bladder, rabbits, smooth muscle

INTRODUCTION

The urinary bladder smooth muscle, similarly to smooth muscles of other organs and systems, possesses its own steady place in morphological as well as functional character of the organism. Its characteristics influence the behavior of not only urinary bladder but of the whole body, as the ability to cumulate urine and consecutively release it belongs to the basic social needs. Only a normal and coordinated bladder function can maintain a good social adaptation of an individual. Any changes in this basic need can disturb its integration and social positioning and so could lead to significant decrease of the quality of life. Therefore, it is very necessary to know all the mechanisms participating in filling of the urinary bladder, voiding and in case of impairment to be able to eliminate it.

In clinical practice, we meet the problems of hyperresponsiveness or hyperreactivity of smooth muscle in various organ systems, like respiratory system, gastrointestinal tract, skin, as well as urinary system. Diseases of the lower urinary tract belong to the most frequent disorders at all. They include a wide range of incontinencies, as well as disorders in urination, which in serious cases can cause an impairment of the kidneys or renal failure. One of these disorders is also hyperreactivity of the urinary bladder smooth muscle, leading to incontinence and so to the decreasing quality of life.
The modulation of contraction is possible by two basic mechanisms - inhibition of contraction (anticholinergic drugs) or induction of relaxation (sympathomimetics, calcium channel blockers). Both of these mechanisms work on the synaptic level.

Acetylcholine, as a major neurotransmitter of parasympathetic nervous system, mediates its contractile responses through muscarinic receptors. Nowadays are known five pharmacologically different muscarinic receptors subtypes – M1-M5, from which M2 and M3 receptors mostly occur. Their stimulation leads to two different pathways. M3 receptor acts through activation of PLC, AC – adenylate cyclase, IP3 – inositol triphosphate, DAG – diacylglycerol, cAMP – cyclic adenosin monophosphate (Fig. 1).

M1 receptor acts through G protein that inhibits adenylate cyclase. This inhibition leads to lowering of the formation of cAMP from ATP. The already created cAMP is dissociated into AMP by enzyme phosphodiesterase. Decrease of intracellular level of cAMP leads to the increase of intracellular calcium and to contraction (Fig. 2) (4).

The aim of the presented study was to examine the influence of aminophylline as a representative of xanthine derivatives (inhibitor of enzyme phosphodiesterase) on the mechanism of contraction of the urinary bladder smooth muscle. In literature, there are very rare references about their interactions in urinary bladder smooth muscle (Ach – acetylcholine, NA – norepinephrine, M – muscarinic receptor, β – beta adrenoceptor, PLC – phospholipase C, AC – adenylate cyclase, IP3 – inositol triphosphate, DAG – diacylglycerol, cAMP – cyclic adenosin monophosphate).

METHODS

The reactivity of urinary bladder smooth muscle was estimated by in vitro method (8,9,10). The detrusor samples of 11 rabbits (weighting 1500-2500g) were used. The samples were cut into small strips (2 x 2 x 15 mm), mounted between two hooks and placed into a 30 ml organ chamber containing Krebs-Henseleit’s buffer of the following composition: NaCl 110.00 mmol.l-1, KCl 4.80 mmol.l-1, CaCl2 2.35 mmol.l-1, MgSO4 1.20 mmol.l-1, KHPO4 1.20 mmol.l-1, NaHCO3 25.00 mmol.l-1 and glucose 10.00 mmol.l-1 in glass-distilled water. Organ chambers were maintained at 36.5 ± 0.5 °C and were aerated continuously with a mixture of 95% O2 and 5% CO2, to maintain pH 7.5 ± 0.1. One of the hooks was connected to force transducer (TSR 10G, V˘voj Martin, Slovakia) and amplifier (M1101 SUPR, Mikrotechna Praha, Czech Republic) and tension recordings were made on Line Recorder TZ 4620 (Laboratorní přístroje Praha, Czech Republic). The tissue strips were initially set to 4 g of tension (30 minutes loading phase). After this period, the tension in each strip was readjusted to a baseline of 2 g (30 minutes adaptation phase). During both periods, the tissue strips were washed at 10 minutes intervals. Thereafter cumulative doses of acetylcholine (10-8 to 10-3 mol.l-1, subst. Sigma-Aldrich) were added and continual graphical recording of contractions was carried out. This recording was named “Control”. After 25 minutes of washing up period, 200 µl of aminophylline (Sintofyllin, Hoechst-Biotika) was added into each organ chamber to reach the concentration of aminophylline 10-4 or 10-3 mol.l-1. After a 15 minutes period of incubation the amplitudes of contractions (g / 100g) of urinary bladder smooth muscle strips to the cumulative doses of acetylcholine (10-8 to 10-3 mol.l-1) were recorded. These records were used for evaluation of contractile responses (11).

Non-parametric ANOVA test was used for the statistical analysis and comparison of the „control” recordings (strip contractility after stimulation by only cumulative doses of acetylcholine), with the recordings after adding of aminophylline in concentrations of 10-4 or 10-3 mol.l-1. Results are presented as mean ± standard error of the mean (SEM). A probability level of p < 0.05 was accepted as significant. All experiments were conducted in accordance with basic ethical norms and Helsinki Declaration of 1975, revised in 1983.

RESULTS

Addition of acetylcholine into organ bath with urinary bladder smooth muscle strip in cumulative manner (10-8 to 10-3 mol/l) resulted in dose-dependent increasing of contractile responses in controls.

The recordings after adding of aminophylline (10-4 or 10-3 mol.l-1) showed increasing strength of contraction evoked by cumulative doses of acetylcholine, too, but the strength was not so high as in the “control” (Fig. 3).

We observed significant lowering of contractile responses to acetylcholine in cumulative manner after incubation with aminophylline only in concentration of 10-3 mol.l-1. The concentration of aminophylline of 10-4 mol.l-1 did not cause significant inhibition of contractile responses, but the trend to decrease was observed (Fig. 3).

DISCUSSION

There are various ways and levels how to influence the urinary bladder reactivity. The micturition reflex represents classical reflex with its own receptors, afferent nerves, reflex center, efferent nerves and finally effectors. The receptors are located in and under urothelial layer as well as in urinary bladder smooth muscle. The sensory nerve endings can be irritated by various...
treatment and anticipating of possible adverse effects. Moreover, especially adverse effects and lack of efficiency in lower doses of drugs are the reasons for looking for new therapeutic possibilities. Currently we use the following drugs for the treatment of overactive bladder: a) antimuscarinic drugs, drugs acting on membrane channels, drugs with mixed action, alpha-adrenoceptor antagonists, beta-adrenoceptor agonists, antidepressants, prostaglandin synthesis inhibitors, vasopressin analogues and others (21) (Tab. 1).

In the regulation of urinary bladder smooth muscle a major role is played by parasympathetic system similarly to other organ systems (16), with its muscarinic receptors. The most frequently occurring muscarinic receptors in urinary bladder smooth muscle in various animals and in humans are M<sub>2</sub> and M<sub>3</sub> subtypes. M<sub>2</sub> receptor represents 80% of all of them (in humans) (17), but the major role in the mechanism of contraction during voiding phase possesses M<sub>3</sub> receptor (18,19). M<sub>3</sub> receptors are responsible for the direct contraction of smooth muscle; M<sub>2</sub> receptors block the relaxation caused by sympathetic nervous system. Except of these two receptor subtypes located mainly on the postsynaptic membrane, there are also M<sub>1</sub> and M<sub>2</sub>/M<sub>4</sub> receptors located on the presynaptic membrane. Presynaptic muscarinic receptors cause facilitation (M<sub>1</sub>) or inhibition (M<sub>2</sub>/M<sub>4</sub>) of secretion of acetylcholine dependent on the frequency of stimulation. The result is stronger contraction of the detrusor after stimulation of M<sub>1</sub> receptor and inducing of relaxation after stimulation of M<sub>2</sub>/M<sub>4</sub> receptors (20) (Fig. 1).

Failure to store urine can be due to involuntary detrusor contractions, which may or may not be associated with symptoms of urge, frequency and urge incontinence, the components of the overactive bladder syndrome. The prevalence of these symptoms increases with advancing age. To learn the mechanisms underlying this condition is essential for adequate pharmacological treatment and anticipating of possible adverse effects. Moreover, especially adverse effects and lack of efficiency in lower doses of drugs are the reasons for looking for new therapeutic possibilities. Currently we use the following drugs for the treatment of overactive bladder: a) antimuscarinic drugs, drugs acting on membrane channels, drugs with mixed action, alpha-adrenoceptor antagonists, beta-adrenoceptor agonists, antidepressants, prostaglandin synthesis inhibitors, vasopressin analogues and others (21) (Tab. 1).

Tab. 1 Drugs used for the treatment of overactive bladder according to Andersson et al., 2001 (21)

<table>
<thead>
<tr>
<th>Pharmacological group</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimuscarinic drugs</td>
<td>Tolterodine, Trospium, Propantheline, Atropine, hyoscyamine</td>
</tr>
<tr>
<td></td>
<td>(Darifenacin, solifenacin)</td>
</tr>
<tr>
<td>Drugs acting on membrane channels</td>
<td>Calcium antagonists, Potassium channel openers</td>
</tr>
<tr>
<td>Drugs with mixed actions</td>
<td>Oxybutynin, Proprerine, Dicyclomine, Flavoxate</td>
</tr>
<tr>
<td>Alpha-adrenoceptor antagonists</td>
<td>Afluzosin, Doxazosin, Prazosin, Trazosin, Tamsulosin</td>
</tr>
<tr>
<td>Beta-adrenoceptor agonists</td>
<td>Terbutaline, Clenbuterol, Salbutamol</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Imipramine</td>
</tr>
<tr>
<td>Prostaglandin synthesis inhibitors</td>
<td>Indomethacin, Flurbiprofen</td>
</tr>
<tr>
<td>Vasopressin analogues</td>
<td>Desmopressin</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Baclofen, Capsaicin, Resiniferatoxin</td>
</tr>
</tbody>
</table>

Aminophylline is a xanthine derivative drug and its effects in the influencing of contraction are present mainly on the postsynaptic level. The mechanism of action of xanthine derivatives is in spite of their long clinical use not definitely clear. There are various theories, from which the inhibition of enzyme phosphodiesterase plays a major role (Fig. 4). Inhibition of this enzyme leads to decreased metabolism of cAMP and so to the increase of its intracellular level. Cyclic AMP as a second messenger participates in intracellular regulatory mechanisms and regulates cell contractility. Cyclic AMP activates cAMP-dependent protein kinase A mediating the phosphorylation of myosin-kinase and so reduces the ability to activate myosin. This is not the only mechanism leading to relaxation of smooth muscle, used especially in management of airways obstruction diseases (23). The serum concentrations necessary for inhibition of phosphodiesterase should be too high and are associated with adverse effects. In addition, not all of the xanthine derivatives.

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**Fig. 3** Reactivity of guinea pig urinary bladder smooth muscle after adding of aminophylline in concentrations of 10<sup>-4</sup> and 10<sup>-3</sup> mol.l<sup>-1</sup> to cumulative doses of acetylcholine. The columns represent the mean contraction (g/100 mg) and the standard error of the mean (SEM). One and two asterisks represent significance of difference with p < 0.05, and 0.01, respectively (AMP = aminophylline)

**Fig. 4** Mechanism of action of aminophylline (for explanation see Fig. 2)
are able to relax the smooth muscle. Therefore, other mechanisms were sought. One of them is a competitive antagonism of adenosine receptors and a central action on $A_1$ and $A_2$ receptors. Very interesting is also the anti-inflammatory activity of xanthines used especially in the therapy of bronchial asthma. Another effects, which could participate in relaxation of smooth muscle, are hyperpolarisation of membrane with consecutive opening of $K^+$ channels, influence on intracellular calcium stores and releasing of calcium, on secretion of endogenous mediators, activity of prostaglandins, etc. (22).

For our experiments, we chose aminophylline as a representative of xanthine derivatives routinely used in clinical practice for the treatment of obstructive diseases of airways (asthma, COPD) (23). Pharmacodynamic properties of xanthines are based on their effect on many organ systems (24). They stimulate central nervous system leading to positive stimulation of breathing, tremor, convulsions or vasoconstriction. In cardiovascular system their administration lead to increase of cardiac output, decrease of heart fibrillation and dilatation of peripheral arterys and veins. They increase blood flow through the kidneys, glomerular filtration and thus also diuresis. Especially this effect could be a limiting factor in potential therapeutic ambitions of xanthines, as increased diuresis could enhance the incontinence rate in individuals with clinical predisposition. Xanthines possess weak tocolytic activity. In gastrointestinal tract, they increase secretion of gastric acid, gastrin and glucagon. They relax bile and urinary tract, esophageal sphincter, and bronchial smooth muscle. They increase secretion and release of catecholamines, cortisol, insulin, and growth hormone. In airways, they lead to the increase of mucociliar clearing, secretion of phlegm (mucus) and surfactant (25) as well as to the depression of cough (26). They dilate the pulmonary vessels and so decrease the pulmonary hypertension, vascular permeability and edemas (27,28).

In our experiments, we observed that aminophylline significantly lowered the contractile responses to acetylcholine in cumulative manner after incubation with aminophylline only in concentration $10^{-3}$ mol.l$^{-1}$. The concentration of aminophylline $10^{-4}$ mol.l$^{-1}$ did not cause significant inhibition of contractile responses, but tended to their decrease. The concentration of $10^{-3}$ mol.l$^{-1}$ is relatively high, as the recommended serum concentrations of xanthines are 8-15 µmol.l$^{-1}$. The aim of this study was to determine the possibility of aminophylline to be used as a tool for better aspect on their possible effect on urinary bladder smooth muscle and their possible future therapeutic use.

In conclusion, we can state that aminophylline is an effective inhibitor of urinary bladder smooth muscle contraction in rabbits evoked by acetylcholine in vitro only in higher concentration ($10^{-3}$ mol.l$^{-1}$). These findings are still subjected to further research.

REFERENCES

6. Huddart H, Bayton E, Shanklin J. Influence of some common methylxanthines on contractile responses and calcium predisposition. Xanthines possess weak tocolytic activity. In gastrointestinal tract, they increase secretion of gastric acid, gastrin and glucagon. They relax bile and urinary tract, esophageal sphincter, and bronchial smooth muscle. They increase secretion and release of catecholamines, cortisol, insulin, and growth hormone. In airways, they lead to the increase of mucociliar clearing, secretion of phlegm (mucus) and surfactant (25) as well as to the depression of cough (26). They dilate the pulmonary vessels and so decrease the pulmonary hypertension, vascular permeability and edemas (27,28).
MANOMETRIC PROFILE OF ESOPHAGUS IN CHILDREN WITH BRONCHIAL ASTHMA AND RECURRENT RESPIRATORY DISEASES.

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Abstract

Gastroesophageal reflux (GER) is a distinguished pathomechanism of the bronchial asthma and pulmonary infections. The prevalence of GER ranges from 47 to 64 percent in children with chronic respiratory diseases. Esophageal manometry is a diagnostic test that measures intraluminal pressures and coordination of pressure activity of the muscles of the esophagus. It provides both qualitative and quantitative assessment of esophageal pressures, coordination and motility. The aim of the study was to compare esophageal manometric profile in children with positive 24-hour pH study (group B) to children with respiratory complications of gastroesophageal reflux disease (GERD) (group C). Results of manometric profiles from children with negative 24-hour pH study (group A) were used as standard manometric profiles of the esophagus.

The water infusion system was used to demonstrate the manometric profile of the esophagus. The results of the manometric profiles from groups A and B were used from the previous study at the Clinic of Pediatric Surgery in Martin. The only statistically significant difference in the parameters of the lower esophageal sphincter (LES) was in total length of the LES – group B: 3.6 cm and group C: 2.75 cm, group A: 3.6 cm. The intrabolominal LES length, resting LES pressure (LESP) and LES relaxation (LESR) were not significantly different. The parameters of the distal esophagus (DE): percentage of the peristaltic contraction in group A (49.95%) than in group B (60%) and group B (95.4%). Also, group C has a higher presence in non-coordinated contraction of DE than group B. No significant difference was in the amplitude of contraction.

Our study has confirmed the importance of the gastroesophageal reflux disease for the etiology of chronic pulmonary infections and bronchial asthma. Patients with these infections have manometric parameters of the esophagus similar to that of 24-hour pH study of the confirmed GERD patients.

Key words: esophageal manometry, GERD, pulmonary infections

Introduction

The observation that pulmonary disease might, in some unknown way, be influenced by a malfunction of the gut, was first recorded in the holy books. In the Talmud the treatment for unwanted pulmonary symptoms usually involved some types of food. Coughing was treated with a fish oil drink, and asthma was treated with three wheat cakes soaked in honey followed by an ingestion of undiluted wine. In 1802, William Heberden wrote that in asthmatics, "the breath is shorter and more difficult after a meal." In 1892, in The Principles and Practice of Medicine, Sir William Osler [17] wrote in his textbook that "severe paroxysms of asthma may be induced by overloading the stomach, or by taking certain articles of food." In this century, the story has begun to unravel, and the relationship between gastroesophageal reflux (GER) and pulmonary abnormalities has become more clear. In 1934, a study by Bray reported that in some patients dietary indiscretion could lead to asthmatic attacks. He believed that late-evening overindulgence caused gastric distention and led to reflex-mediated bronchoconstriction via the vagus nerve. In 1962, Kennedy opened a new era by suggesting that "silent" GER may be an important but little known cause of pulmonary complications.

In a review on pulmonary complications of esophageal disease, Belsey reported that patients with GER were liable to severe, progressive and disabling pulmonary damage. The results of a survey of 636 patients referred for surgical correction of severely symptomatic GER. More than 60 percent of these patients had symptoms of pulmonary diseases coexisting with the GER. The prevalence of GER in patients with respiratory diseases is well described in numerous studies. It ranges from 47 to 64 percent, depending on the pulmonary diagnosis and the diagnostic parameter used to define GER.

The mechanisms by which GER might induce such pulmonary abnormalities as bronchitis, asthma, pneumonitis, and pulmonary fibrosis have been a subject of debate. Two different mechanisms for GER-induced symptoms have been postulated: 1/activation of a GER-induced vagal reflex arc (vagal mediation) from esophagus to the lungs, resulting in bronchoconstriction 2/microaspiration of gastric contents into the lung, resulting in an exudative mucosal reaction.

Evidence for vagal mediation (reflex theory) was demonstrated by many studies. Four studies attempted to demonstrate vagal mediation of bronchoconstriction by using acid esophageal infusion in asthmatic patients. This study by Mansfield and colleagues [14] demonstrated increased airway flow resistance that rapidly reversed when reflux symptoms were relieved by antacids. These pulmonary changes occurred even during maximal bronchodilator therapy. The changes were statistically significant only for the sensitive measurements of total respiratory resistance and flow at 25 percent of vital capacity. In a later study [15] in dogs they reported a similar fall in respiratory conductance that disappeared with bilateral interruption of the vagal nerves. Using 24-hour pH monitoring, Herbst and co-workers identified 14 infants in whom GER caused apnea. Cessation of apnea and improvement in pulmonary disease occurred in 8 infants after pharmacotherapy and in 6 infants after surgical correction of GER. Instillation of dilute HCl into esophagus of these infants reproduced the apnea. The mechanism by which GER caused apnea is unclear, but the investigators suggested an esophageal pulmonary reflex. Another study by Davis and associates [10] further emphasized a role for GER in causing bronchospasm by infusing acid into the distal esophagus of asthmatic children during sleep. Bronchoconstriction developed in all 4 children with a positive response to the esophageal acid infusion (Bernstein test), but none of the 5 with a negative response. All of the respiratory abnormalities occurred during 4 to 5 am infusion but not during the midnight infusion. The authors suggested that a GER-induced exacerbation of asthma required several factors: 1/ reflux of gastric acid into the esophagus, 2/acid-sensitive esophagus (positive Bernstein test response) and 3/ a low nocturnal threshold to bronchoconstrictive stimuli. Despite the elegance of these studies, the clinical significance of the findings is highly questionable, especially since clinically detectable bronchospasm did not occur. A recent clinical study using dual-electrode ambulatory pH monitoring added support to the reflex theory. Abnormal distal esophageal acid exposure was prevalent in patients with chronic cough (50%), asthma (44%) and unexplained chest pain (54%). The prevalence of abnormal proximal esophageal (20 cm above the LES) reflux was significantly higher in chest pain patients without pulmonary complaints (44%) than in patients with either asthma (24%) or chronic cough (11%).

Abnormal esophageal acid exposure is thought to mediate induction of bronchospasm. Davis and associates [16] used dual-electrode ambulatory pH monitoring and found that the prevalence of GER was significantly higher in children with recurrent cough (50%), asthma (44%) and unexplained chest pain (54%). The prevalence rates were significantly higher than the 19 percent prevalence of hiatus hernia and 5 percent prevalence of barium reflux seen in the matched control group. In addition to barium studies, evidence of microaspiration as a cause of asthma is supported by other methods. Danus and colleagues [9] used cineradiography and esophageal manometry to demonstrate GER in 60 percent of children with recurrent bronchitis, and Euler and associates [12] used esophageal manometry, pH testing and endoscopy to demonstrate GER in 63 percent of children with chronic asthma or recurrent pneumonia.
Esophageal manometry is a diagnostic test that measures intraluminal pressures and coordination of pressure activity of the muscles of the esophagus. It provides both qualitative and quantitative assessment of esophageal pressures, coordination and motility. Manometric studies are used in the assessment of patients with symptoms suggestive of esophageal origin such as dysphagia, odynophagia and noncardiac chest pain. A manometric study is also indicated prior to antireflux surgery and in assessing possible esophageal involvement in systemic disorders such as scleroderma and chronic idiopathic pneumonia [4].

The catheter is a specially designed, long, flexible tube. It has four capillary tubes around a larger central tube with an overall diameter of 4.5 mm. Each lumen is connected to an external transducer. The infusion pump perfuses the capillary tubes with water at a constant rate of 5 ml/min. This system consisting of a catheter composed of small capillary tubes, a low-compliance hydraulic capillary infusion pump and external transducer is called water infusion system. The computer receives the electrical signal from the transducers and produces a graphic record that is easy to read, measure and interpret. Software that we created transforms the esophageal pressures into the 2D model that simulates the esophageal peristalsis.

The esophageal manometry study is performed while patient is awake and alert; therefore, the cooperation and comfort of the patient are essential for a good study. The patient should have fasted for at least 6 hours. Medications that might alter normal esophageal function should be discontinued at least 48 hours before the study. These includes nitrates, calcium channel blockers, anticholinergics, H2-blockers, PPI, promotility agents and sedatives. Intubation with the manometry catheter is generally the most uncomfortable part of the entire study. Viscous lidocaine (Mesocaine gel) or a similar topical anesthetic can be applied to the tip of the catheter if necessary. The patient should be seated comfortably and should remove any eyeglasses or dentures. The tip of the catheter is inserted into the nose and moving slowly straight back. As it drops into the back of the throat, the gag reflex will be stimulated. Having the patient sip some water and bend the neck forward will facilitate passage of the catheter into the esophagus. Then advance to the 40-45 cm level (depends on the age of the child) and tape it in place. At this point the recording sites are in the stomach. Manometric assessment of the lower esophageal sphincter (LES) is aimed at measuring the resting pressure (LESP) of the sphincter and assessing relaxation of the sphincter during swallowing. The LES is first identified in the proximal channel by the recording sites are in the stomach. Manometric assessment of the lower esophageal sphincter (LES) is aimed at measuring the resting pressure (LESP) of the sphincter and assessing relaxation (LESR) of the sphincter during swallowing. The LES is first identified in the proximal channel by an increase in the respiratory variation, followed by the bottom of the pressure tracing rising above the baseline. As the catheter is advanced, the pressure will increase, and at the point where the catheter enters the stomach, the tracing will show a marked change in configuration, with a fall in pressure during inspiration instead of rise in pressure. This is called the respiratory inversion point (RIP). RIP is a landmark within the sphincter that is used to calculate the ratio of intraabdominal to intrathoracic LES length. This ratio, along with total LES length, may be important parameters in the assessment of the LES as a competent reflux barrier. Measurement of the resting LES pressure (LESP) must take into account the added influence of respirations. Once the resting pressure has been measured with proximal transducer, the distal transducer is placed in the high-pressure zone to evaluate sphincter relaxation during deglutition. Dry swallows often do not induce complete relaxation of the sphincter so we use a 5-ml water bolus. Following a swallow, the pressure should drop approximately to the level of the gastric baseline. Parameters normally evaluated include percent relaxation and the duration of the relaxation. Recent studies proposed that the residual pressure (RP), which is defined as the difference between the lowest pressure achieved during relaxation and the gastric baseline pressure, is a better indicator of function than is percent relaxation since its residual pressure is independent of the resting LES basal pressure [7].

Low LES pressure can be associated with gastroesophageal reflux disease (GERD), whereas abnormal high LES pressures are often associated with symptoms of dysphagia or noncardiac chest pain. Failure of the sphincter to relax adequately contributes to symptoms of dysphagia and is usually associated with diffuse esophageal spasm and achalasia. Manometric studies of the esophageal body are used to assess the strength and duration of the muscular contractions, to evaluate peristaltic activity, and to detect any motility abnormalities. A complete evaluation should include measurements of both the smooth muscle of the distal esophagus and the striated muscle of the proximal esophagus. Measures are made of at least the following peristaltic parameters: amplitude, duration and velocity. Amplitude is a measure of strength of the contraction and is expressed in mmHg. In children it is about 30 – 80 mmHg. Duration of the contraction is expressed in seconds. Velocity is a rate of progression of the contraction down the esophagus and is expressed in centimeters per second. In children the velocity in proximal esophagus is about 3.0 ± 0.6 cm/sec, in the distal esophagus it is 3.5 ± 0.9 cm/sec. It should not be higher than 20 cm/sec because of the dismotility of the esophagus. Initially esophageal peristalsis was evaluated with dry swallows. However, it soon became apparent that after meal stimulation by a liquid bolus was important for reproducible and accurate quantitative assessment of the peristaltic sequence [6]. Normal peristalsis in the distal esophagus is an orderly, sequential contraction down the esophagus, with amplitude, duration and velocity in normal range. Wet swallows in the proximal esophagus produce a somewhat different appearance. The contraction in the striated-muscle segments is usually sharper, with shorter duration. If a transducer is placed in the transition zone between the striated- and smooth-muscle portions of the esophagus, no or at best very low contraction will be seen.

A manometric evaluation of the upper esophageal sphincter (UES) and pharynx includes a determination of the resting pressure of the UES, the relaxation of the UES, pharyngeal contraction and peristalsis, and an assessment of the coordination between UES relaxation and the pharyngeal contraction. The UES and pharyngeal region differs from the body of the esophagus in several ways that markedly affect the manner in which manometry must be performed. They are composed of striated muscle, therefore, the muscular contractions and responses are much more rapid than those in the smooth-muscle distal esophagus. The second difference that affects UES-pharyngeal manometry concerns the anatomy of the UES. The highest pressures are

**METHODS**

![Fig. 1 Schematic representation of pressure parameters measurable during lower esophageal sphincter (LES) relaxation. Resting LES pressure (LESP) is the pressure rise measured from gastric pressure; residual pressure (RP) is the difference between the pressure at the nadir of the relaxation and gastric pressure; LES relaxation (LESR) is the difference between LESP and RP, expressed as a percentage. Duration and area of the relaxation are also shown.](image1)

![Fig. 2 Manometric profile of esophagus – healthy child.](image2)

![Fig. 3 Manometric profile of esophagus a patient with GERD and asthma bronchiale.](image3)
recorded from the anterior and posterior directions, and the lowest from the lateral direction. The asymmetry of the UES pressure profile has been resolved by the development of a circumferential sphincter transducer, that has allowed for accurate sphincter measurements without the need to control catheter orientation. The resting UES pressure in children is 30 - 80 mmHg.

RESULTS

In the previous study at the Clinic of Pediatric Surgery in Martin the manometric profile of the esophagus in a file of 40 children was done. Their average age was 9.7 ± 3.3 years. This file was divided in 2 groups. Group A: 15 children with negative 24-hour pH monitoring, and group B: with positive 24-hour pH monitoring. In our own study we had a group of 16 pediatric patients with pulmonary complications of GERD. 7 children had confirmed GERD by pH monitoring, in 2 cases the Wasser-Siphon test was positive, in 1 child gastrofibroscopic view of reflux esophagitis and 2 children had also the esophageal symptoms of GERD. We compared the manometric profiles of the esophagus of group B with group C. The parameters of the lower esophageal sphincter (LES), total LES length, intraabdominal LES length, resting LES pressure (LESP) and LES relaxation (LESR) are in Table 1. The control group A shows the normal rates for LES parameters. Significant difference was only in total LES length - group B: 3.6 ± 0.86 cm and group C: 2.75 ± 0.75 cm. The intraabdominal LES length was approximately similar, LESP in group C was lower (7.3 ± 3.98 mmHg) than LESP in group B (7.9 ± 4.01 mmHg) but not significantly, and LESR was not statistically significant too. We can see, that the results of the LES parameters of group C are approaching the parameters of group B.

Table 1. Parameters of the LES

<table>
<thead>
<tr>
<th>Group A – neg. 24-hour monitoring</th>
<th>Group B – pos. 24-hour monitoring</th>
<th>Group C – GERD + pulmonary diseases</th>
<th>p (B-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LES length (cm)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3.6 SD 1.1</td>
<td>3.6 SD 0.86</td>
<td>2.75 SD 0.75</td>
<td></td>
</tr>
<tr>
<td>Intraabdominal LES length (cm)</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2.7 SD 0.97</td>
<td>2.1 SD 0.60</td>
<td>2.1 SD 0.75</td>
<td></td>
</tr>
<tr>
<td>LESP</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>13.1 SD 6.35</td>
<td>7.9 SD 4.01</td>
<td>7.3 SD 3.98</td>
<td></td>
</tr>
<tr>
<td>LESR</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>139.4 SD 46.66</td>
<td>168.4 SD 84.5</td>
<td>138.9 SD 57.85</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of the peristaltic contraction was actually lower in group C (43.75%) than in group B (68%), group A (93.4%). Also, group C (<30%: 6 children, 30 – 50%: 1 child, >50%: 15 patients) has higher representation in non-coordinated contraction of DE than group B (30%: 1 child, 30 – 50%: 7 children, >50%: 17 patients). There was no significant difference in the amplitude of the contraction, they were approximately similar – group B / 32.7 mmHg/ and group C /33.0 mmHg/.

REFERENCES

5. Castell JA, Castell DO. Modern solid state computerized manometry of the pharyngoesophageal segment. Dynphaga 1993; 8:270
15. Mansfield LE, Stein MR. Gastroesophageal reflux and asthma: a possible reflex mechanism. Ann Allergy 1978; 41:224

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Abstract

Recently, spectral analysis of the heart rate variability is regarded as the most accurate method for heart regulation and autonomous nervous system (ANS) study. The aim of this work was to determine the development of the heart rate variability in the first three days of life and gender differences of this phenomenon. The values were measured in 56 healthy term newborns (26 girls, 30 boys). The measurements were taken during the first and third day of life in supine position, in semi-verticilization (45 degrees) and in laying position again.

The results show that between the first and third day of life there is a significant increase in the parameters of heart rate variability reflecting regulation ability of the heart and both sympathetic and parasympathetic ANS divisions. The heart rate variability is higher in newborn boys than in girls, the gender differences being more marked in the third day of life.

Key words: autonomous nervous system, heart rate variability, newborn, spectral analysis

INTRODUCTION

Spectral analysis of the heart rate variability (HRV) is regarded as the most accurate method for heart regulation and autonomous nervous system (ANS) study. The HRV can be seen on fluctuations of the R-R intervals on ECG record. These intervals are variable in length - duration and they can be described by oscillations in frequencies and amplitudes. The variability of the R-R intervals depends on many factors (respiratory sinus arrhythmia, baroreflexes - changes in blood pressure, changes in the activity of ANS, endocrine systems, state of arousal, mental activity, etc.). The analysis of the R-R interval variability provides information about the control of heart activity and the ability of the heart to react to these regulatory inputs (7).

Study of the HRV started long time ago. Hon and Lee (5) published key work on this topic in fetuses in 1965. Recently, the HRV analysis is mostly used in fetuses as a part of routine cardiotocographic examination. Wolf et al. (25) showed in their work published in 1978 an increased risk of mortality following myocardial infarction in adult patients with a diminished heart rate variability. In 1981, Akselrod et al. (1) introduced spectral analysis as a method for HRV quantification and they determined the dependence of spectral power in the individual frequency bands on activities of individual divisions of the ANS. Spectral analysis became an accepted examination method in adults (13). The Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology was established, and in 1996 published guidelines (22) called “Heart rate variability. Standards of measurement, physiological interpretation and clinical use”. Recently, nonconventional and nonlinear mathematical methods in biosignal analysis including the HRV are used for gaining information about cardiovascular controlling system in physiological and pathological conditions (9-11).

The knowledge of HRV in newborns is much smaller than in adults and preschool children. The HRV parameters changes in boys between the first and third day of life are in Table 3. There were significant differences - increases in most of the HRV parameters between the first and third day of life. We found the following significant differences: Total Power (+), MSSD (+), Power VLF(+), Power LF(+), Power HF (+), PSD VLF(+), PSD LF(+), PSD HF(+), VLF/HF(+), CCV VLF(+), CCV LF (+), CCV HF (+), RR in I3(+), . PSD VLF in I3(+). An increase in almost all parameters was observed, the only fall was in the ratio VLF/HF.

The values of the examined newborns were informed written consent. The study was approved by the Ethical Committee and the examination was performed according to the Helsinki Declaration.

RESULTS

a) Changes of the HRV during the first three days of life

Duration of RR intervals, values of MSSD and Total Power (TP) are given in Table 1. The comparison of the HRV parameters from the first and third day (Z1 and Z2) in the sum of all intervals (i1, i2, i3) and then between the intervals regardless of the gender is summarized in Table 2. Significant differences were found between most of the HRV parameters both in the recordings Z1,Z2 with combined intervals (i1 + i2 + i3) and in individual parameters i1,i2:i3:i3. The significant (p<0.05) differences of HRV (+increase, - decrease) were in the parameters: Total Power (+), MSSD (+), RR (+), Power VLF (+), Power LF (+), Power HF (+), PSD VLF (+), PSD LF (+), PSD HF (+), VLF/HF (+), CCV VLF (+), CCV LF (+), CCV HF (+), RR in I3 (+), . PSD VLF in I3 (+). An increase in almost all parameters was observed, the only fall was in the ratio VLF/HF.

The HRV parameters changes in boys between the first and third day of life are in the Table 3. There were significant differences - increases in most of the HRV parameters between the first and third day of life. We found the following significant differences: Total Power (+), MSSD (+),
Table 1. Physiological values of the RR intervals, MSSD and Total Power of the heart rate variability in the newborns in the 1st and 3rd postnatal days. The values are given as median (Me) and difference (D) of the maximal and minimal values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole cohort</th>
<th>Boys</th>
<th>Girls</th>
<th>Whole cohort</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me =</td>
<td></td>
<td>486</td>
<td>504</td>
<td>477</td>
<td>507</td>
<td>533</td>
</tr>
<tr>
<td>D =</td>
<td></td>
<td>226</td>
<td>224</td>
<td>182</td>
<td>286</td>
<td>286</td>
</tr>
<tr>
<td>Me =</td>
<td></td>
<td>184</td>
<td>169</td>
<td>159</td>
<td>343</td>
<td>372</td>
</tr>
<tr>
<td>MSSD D =</td>
<td></td>
<td>7472</td>
<td>7470</td>
<td>2494</td>
<td>9372</td>
<td>9372</td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td>336</td>
<td>362</td>
<td>276</td>
<td>652</td>
<td>703</td>
</tr>
<tr>
<td>Me =</td>
<td></td>
<td>2707</td>
<td>2687</td>
<td>2210</td>
<td>4918</td>
<td>3750</td>
</tr>
<tr>
<td>D =</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Relation between parameters of HRV between the recordings from the first day (Z1) and third day (Z3) regardless of sexes. Boldly printed values denote p<0.05.

<table>
<thead>
<tr>
<th>Whole cohort</th>
<th>Whole cohort</th>
<th>Whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1 : Z2</td>
<td>1 : i2 + i3</td>
<td></td>
</tr>
<tr>
<td>i1</td>
<td>0.012</td>
<td>0.0001</td>
</tr>
<tr>
<td>i2</td>
<td>0.0002</td>
<td>0.0003</td>
</tr>
<tr>
<td>i3</td>
<td>0.0001</td>
<td>0.071</td>
</tr>
<tr>
<td>POWERVLF</td>
<td>0.653</td>
<td>0.74</td>
</tr>
<tr>
<td>POWERLF</td>
<td>0.18</td>
<td>0.57</td>
</tr>
<tr>
<td>POWERHF</td>
<td>0.205</td>
<td>0.474</td>
</tr>
<tr>
<td>VLF HF</td>
<td>0.346</td>
<td>0.542</td>
</tr>
<tr>
<td>LF HF</td>
<td>0.934</td>
<td>0.564</td>
</tr>
<tr>
<td>VLF LF</td>
<td>0.055</td>
<td>0.76</td>
</tr>
<tr>
<td>RR</td>
<td>0.0002</td>
<td>0.06</td>
</tr>
<tr>
<td>MCC VLF</td>
<td>0.0003</td>
<td>0.0004</td>
</tr>
<tr>
<td>MCC LF</td>
<td>0.0006</td>
<td>0.0002</td>
</tr>
<tr>
<td>MCC HF</td>
<td>0.0008</td>
<td>0.01</td>
</tr>
<tr>
<td>REL VLF</td>
<td>0.207</td>
<td>0.178</td>
</tr>
<tr>
<td>REL LF</td>
<td>0.04</td>
<td>0.566</td>
</tr>
<tr>
<td>REL HF</td>
<td>0.584</td>
<td>0.739</td>
</tr>
<tr>
<td>MSSD</td>
<td>0.0001</td>
<td>0.16</td>
</tr>
<tr>
<td>TOTALPWR</td>
<td>0.0004</td>
<td>0.003</td>
</tr>
</tbody>
</table>

List of abbrevations used in the tables:
Z1 – first day of life recording, Z2 – third day of life recording, i1 – first supine measurement, i2 – measurement in semi-verticalisation, i3 – second supine measurement, POWER VLF – spectral power in very low frequency band (ms²), POWER LF – spectral power in low frequency band (ms²), POWER HF – spectral power in high frequency band (ms²), PSD VLF – power spectral density in very low frequency band, PSD LF – power spectral density in low frequency band, PSD HF – power spectral density in high frequency band, FREQ VLF – modal frequency in very low frequency band, FREQ LF – modal frequency in low frequency band, FREQ HF – modal frequency in high frequency band, VLF / HF – power ratios of very low frequency and high frequency band, LF / HF – power ratios of low frequency and high frequency band, VLF / LF – power ratios of very low frequency and low frequency band, RR – the distance of R-R intervals (ms), CCV VLF – coefficient of variation in very low frequency band, CCV LF – coefficient of variation in low frequency band, CCV HF – coefficient of variation in high frequency band, REL. P VLF – relative power in very low frequency band, REL. P LF – relative power in low frequency band, REL. P HF – relative power in high frequency band MSSD - mean squared successive difference (ms²), Total Power (ms²)
Table 4. Differences between sexes and in the whole cohort in the first and third day of life. Boldly printed values denote p<0.05.

<table>
<thead>
<tr>
<th>Whole cohort</th>
<th>RECORDING 1</th>
<th>RECORDING 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z1</td>
<td>Z2</td>
</tr>
<tr>
<td>POWERVLF</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>POWERLF</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>POWERHF</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>PSD VLF</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>PSD LF</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>PSD HF</td>
<td>0.089</td>
<td></td>
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<tr>
<td>FREQ VLF</td>
<td>0.223</td>
<td></td>
</tr>
<tr>
<td>FREQ LF</td>
<td>0.379</td>
<td></td>
</tr>
<tr>
<td>FREQ HF</td>
<td>0.897</td>
<td></td>
</tr>
<tr>
<td>VLF HF</td>
<td>0.752</td>
<td></td>
</tr>
<tr>
<td>LF HF</td>
<td>0.818</td>
<td></td>
</tr>
<tr>
<td>VLF LF</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>0.0003</td>
<td>0.0005</td>
</tr>
<tr>
<td>CCV VLF</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td>CCV LF</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>CCV HF</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>REL VLF</td>
<td>0.644</td>
<td></td>
</tr>
<tr>
<td>REL LF</td>
<td>0.241</td>
<td></td>
</tr>
<tr>
<td>REL HF</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>MSSD</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>TOTALPWR</td>
<td>0.008</td>
<td>0.01</td>
</tr>
</tbody>
</table>

DISCUSSION

Theoretical and practical aspects of HRV have been studied in neonatology since the 1970’s. The first author who studied HRV in newborns was Kero (12). He found that the lower is the gestational age and birth weight the smaller is the heart rate variability probably as a result of the ANS centres immaturity.

The maturity of sympathetics and parasympathetics and the resulting control of heart are at birth not complete. According to Javorova (10), the activity and influence of the parasympathetics on a newborn’s heart is small which results in a higher heart rate and smaller heart rate fluctuations. HRV decreases during the first month of postnatal life with a subsequent rise in the following months. A decreased maturity mainly in preterm newborns was shown e.g. by Drouin et al. (3). During sleep a higher frequency component becomes more prominent in term neonates, which is not observed in preterm ones. This is probably as a result of the lack of maturity of sleep organisation and parasympathetic system in preterms (24). Patzak (17) and Mehta (14) examined physiological values of term neonates. Changes of HRV’ were shown during different phases of sleep in preterm neonates (4). There are also differences depending on supine or prone position during sleep (20). Following analysis of HRV it was shown that term neonates do not have 24-hour sleep cycles, but three-hourly cycles. Twelve-hourly cycles develop around the 15th to 30th day of life (22). Studies of pathological states concentrate on separate problems rather than on a complex view (15, 19 and others).

a) Changes of the heart rate variability (HRV) during the first three days of life

The measurements were taken during the first and third day of life and comparison of the results – differences reflect continuous maturation of the ANS. Significant differences were found between the majority of the parameters of HRV. Patzak et al. (17) studied the spectral analysis of HRV in the first and fifth day of life (and up to 6 months of life) and their results as well as those of other authors (16, 24) support our findings.

We found an increase of the R-R intervals, that means progressive slowing down of the heart rate. From the majority of significantly changed parameters of HRV follows that during the first three days of postnatal life the activity of both ANS divisions increased. The increase in HF band activity is comparable with that of Patzak (17).

Our findings showed a significant difference in the HRV parameters during the first and third day. The HRV parameters both in the sympathetic and parasympathetic component were increased during the early postnatal period.

An interesting finding is the decrease of the VLF/HF ratio. We suggest the decrease is a result of the enhancements of parasympathetic activity seen on prolongation of the R-R intervals (low-
ering of heart rate). We did not find any evident signs of respiratory sinus arrhythmia (RSA). This is probably due to a higher heart and respiratory rate and smaller tidal volumes in newborns. 

b) Gender differences of the HRV

In our study, the HRV was higher in boys compared to girls in all measurements. The differences were more pronounced in the third day of life than in the first day. These findings are original and to a certain extent surprising. Many functions are better developed in newborn girls than in boys (e.g. production and metabolism of surfactant) (6).

We did not find any work comparing the differences of HRV between boys and girls in the newborn period. However, a similar trend can be seen in young population aged 15–19 years. HRV parameters increase in boys during the mentioned four years, though there were no changes in HRV parameters in girls (23).

In conclusion, the heart rate variability parameters were determined in healthy mature newborns in early period of their postnatal life. HRV examinations revealed faster development of chronotropic regulation of the heart activity during the first three postnatal days in boys in comparison to girls. Determination of the HRV parameters provides accurate and valuable method for examination of the heart rate regulation even in the newborn’s period.

REFERENCES


The pathogenesis and occurrence of four malignancies in our patient are discussed. The signs of diffuse peritonitis and circulatory failure. The autopsy revealed obturating rectosigmoid adenocarcinoma. Months later fibrogastroscopy and histology revealed gastric adenocarcinoma and the patient died 6 months later with liposarcoma. Four years later bone marrow biopsy confirmed HCL. The patient was treated with Leustatin s. c. (CD20, LCA, DBA44), bioptic samples of paratesticular and gastric tumor and autoptic material.

The quadruple malignancy in our patient is very rare.

Aims: We present the case of a patient with hairy cell leukemia (HCL) treated with Leustatin, with the development of three secondary malignancies.

Methods: We examined bone marrow biopsy samples by standard morphological and immunohistochemical methods (CD20, LCA, DBA44), bioptic samples of paratesticular and gastric tumor and autopic material.

Results and Discussion: Splenomegaly was described in patient already at the first diagnosis of paratesticular myxoid liposarcoma. Four years later bone marrow biopsy confirmed HCL. The patient was treated with Leustatin s. c. 15 months later fibrogastroscopy and histology revealed gastric adenocarcinoma and the patient died 6 months later with the signs of diffuse peritonitis and circulatory failure. The autopsy revealed obstructing rectosigmoid adenocarcinoma. The pathogenesis and occurrence of four malignancies in our patient are discussed.

Conclusion: Our finding of multiple malignancies in the course of HCL is in accordance with literary data, however, the quadruple malignancy in our patient is very rare.

Key words: hairy cell leukemia, multiple neoplasms, leustatin

INTRODUCTION

Buroncle, Wiseman and Doan /1/ published in 1958 a unique clinical entity under the name „leukemic reticuloendotheliosis“, currently known as „hairy cell leukemia“ (HCL). HCL is the variant of chronic lymphocytic leukemia with typical fine hairy, filamentous cytoplasmic proj-
inceptions. The surface of the neoplastic cells. A B lymphocyte lineage is almost always demon-
strated. This disease affects mostly patients of male sex and represents only 2 % of all leukemias. About 25 % of patients are initially asymptomatic. The suspicion of HCL is suggested by the finding of isolated splenomegaly or cytopenia. Sometimes the first sign of the disease is abdominal fullness owing to splenomegaly, bleeding, recurrent infection or nonspecific conditions, including weight loss, fatigue and weakness. The most frequent cause of death is infection /2/. The diagnosis of HCL is possible by demonstration of hairy cells in Gimsa stained blood smears, the tartarate-resistant acid phosphatase positive stain in tumor cells and distinctive histopatho-
logic finding in bone marrow biopsy with positive reaction to leukemic cells with DBA-44 anti-
body. CD 20 antigen is mostly also expressed /3/. Evaluation of bone marrow biopsy is a „basic stone“ of the diagnosis HCL. The majority of patients has hypercellular bone marrow, others have only focal infiltration with hairy cells. The groups of hairy cells have distinct appearance in low power microscopical evaluation.

Their cytoplasm is abundant water-clear to cosinophilic which creates a perinuclear halo, so there are no contacts of nuclei in thin histological cuts („mosaic-like“ pattern). Hairy cells have blad nuclei in high power magnification, homogenous, round to oval, bilobated or indented, with grooves. Nucleoli are not prominent and mitotic figures are sparse. Silver stain demonstra-
strates medica reticular fibrosis, but dense collagenous fibrosis is absent /2/. Prior to modern therapy, the only treatment available was splenectomy. The introduction of interferon alpha, 2-
round to elongated or stellate cells and dispersed lipoblasts and myxoid hyaluronic acid rich (positive with alcian blue stain) stroma with characteristic prominent plexiform vasculature predominated /Fig. 1/. Mitotic figures were minimal. There was more prominent cytonuclear atypia in some areas with mononuclear and multinuclear lipoblasts. More mature adipose tissue and hemorrhages were also present. The diagnosis of paratesticular myxoid liposarcoma was established. Testis was without tumorous changes.

Recurrent tumors had infiltrative growth and similar histomorphology, but their cellularity was higher, had more numerous regressive changes and transition to round cell liposarcoma was evident in some areas.

Bone marrow biopsy samples were characteristic with the finding of irregular cellularity in first biopsy. Areas of moderate hypoplasia predominated. There was mild hypercellularity in some regions. The confluent interstitial small cell lymphoid infiltration was present in all areas with reduction of normal hemopoesis. Lymphoid cells had small round to ovoid nuclei, cosinophilic to clear cytoplasm. Immunohistochemically, these cells were DBA 44 and CD20 positive and alphanaphtholchloracetesterase negative. Histomorphological and immunohistochemical findings in bone marrow biopsy respond to the diagnosis of HCL with the range of infiltration 50 % (Fig. 2). Diffuse mild reticular fibrosis was also present.

The second restaging bone marrow biopsy revealed discontinuous interstitial lymphoid infiltration (DBA 44+, CD20+), responding to minimal residual infiltration, in range to 5 %.

The structures of intestinal adenocarcinoma grade II-III were present in bioptic samples stripped by endoscopic forceps from ulcerous lesion localised in mediogastric region (Fig. 3). Grossly, there was central ulcerated tumor with elevated margins and hard consistency, 3 cm in maximal diameter in gastric postoperative preparation. Microscopically, tumor had identical structure as in biopsy, but admixture of signet ring cells carcinoma and infiltration through all gastric layers was also evident.

Fig. 1. Paratesticular tumor – myxoid liposarcoma. Small to medium size tumorous cells embedded in myxoid stroma with typical plexiform vasculature (hematoxylin and eosin, x 240)

Fig. 2. Bone marrow with characteristic infiltration by hairy cells (hematoxylin and eosin, x 480)

Fig. 3. Intestinal type of gastric adenocarcinoma in bioptic samples (hematoxylin and eosin, x 480).
Fig 4. Colonic tubular adenocarcinoma of in autopsic sample (hematoxylin and eosin, x 480).

Autopsy findings. Grossly, there were constituent pathological changes in abdominal cavity with sodden brown contents of large bowel. Intestinal tags were markedly dilated, parietal and visceral peritoneum was the sick, congested, with sheetings of stool. Rectum was empty. There was a cauliflower tumor in a distance of 10 cm from the anus, bulging and obstructing the bowels lumen and outgrowing to posterior vesical wall. Its maximal diameter was 8 cm. The large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor. The gray region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the large bowel ante tumor was dilated and overcrowded with stool. Perforation was found in the region of colon transversum with a 3 cm diameter. Bleedings were found around perforation in the colonic wall and mucosa around the perforation. The gastric stump had no tumor.

DISCUSSION

Frequency of secondary neoplasms in patients with HCL is 5 – 9 % according to various reports /4/. From Au WY at al. /7/ series of 117 followed patients treated by splenectomy, interferon-alpha, deoxycoformycin and cladribin. 10.2 % patients had diagnosed secondary malignancies preceding HCL, 2.6 % concurrent with HCL and 21.3 % after therapy. Kurzrock at al. /13/ observed in a series of 350 patients with HCL incidence of secondary malignancy in 7.4 %. This incidence did not reach statistical significance and was not associated with the therapy. Alan Saven at al. /14/ in the long term followed up 358 patients with HCL. Secondary malignancies occurred in 27 patients (8 %).

Therapy is one of the possible factors contributing to the incidence of subsequent malignancies in patients with HCL. Double risk of secondary malignancies was recorded in patients with HCL treated with nucleoside /9/. Because leustatin (cladribine) is powerfully immunosuppressive, reducing CD4 lymphocytes for up to 2 years, it was initially speculated that cladribine might be responsible for an increased incidence of secondary cancers. In addition, the phosphorylated derivative of cladribine (2-chlorodeoxyadenosine-5´-triphosphate), impairs DNA synthesis through its preferential use by DNA polymerase and by retardation of DNA chain elongation. Its incorporation into the DNA of the vulnerable lymphocytes is potentially mutagenic. It is most intriguing that only 1 of 27 patients with second malignancies had a hematopoietic cancer, the rest being solid tumors, because the prolonged immunosuppression is generally associated with an increased incidence of lymphoid cancers /14,15/. Increased risk of secondary malignancies in patients treated by interferon-alpha is also controversial. Secondary neoplasms are more frequently haemic in these patients compared to those ones treated by nucleoside /16/. The study of Pawson at al. /17/, however, advised of increased incidence of secondary malignancies in patients with HCL treated by interferon-alpha2b, that gives incidence of second neoplasms in 18.8 %. This indicates to a possible role of interferon-alpha in pathogenesis of these second malignancies.

The distinctive feature of HCL is a disturbance of immune T-cell lymphocyte dependent response with disturbed function of natural killers. Lower rate of CD4+ and CD45RO+ cells is indicated in patients with active HCL compared to healthy persons. It is in contrast with the high number of these cells in chronic lymphocytic leukemia, myeloma multiplex and Waldenstrom macroglobulinemia. The hairy cells probably produce factors with the ability to interfere with some specific functions of T-lymphocytes or to inhibit their immune response. Hairy cells spontaneously produce interleukin 10 (IL-10). The level IL-10 is markedly higher in patients with HCL. IL-10 considerably inhibits expression and production of IL-2, interferon-gamma, TNF-alpha by activated T-lymphocytes. It si probable that IL-10 contributes to the suppression of immune response by T-lymphocytes. Suppressive role of high level of TNF-alpha in bone marrow and peripheral blood is also probable /4/.

We have noticed intriguing incidence of multiple nonhematological malignant neoplasms in patient who was treated by leustatin because of HCL. Our finding of multiple malignancies in HCL is in accordance with the results of reports as listed above, but here described malignant neoplastic „quadruplicity” in one patient is very unusual. An interesting and significant fact is that our patient had more striking splenomegaly diagnosed in CT examination of abdomen already in time of the diagnosis of paratesticular tumor as the first malignancy. It is very feasi-ble that the patient had also HCL much earlier. Infiltration of bone marrow, spleen and eventu-ally lymph nodes by hairy cell could have had constituent influence on the suppression of immune activity, too.

Described incidence of the multiple secondary malignancies can have immediate connection with leukemic infiltration of immunocompetent organs but can be also a consequence of leustatin treatment. too.

REFERENCES


