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Contents

Editorial

Calkovska A., Javorka K.

6

Evaluation of surfactant function: in vitro and in vivo techniques in experimental respirology

Calkovska A., Mokra D.

13

Anti-inflammatory drugs in the treatment of meconium aspiration syndrome

Mokra D., Mokry J., Calkovska A.

22

Effects of elevated body temperature on control of breathing

Zila I., Calkovska A.

29

Quantification of nonlinear features in cardiovascular signals

Javorka M., Tonhajzerova I., Turianikova Z., Chladekova L., Javorka K., Calkovska A.

39

Time irreversibility of heart rate oscillations during orthostasis

Chladekova L., Turianikova Z., Tonhajzerova I., Calkovska A., Javorka M.

44

Cardiac vagal control in depression and attention deficit/hyperactivity disorder

Tonhajzerova I., Ondrejka I., Javorka K., Calkovska A., Javorka M.

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Editorial

This supplement of Acta Medica Martiniana is devoted to current research at the Department of Physiology that is supported by Centre of Excellence for Perinatalogical Research and Centre for Experimental and Clinical Respiriology.

Research at the Department of Physiology goes down to 1969. Since that time it has been focused on respiratory reflexes, mainly cough and aspiration reflex as well as on cardiorespiratory relations that have been studied on experimental animals. The close co-operation with clinical, and especially pediatric departments, affords the opportunity to study ontogenic aspects of respiratory and cardiovascular reflexes in humans, particularly in preterm newborns.

In last decades the research dissociated into two branches: experimental and clinical-physiological. The direction of basic research became strongly influenced by the experiences earned in Surfactant Research Laboratory at Karolinska Institute in Stockholm that put pulmonary surfactant and models of respiratory diseases related to surfactant deficiency in the centre of interest.

In this issue of Acta Medica Martiniana experimental research is represented by three papers. First paper deals with evaluation of surfactant function and presents the possibilities and importance of surfactant assessment in bubble and capillary surfactometers as well as in animal models. Beside exogenous surfactant, experimental models of neonatal diseases can be used to test other treatment modalities. For instance, experimental meconium aspiration allows the study of the pathomechanisms participating in respiratory failure in MAS and the effect of various anti-inflammatory drugs. As shown in second paper, the understanding of the processes at cellular and molecular levels activated by meconium aspiration is the major pre-condition of its causal therapy. The third paper presents a traditional topic on interactions of respiratory and thermoregulatory systems and analyzes possible mechanisms of breathing alterations during elevated body temperature.

Clinical-physiological research deals with the control and dysregulation of cardiovascular system in different age groups in health or disease. Data are collected by modern methods represented by registration of variability of cardiovascular parameters, mainly R-R intervals (heart rate variability) as well as continuous monitoring of blood pressure by non-invasive techniques of Finometer or Portapres. Beside the commonly used methods of spectral analysis the behaviour of cardiovascular parameters is assessed by methods of non-linear dynamics that disclose the new aspects of cardiovascular control.

Two first contributions in this series are based on new analytical and mathematical methods to identify cardiovascular dysregulation. First paper gives a view on trends in the research of cardiovascular complexity as a part of complex system – a human body. A reduced measure of complexity of cardiovascular control contributes to pathological dynamics in the system and to non-physiological reactivity. New methods described offer a fast way to identify the regulatory disorders. The second paper focuses on the time irreversibility of the heart rate variability. Three indexes are compared that enable by different ways to quantify the measure of irreversibility during standard orthostatic test. The last paper gives the information on the influence of autonomic nervous system, mainly parasympathetic part, on the heart activity in the light of the Porges polyvagal theory. The authors focused on cardiac vagal control in young patients with depression, attention deficit/hyperreactivity disorders (ADHD) and discuss the pathomechanisms.

Clinical-physiological, and also experimental research depends on cooperation with the clinics of Jessenius Faculty of Medicine and University Hospital Martin. Since 2009 this cooperation is covered by the Centers of Excellence. Department of Physiology cooperates primarily with the Clinic of Neonatology, Clinic of Gynecology and Obstetrics and Clinic of Children and Adolescents, as well as with the Clinic of Psychiatry of Jessenius Faculty of Medicine and University Hospital Martin. Such a team work facilitates the research of current medical problems with results being summarized in this supplement.

ANDREA CALKOVSKA
KAMIL JAVORKA

EVALUATION OF SURFACTANT FUNCTION: *IN VITRO* AND *IN VIVO* TECHNIQUES IN EXPERIMENTAL RESPIROLOGY

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ABSTRACT

Pulmonary surfactant is present as the thin film of surface active material in the terminal airspaces and conducting airways. The major function of the surfactant film is to reduce the surface tension at the alveolar surface. Deficiency or dysfunction of pulmonary surfactant cause severe respiratory diseases that make the study of pulmonary surfactant not only of physiological but also of clinical importance.

There are three main categories of methods for assessing the properties of pulmonary surfactant: *in vitro*, *in situ* and *in vivo* techniques. Pulsating bubble surfactometer (PBS) and captive bubble surfactometer (CBS) enables to study surfactant properties at spherical air-liquid interphase. Capillary surfactometer in contrast to alveolar models mimics the human terminal airways and evaluates surface properties required for airway patency. Each of above-mentioned methods enables to study exogenous surfactants, as well as surface activity of lavage fluids or tracheal aspirates.

Biophysical characteristics should be reflected by lung compliance and, as a consequence, by improved blood oxygenation. Various animal models have been developed to evaluate the efficacy of surfactant replacement therapy on preterm and term animals. *In vivo* models can be divided in those, primarily involving surfactant deficiency, such as premature animal model, and those with secondary surfactant dysfunction or inactivation, such as meconium or acid aspiration models.

This review is restricted to the *in vivo* and *in vitro* techniques handled by the authors in their research performed within the last years at both domestic and external laboratories.

Key words: Pulmonary surfactant, Surface activity, Surfactometer, Animal models

INTRODUCTION

Pulmonary surfactant is present as the thin film of surface active material in the terminal airspaces and conducting airways. The major function of the surfactant film is to reduce the surface tension at the alveolar surface. First, pulmonary surfactant reduces the energy required to inflate the lungs thereby increasing pulmonary compliance, second, by increasing elastic recoil, pulmonary surfactant reduces the likelihood of alveolar collapse during expiration. Deficiency or dysfunction of pulmonary surfactant causes severe respiratory disease. Exogenous surfactant replacement therapy, in which either synthetic or modified natural pulmonary surfactant is delivered into the patients' lungs, has been established as a standard therapeutic intervention for patients with neonatal respiratory distress syndrome (RDS). Beside neonatal RDS, surfactant replacement therapy can also be helpful in other forms of lung disease in paediatric or adult patients, in which endogenous surfactant is inactivated by aspirated material or leakage of plasma proteins into the airspaces. Thus, with less or more success, exogenous pulmonary surfactant is also used in acute respiratory distress syndrome (ARDS), meconium aspiration syndrome (MAS), aspiration pneumonia and other forms of lung injury.

Methods for assessing the properties of pulmonary surfactant fall into three categories: *in vitro*, *in situ* and *in vivo* techniques. This review deals with the techniques used by the authors in the research done within the last years in laboratories of domestic and foreign institutions.

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IN VITRO METHODS FOR EVALUATING PULMONARY SURFACTANT

At least three physical properties of the surfactant system are essential for normal lung function, especially in the neonatal period. These are: 1) rapid adsorption from the hypophase, 2) low film compressibility with a drop in surface tension to very low values during surface compression and 3) effective replenishment of squeezed-out film constituents during surface expansion (1). Several systems have been used to determine the surface activity of surfactant materials. In this chapter, pulsating bubble surfactometer, captive bubble surfactometer and capillary surfactometer are discussed. Each of them enables to study exogenous natural or modified natural surfactants or newly developed synthetic surfactants, as well as surface activity of lavage fluid, tracheal or gastric aspirates after appropriate processing.

Pulsating bubble surfactometry

Pulsating bubble surfactometer was developed by Göran Enhörning and first published in 1977 (2). It enables to study surfactant properties at spherical air-liquid interphase. With this technique, approximately 20 μl of test fluid is filled in a plastic sample chamber. An air bubble is connected to the ambient air by a chimney. A bubble of minimal radius ($r=0.4$ mm) is created at 37 °C and it is maintained at minimal size for one to several minutes. Next, pulsation is started usually at a cycling rate of 20-40 rpm. The bubble radius then varies between 0.4-0.55 mm (corresponding to an area compression of 50 %). Pressure across the bubble wall is recorded during the 5th cycle and after 1, 2 and 5 min of pulsation with a microprocessor and values for surface tension at maximum and minimum bubble size (Fig.1) are calculated according to the Laplace equation (e.g. 3). With this method, there are still problems with surface leaks. The method is not suitable to investigate the film stability at minimum surface tension in the non-pulsating mode. The pulsating bubble system is very convenient for examining the surface activity of samples under dynamic compression, as leakage is less of problem if relatively high cycling frequencies are chosen (1).

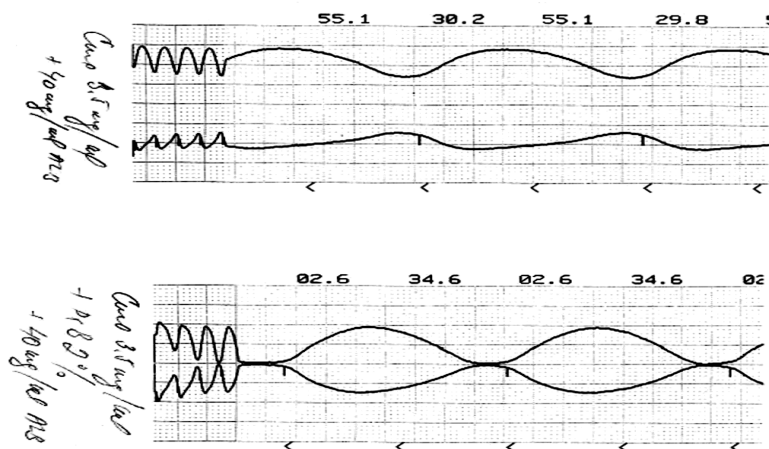


Fig. 1. Original recording from the pulsating bubble surfactometer reflecting different surface activity of the sample. Natural modified surfactant sensitive to inhibitor (up) and resistant to inhibitor (down). Differences in recordings are due to inability of inactivated surfactant to reach low surface tension at dynamic cycling. (Recordings were obtained during the study 3)

Captive bubble surfactometry

Captive bubble surfactometer (CBS) was introduced in 1989 as an air-tight, leakage-proof alternative to pulsating bubble surfactometer (4). The test chamber is initially filled with 10% sucrose in saline. Two μl of surfactant are injected into the sample chamber and allowed to migrate by buoyance to the 1% agarose gel ceiling. An air bubble is then placed under the ceiling in contact with the surfactant preparation, and surface tension is measured from the time of bubble insertion. After 5 min of adsorption, the sample chamber is sealed, and the quasi-static cycling is initiated. Bubble volume is controlled by varying the pressure in the sample chamber. As bubble volume is reduced, the surface area is reduced and the surface tension of the surfactant film at the bubble surface falls. The bubble shape changes depending on surface tension, from more spherical to an oval shape. As the surface tension falls towards zero, a bubble shape assumes a thin disk. Minimum and maximum surface tension (Fig.2; 5), volume and area of compression needed to reach γ_{min} of 5 mN/m (area %) are calculated from the bubble height and diameter, from video images (6). The bubble is compressed stepwise until the surface tension < 5 mN/m is reached or to 50% area compression and thereafter expanded to the initial size. This manoeuvre is done repeatedly.

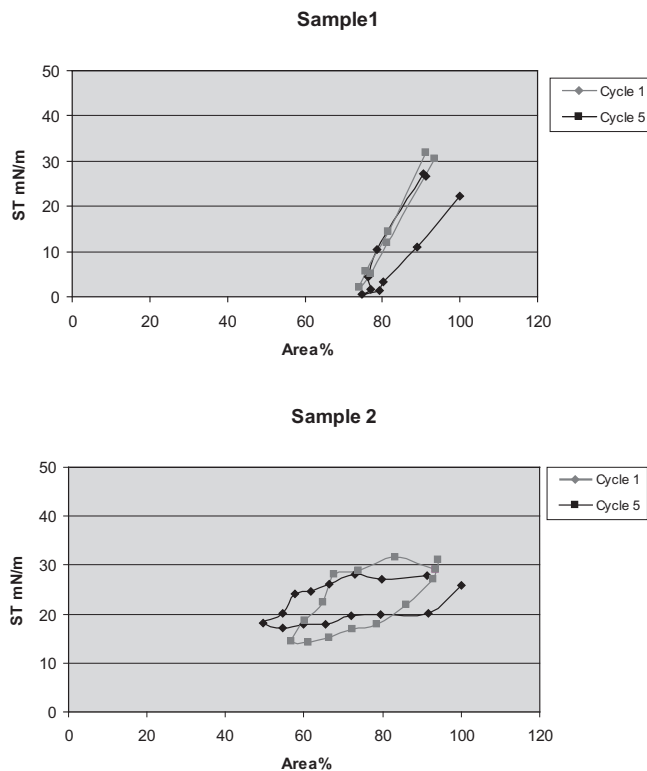


Fig. 2. Data obtained by measurement with captive bubble surfactometer. Upper figure represents active surfactant reaching minimum surface tension $\cong 0$ mN/m, lower figure shows inactivated sample unable to reach low minimum surface tension upon compression. (Recordings were obtained during the study 5)

Abbreviations: ST – surface tension (mN/m); Area % - surface area compression needed to reach surface tension ≤ 5 mN/m, Cycle 1 and Cycle 5 – first and last cycles of compression and expansion during measurement

Capillary surfactometry

Capillary surfactometer is relatively new tool in the study of pulmonary surfactants (7). This model, in contrast to previous „alveolar“ models, mimics the human terminal airways. It evaluates surface properties required for airway patency. The sample at a volume of only 0.5 μl is introduced into the narrow section of a glass capillary. At one end the capillary is connected to a bellows and a pressure transducer. When the bellows is slowly compressed, pressure is raised and recorded. The increasing pressure causes the sample to be extruded from the narrow section of the capillary.

As air gets through, pressure is abruptly lowered. If the sample contains well functioning pulmonary surfactant the sample liquid will not return to the narrow section. The steady airflow obtained by the continuous compression of the bellows will meet no resistance and the pressure recorded will be zero. On the other hand, at less active pulmonary surfactant, the sample liquid will return repeatedly. Ability to maintain the patency of the capillary (terminal conducting airways) is assessed by percentage of the total time (120 s) for which the capillary is open (Fig.3, 8).

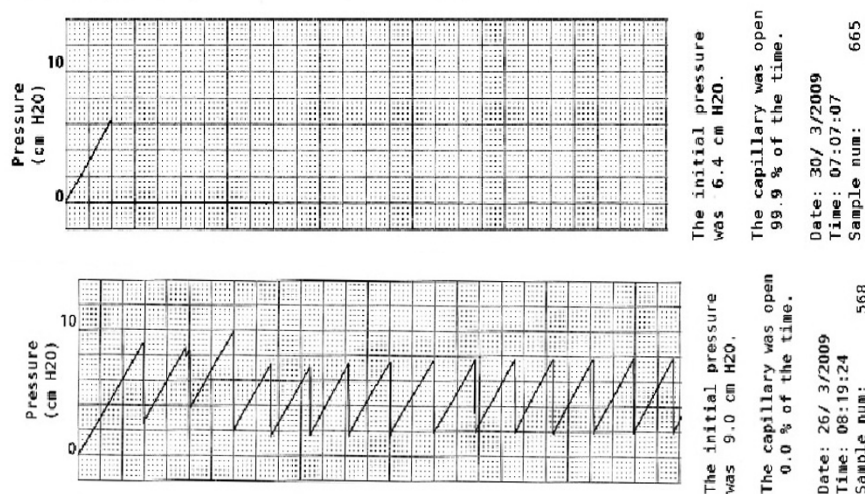


Fig. 3. Active surfactant does not return back to the narrow part of the capillary and thus, the capillary is open during the whole period of measurement (up); sample with inactivated surfactant requires repetitive generation of the pressure to open the capillary in effort to keep it patent (down) (from 8)

ANIMAL MODELS FOR EVALUATION OF EXOGENOUS SURFACTANTS

Biophysical characteristics of pulmonary surfactant should be reflected by lung compliance and, as a consequence, by improved blood oxygenation. However, the *in vivo* situation is more complicated since the physiological response is not only determined by the biophysical properties of the surfactant, but also by how the material is delivered to the terminal airspaces and how it is metabolized once deposited (9).

Various animal models have been developed to evaluate the efficacy of surfactant replacement therapy on preterm and term animals. *In vivo* models can be divided in those, primarily involving the surfactant deficiency, such as premature animal model, and those with secondary surfactant dysfunction or inactivation, such as meconium or acid aspiration models.

The immature newborn rabbit model

Respiratory distress syndrome (RDS) is typically found in prematurely born infants. Key pathophysiological features include low lung compliance, low lung volumes and inadequate oxygenation. A relevant experimental model of neonatal RDS should be based on premature animals that do not have adequate amounts of surfactant in the fetal lung liquid and therefore fail to maintain proper lung aeration after birth. The animals should develop morphological evidence of hyaline membrane disease if allowed to breathe spontaneously or subjected to artificial ventilation. These criteria are met by several animal models, the most widely used prematurely delivered rabbits and lambs (10).

At 27 days of gestation (term=31 days), the fetal rabbit lung is nearly devoid of endogenous surfactant and the animals fail to develop adequate functional residual capacity. The animals are tracheotomized at birth and randomly allocated to different treatment groups. Up to ten animals are simultaneously kept in plethysmograph boxes at 37 °C and ventilated in parallel with 100% oxygen. Depending on the protocol, different ventilatory strategies can be used. All animals are either ventilated with the same peak inspiratory pressure (PIP) with tidal volumes depending on lung function. As other modality, PIP and thus tidal volume are individualized for each animal. Beside the basic parameters of lung functions (V_T , lung – thorax compliance), it is possible to evaluate *post mortem* lung gas volumes as an equivalent to functional residual capacity, and to do histological or morphometric studies (e.g. 5, 11). As an alternative lung lavage can be performed.

In this animal model it was shown for the first time that neonatal RDS can be prevented by surfactant replacement via airways (12) and these encouraging results stimulated the first clinical pilot studies of surfactant replacement therapy for neonatal RDS (13). Moreover, this model can be a base for e.g. neonatal meconium aspiration model (14) or neonatal pneumonia model (15, 16). This model has also been utilized extensively to test exogenous surfactants. In those studies the level of positive end-expiratory pressure (PEEP) should be carefully standardized. The use of PEEP during mechanical ventilation influences several parameters of surfactant function, including dynamic lung compliance and lung permeability, and these effects may differ for different surfactants.

One of the advantages of the preterm rabbit model is the size of the preterm rabbit lung which ensures optimal surfactant distribution through the lung with a relatively simple instillation technique. Moreover, the pregnant rabbit doe usually carries multiple fetuses – ten or more in each litter that makes possible to do the measurements relatively fast by using a large number of animals simultaneously (10, 17). On the other hand, the small size of these experimental animals (average body weight about 30 g at a gestational age of 27 days) precludes more invasive pathological studies (e.g. intravascular administration).

Surfactant-depletion model by lung lavage

Surfactant-depletion experimental model was developed in adult guinea-pigs (18). It is a rather clean model of surfactant depletion, involving no other initial insult to the pulmonary parenchyma. In this model, severe respiratory insufficiency is defined as a fall in PaO_2 below 60 mmHg during appropriate artificial ventilation with 100% oxygen. This method involves connecting the animal to a mechanical ventilator and subsequently lavaging the lung with saline to remove the endogenous surfactant. Because the lavage procedure can evoke the secretion of intracellular surfactant, it should be repeated several times at regular intervals. Blood gases, parameters of lung mechanics, and histologic and electron microscopic findings indicated that the lavage procedure induces a condition similar to the adult respiratory distress syndrome. Already the first lavage results in significant reduction of lung-thorax compliance and repeated lavage in a perturbation of lung functions persisting for at least 8 h. The most relevant outcome of surfactant function in these studies will be blood oxygenation (17). The original protocol has been modified slightly by other investigators. The lung lavage models are useful for a variety of experimental purposes, including testing of surfactant preparations, evaluation of pharmacological agents stimulating synthesis and

secretion of alveolar phospholipids and studies on the significance of various ventilator settings. In our studies, we used lung-lavaged adult rabbits to test the delivery method of exogenous surfactant and the effect of ventilation strategy after treatment (19).

Model of meconium aspiration

Surfactant function may be disturbed in several forms of neonatal lung disease other than RDS. For example, surfactant may become inactivated by aspirated meconium or by leaking plasma proteins in inflammatory conditions characterized by increased alveolar permeability. The first experimental demonstration of meconium aspiration was reported by Gooding et al. (20) who injected human meconium into the trachea of newborn dogs. The presence of aspirated meconium in peripheral airspaces was verified by radiological and histological examination. Surfactant inhibition by meconium in experimental animals was first demonstrated by Chen et al. (21), who studied the quasi-static pressure-volume characteristic of the lungs after airway instillation of 10% human meconium. Since then only six years were needed to publish results on first severely ill babies with meconium aspiration syndrome (MAS) treated with exogenous surfactant (22).

Surfactant dysfunction by meconium was proved in various animal models that have been used to evaluate the efficacy of different ventilatory strategies, surfactant replacement and other pharmacological therapy. This model is extensively used by our group (e.g. 23). Today, due to technical difficulty with newborn animals, the adult ones are favoured. Usually, human meconium from healthy full-term newborn infants is used. It is pooled, lyophilized and stored frozen. Prior administration, meconium is diluted by saline to appropriate concentration and injected intratracheally into anesthetized and paralyzed animals into right and left position. Animals are then artificially ventilated. Respiratory failure is defined as a $\geq 30\%$ decrease in dynamic lung-thorax compliance and $\text{PaO}_2 \leq 10 \text{ kPa}$ at $\text{FiO}_2 1.0$. Pathophysiological and morphological consequences in this model of newborn MAS are due to combination of airway obstruction, chemical pneumonitis and surfactant inactivation (14, 24).

The model imitates to a great extent the aspiration of meconium in a newborn, however, the interpretation of results must be done carefully. Animals are usually healthy before meconium instillation that does not correspond to hypoxic human newborn and moreover, dynamics of spontaneous meconium aspiration differs from that after syringe instillation. In spite of these concerns, experimental model of meconium aspiration is a valuable tool in evaluation of many aspects of clinical MAS therapy (for review, see 25).

CONCLUDING REMARKS

In vitro modelling provides results that can be obtained faster than in animal studies. It is also less expensive and enables larger flexibility of experimental conditions. The problem is that not all *in vivo* studies confirm the previous *in vitro* data. *In vivo* the situation is more complex since the physiological response is not only determined by the biophysical properties of the surfactant, but also by many factors arising from the living organism.

Moreover, stimulating results obtained in animal experiments are not always reproducible in clinical trials. Firstly, most animal studies have reported on respiratory failure caused by direct injury whereas clinical acute lung injury is caused often by indirect insult. Secondly, in animals, surfactant is usually administered in optimal timing whereas in patients this treatment tends to be late. Finally, most of the animal studies are terminated within several hours and thus, they cannot evaluate long-term effects of surfactant therapy. In spite of all these concerns *in vitro* and *in vivo* modelling has a constant position in the surfactant research.

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ANTI-INFLAMMATORY DRUGS IN THE TREATMENT OF MECONIUM ASPIRATION SYNDROME

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Abstract

Meconium aspiration syndrome (MAS) is a major cause of respiratory distress in both the term and post-term neonates. Obstruction of the airways, dysfunction of pulmonary surfactant, inflammation, lung edema, pulmonary vasoconstriction and bronchoconstriction participate in the pathogenesis of this disorder. Since the inflammatory changes associated with meconium aspiration cause a severe impairment of the lung parenchyma including surfactant and influence the reactivity of both vascular and airway smooth muscle, administration of anti-inflammatory drugs may be of benefit also in the management of MAS. This article reviews effects of various anti-inflammatory drugs used in experimental models of MAS as well as in the treatment of newborns with meconium aspiration.

Key words: meconium aspiration syndrome, inflammation, anti-inflammatory drugs, newborn, animal model

Meconium aspiration syndrome (MAS)

MAS is a serious disease in both the term and post-term newborns. Obstruction of the airways by aspirated meconium with subsequent alveolar atelectasis behind the plug and air-trapping, inactivation of pulmonary surfactant, inflammation, edema, pulmonary vasoconstriction are often leading to persistent pulmonary hypertension (PPHN), and bronchoconstriction participate in the pathogenesis of MAS (Figure 1). Because meconium-induced inflammation with its multiple impacts on the lungs plays more important role than was previously thought, various anti-inflammatory drugs have been tested in the treatment of MAS. This article reviews inflammatory changes in MAS as a rationale for anti-inflammatory treatment and introduces anti-inflammatory drugs mostly used in the treatment of MAS.

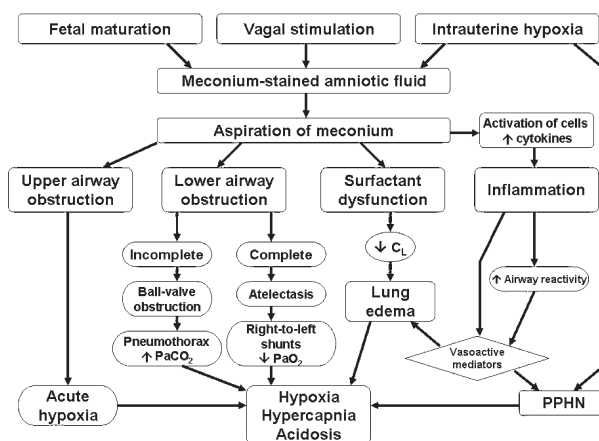


Fig. 1. Scheme of pathomechanisms participating in MAS.

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Inflammation in MAS

Meconium is a source of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α or interleukins (IL-1 β , IL-6, IL-8) (1), which may directly or indirectly through the cytokines produced by neutrophils (2), macrophages (3) and epithelial cells injure the lungs. Neutrophils are activated by macrophages and cytokines, stimulating adhesion of neutrophils on the endothelium. Besides this, meconium itself potentiates chemotactic activity of neutrophils (4). Within several hours, instillation of meconium causes an accumulation of neutrophils in the lungs, while their number in the peripheral blood decreases (5-7).

Activated neutrophils and macrophages may damage the lungs by different ways. Production of pro-inflammatory substances (TNF α , interleukins, prostaglandins, leukotrienes), activation of complement, activation of coagulation cascade, release of platelet activating factor (PAF) and vasoactive substances may finally lead to destruction of capillary endothelium and basement membranes. Injury of alveolocapillary membrane results in leak of liquid, plasma proteins and cells into the interstitium and later also into the alveolar spaces. Closure of capillaries by fibrin and cellular debris participates in the development of pulmonary hypertension, a frequent complication observed in MAS (8).

Together with mediators, proteolytic enzymes are released from the neutrophilic granules. Proteases, e.g. elastase, may destruct the membranes and surfactant proteins, and stimulate the synthesis of bioactive substances and increase endothelial permeability (9). Activated leukocytes produce also reactive nitrogen and oxygen species (RONS) with cytotoxic effect. Peroxidation of unsaturated free fatty acids causes a loss of the functional integrity of membranes and increase of capillary permeability. Oxidation stress finally results in vasoconstriction, bronchoconstriction, platelet aggregation, increased cellular apoptosis (10) and injury of cerebral structures (11).

Activated cells produce high amounts of phospholipase A₂ (PLA₂), too. In addition, meconium itself contains pancreatic PLA₂, which may directly or through the stimulation of arachidonic acid metabolites injure the lung epithelium, endothelium and surfactant and participate in intensified apoptosis (12). Arachidonic acid released from membrane lipids under stimulation of PLA₂ is a precursor for synthesis of both cyclooxygenase (COX) and lipoxygenase products, e.g. for thromboxan A₂ (TXA₂) increasing pulmonary vascular resistance (13), or for leukotrienes causing bronchoconstriction (14), both potentiating microvascular permeability. PLA₂ stimulates also the production of PAF, which participates in pulmonary hypertension, increased capillary permeability and bronchoconstriction, as well as in aggregation and degranulation of neutrophils, macrophages and platelets (15).

In addition, cytokines enhance expression of inducible NO synthase (iNOS) (16, 17) and production of NO (18). Excessive amounts of produced NO via iNOS then lead to increased formation of reactive nitrogen species (e.g. peroxynitrite) and finally increase permeability of alveolocapillary membrane and lung injury (19). Furthermore, cytokines increase production of endothelin-1 (ET-1), a potent vasoconstrictor stimulating proliferation of smooth muscle (20). Inflammation and release of bronchoactive substances (e.g. leukotrienes, PAF etc.) are probably responsible also for increased airway reactivity in MAS (7).

Anti-inflammatory treatment in MAS

Regarding the above mentioned knowledge on the role of inflammation in the pathogenesis of MAS, several anti-inflammatory drugs were administered in experimental models of MAS as well as in the treatment of newborns with meconium aspiration. Their pharmacological action and effects in MAS is reviewed in further subsections.

Glucocorticoids

Potent anti-inflammatory activity of glucocorticoids (GCs) results from reducing the migration and activation of neutrophils, eosinophils, mononuclears, and other cells (endothelial, epithelial etc.) as well as from modulation of chemotaxis and action of mediators released from the activated cells. In addition, by stabilization of cell membranes and by decreased production of pro-inflammatory and vasoactive substances GCs reduce microvascular permeability. Furthermore, directly modulating pulmonary vasomotoric tone GCs diminish pulmonary vasoconstriction and inhibit fibrogenesis (22).

GCs possess both genomic and non-genomic mechanisms of the action. In genomic action, GCs penetrating into the cytoplasm interact with glucocorticoid receptor (GR). The activated complex moves into the nucleus and binds to specific nuclear sequence of DNA (glucocorticoid responsive element, GRE). When GC-GR complex interacts with negative responsive element (GRE⁻), inhibition of transcription factors including nuclear factor (NF)- κ B and protein activator (AP)-1 inhibits an expression of pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF α etc.), enzymes (PLA $_2$, COX-2, iNOS etc.) and other biologically active substances such as PAF, ET-1 etc. (22, 23). On the other hand, interaction of GC-GR complex with positive responsive element of DNA (GRE⁺) increases transcription of lipocortine-1 in leukocytes, which inhibits activity of PLA $_2$, and thereby decreases production of arachidonic acid and its metabolites as well as of PAF (23).

Besides genomically mediated mechanisms, GCs act also through nongenomically-mediated ones, which are responsible for rapid GCs action until the effects mediated by genomic mechanisms occur (24). GCs may exert effects on various cells modulating hormone secretion, neuronal excitability, ion cycling, saccharide metabolism, and other processes within seconds or minutes (24, 25). Rapid effects of GCs are presumably responsible for inhibition of airway hyperreactivity (26), as well as for cardiovascular changes (27) or improved respiratory parameters (28, 29) in animals with MAS observed within minutes after GCs administration.

In *in vitro* studies, GCs suppressed meconium-induced expression of COX-2 and iNOS in macrophages, epithelial and endothelial cells (16, 17), as well as production of NO and TXA $_2$ in epithelial cells (18, 30).

In animals with MAS, GCs inhibited expression of PLA $_2$ in the lungs (6, 31). Interruption of cytokine cascade and inhibition of chemotaxis by GCs resulted in reduced neutrophil influx into the lungs with simultaneous increase in leukocyte count in the blood (5-7). Although treatment with hydrocortisone in newborn rabbits (32) and in newborns with MAS (33) led to controversial results, higher doses of more potent GCs in later experiments were of benefit. Administration of methylprednisolone (5) or prednisolone (34) effectively improved the lung functions. Similarly, pretreatment and early treatment with dexamethasone reduced pulmonary vasoconstriction and improved oxygenation (6, 35). In rabbits, dexamethasone administered 30 minutes after meconium instillation enhanced gas exchange, reduced ventilatory pressures and decreased number of neutrophils, edema formation, and oxidative lung injury, and alleviated meconium-induced airway hyperresponsiveness to histamine (7, 28). Similarly in newborns with MAS, dexamethasone decreased number of leukocytes in tracheal aspirate and levels of several cytokines, improved lung functions and facilitated weaning from the ventilator (14, 36).

Taken together, neonates with severe MAS could benefit from systemic GCs. However, timing of administration is critical for ideal response, as the changes associated with meconium aspiration become severe very early. Nevertheless, repetitive administration of GCs may increase the effectiveness of the treatment. Administration of dexamethasone in two doses enhanced gas exchange and reduced oxygen requirements in piglets (14) and in rabbits with MAS (28). In newborns with MAS, dexamethasone given for several days in a reducing schedule improved lung functions and facilitated weaning from the ventilator (36). Thus, GCs may be effective also in well-established MAS, but repetitive doses should be used.

On the other hand, systemic administration of GCs may be associated with various adverse effects. In our experiments single-dose, but especially two-dose dexamethasone increased blood pressure, decreased heart rate, increased heart rate variability, and increased incidence of cardiac arrhythmia in meconium-instilled rabbits (27).

Nevertheless, occurrence of adverse effects of GCs depends on the properties of individual GCs, duration and dosing, as well as on the route of administration. For example, local administration may eliminate the side effects of GCs. In meconium-instilled rabbits, we have administered budesonide directly into the jet of ventilator during inspersion regime of high-frequency jet ventilation (inspiration time 20 %). Budesonide improved oxygenation and decreased pulmonary shunting, edema formation, neutrophil count in the lungs and markers of oxidative injury compared to non-treated group (29), with less cardiovascular side effects. In other study, shortened duration of oxygenotherapy and hospitalization, improved x-ray of the lungs without increased incidence of sepsis after both systemic (methylprednisolone) and nebulized (budecort) GCs were observed in newborns with MAS (37, 38).

Adverse effects of GCs may be reduced also by combined administration with drugs with similar or additive effects, e.g. with methylxanthines or antioxidants. In our study, intratracheal budesonide followed by intravenous aminophylline showed more pronounced improvement in lung functions than single aminophylline (39).

Inhibitors of phosphodiesterase

Phosphodiesterases (PDE) are a superfamily of enzymes degrading cAMP and cGMP. From up to now 11 PDE families identified, predominantly PDE3, PDE4, and PDE5 isoforms may be involved in MAS due to their pro-inflammatory and smooth muscle contraction activity (40). Therefore, non-selective (methylxanthines) as well as selective inhibitors of PDE could be potentially used in the therapy of MAS.

Non-selective PDE inhibitors (methylxanthines)

Methylxanthines improve respiratory and hemodynamic parameters by their vasodilation, bronchodilation, and anti-inflammatory effects. Decreasing PDE activity, methylxanthines increase intracellular cAMP and cGMP, decrease the concentrations of intracellular calcium, acetylcholine and monoamines, and reduce the releasing and action of various mediators. In addition, methylxanthines via antagonizing adenosine receptors improve the immune activity of mastocytes and basophils, increase surfactant secretion and mucociliary transport, and enhance the up-take of ROS (41, 42). Furthermore, in low plasma concentrations methylxanthines exert anti-inflammatory action resulting from direct activation of histone deacetylase activity leading to reduced transcription of inflammatory genes (42).

In *in vitro* incubation with meconium, pentoxifylline inhibited degranulation of polymorphonuclears and decreased the production of TNF_α (43). In piglets with MAS, pentoxifylline prevented local ventilatory perturbations as well as increase in macrophage count of BAL fluid, TNF_α and protein concentrations in the lungs, but had no significant effect on the lung neutrophil accumulation (44).

Other methylxanthine derivative - aminophylline - improved gas exchange, reduced lung edema and number of neutrophils in the lungs and decreased oxidative lung injury and airway hyperreactivity to histamine in a rabbit model of MAS (45). Comparing two different doses of aminophylline we found that higher-dose aminophylline (2 mg/kg) had stronger effect on pulmonary functions, lung edema and number of neutrophils in the lungs than lower-dose aminophylline. Nevertheless, lower-dose aminophylline more effectively diminished protein oxidation in the lungs and lung tissue reactivity to histamine (45).

Selective PDE inhibitors

As mentioned above, particularly the activity of PDE3, PDE4 and PDE5 may be presumed in meconium-induced inflammation and pulmonary vaso- and bronchoconstrict-

tion. In piglets with MAS, PDE5 inhibitor sildenafil reversed an increase in pulmonary vascular resistance within 1 hour of the treatment, without affecting the systemic hemodynamics (46). Milrinone, a selective PDE3 inhibitor, improved oxygenation and survival of neonates with MAS (47). In our experiments, PDE3 inhibitor olprinone enhanced pulmonary functions, reduced lung edema and diminished inflammation and oxidative lung injury in meconium-instilled rabbits (48). While PDE3 and PDE5 inhibitors were already tested in MAS, there are no remarks about the use of PDE4 inhibitors in MAS. As they have been proven as efficient in asthma (49), their possible benefits in MAS need to be evaluated.

Nevertheless, possible side effects of PDE inhibitors on cardiovascular functions should be considered. As previously noticed, cardiovascular side effects may be comparable in both selective and non-selective PDE inhibitors (42). In our recent study, administration of both aminophylline and olprinone caused rapid, but short-term increase in blood pressure and heart rate (unpublished observation).

Interactions with renin-angiotensin-aldosterone (RAA) system

Angiotensin II as a product of angiotensin-converting enzyme (ACE) action is responsible for contraction of vascular smooth muscle. Furthermore, angiotensin II is suggested to regulate locally the apoptosis of alveolar epithelial cells and to affect the neutrophil accumulation in the tissue (50). Pretreatment with ACE inhibitor captopril before meconium instillation in newborn rabbits decreased levels of ET-1 and pro-inflammatory cytokines and reduced apoptosis (50). Similarly, pretreatment with a non-specific angiotensin II receptor blocker saralasin prevented an increase in lung tissue myeloperoxidase activity, endothelial monocyte-activating polypeptide, and lung epithelial apoptosis in rats with MAS (51). Therefore, influencing the action of angiotensin II via inhibition of ACE or blockade of angiotensin receptors seems to be promising in the treatment of MAS.

Antioxidants

Since RONS and products of lipid and protein peroxidation impair the lung tissue in MAS, administration of substances with antioxidant properties may be of benefit. Intratracheal administration of recombinant human superoxide dismutase decreased myeloperoxidase activity, NO and 8-isoprostane levels and lung injury score in meconium-instilled rats (52), as well as increased oxygenation and reduced vasoconstriction and oxidative injury in newborn lambs with persistent pulmonary hypertension (53). In our recent experiments, intravenous N-acetylcysteine enhanced gas exchange and reduced inflammation in meconium-instilled rabbits, with negligible cardiovascular side effects (unpublished observation).

Inhibitors of cyclooxygenase

Inhibitors of cyclooxygenase are used for their analgesic, antipyretic, and anti-inflammatory effects. According to their selectivity, they are divided to COX-2 selective and COX non-selective non-steroid anti-inflammatory drugs. In MAS, indomethacin inhibited release of TXA₂ from epithelial cells (30), but did not influence an expression of COX-2 or iNOS in the lungs (16). On the other hand, pretreatment with acetylsalicylic acid prevented the initial pulmonary hypertensive response and reduced release of prostanoids in piglets with meconium aspiration (13). However, these results are insufficient to recommend COX inhibitors for MAS treatment at the moment, although their adverse effect profile (especially of COX-2 selective inhibitors) is more convenient compared to that in GCs.

Endothelin antagonists

Considering the role of ET-1 in the meconium-induced pulmonary vasoconstriction (20), administration of endothelin receptor antagonists may improve the status of meconium-injured newborns. In hypoxia-induced pulmonary hypertension in rats, pretreatment with orally given antagonist of ET-1 receptors ET_A and ET_B bosentan attenuated pulmonary hypertension, right heart hypertrophy, and remodelling of small pulmonary arteries (54). Similarly, intravenous administration of other ET_A receptor blockers lowered pulmonary vascular resistance, and enhanced survival in piglets with MAS (55).

Prostacyclin analogues

Prostacyclin as a potent pulmonary vasodilator may be beneficial particularly in the conditions of hypoxia (56). In addition, inhaled PGI₂ may be well combined with other drugs. For example, inhalation of PGI₂ showed a synergistic effect with PDE inhibitors in experimental pulmonary hypertension (57). In a model of MAS, synthetic analogue of PGI₂ iloprost was combined with dual endothelin A and B receptor blocker tezosentan. Since intravenous tezosentan improved gas exchange and hemodynamics, inhaled iloprost enhanced only gas exchange reducing intrapulmonary shunts (58).

Exogenous surfactant

Pulmonary surfactant may protect the lungs also from the inflammation modulating the peroxidation, nitric oxide, PLA₂, arachidonic acid metabolites, and cytokines (59). Similar effects may be theoretically observed also in exogenous surfactants containing surfactant proteins A and/or D (60). Anyway, considering other favourable effects of surfactant, its anti-inflammatory properties seem to be of minor importance in MAS.

Drugs potentially beneficial in MAS

Considering similar pathogenesis, several other drugs being successfully tested or used in the treatment of persistent pulmonary hypertension or acute lung injury may be potentially beneficial also in MAS, e.g. inhibitors of complement, cytokine monoclonal antibodies, inhibitors of proteolytic enzymes, anticoagulants, or calcium-channel blockers.

Conclusion

Advances in our understanding of the pathogenesis of MAS lead to the development of novel approaches focusing on pulmonary inflammation and oxidative injury. Wide variety of anti-inflammatory drugs acting on different levels of inflammatory cascade may alone or in combination with exogenous surfactant and vasodilators potentially improve the clinical status and survival of newborns with MAS. Nevertheless, effects of anti-inflammatory drugs including their side effects in meconium-induced lung injury should be tested thoroughly in experimental and clinical conditions till their use may be recommended.

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EFFECTS OF ELEVATED BODY TEMPERATURE ON CONTROL OF BREATHING

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Abstract

Changes in body temperature can be evoked mainly by alterations in the peripheral temperature, or modified by shifts in the central body temperature. Two conditions can lead to abnormal elevation of body temperature: hyperthermia or fever. As regards respiratory system, exposure to heat stress is accompanied by marked alterations in breathing, especially by an increase in ventilation. Ventilation rises due to an increase in central output from hypothalamus or brainstem, an increase in peripheral output via skin temperature receptors, an increase in central or/and peripheral chemoreceptor output or sensitivity and can be also mediated through changes in thermoregulatory mechanisms.

This review summarizes results of previous studies as well as of experiments done in our laboratory in order to elucidate the mechanisms included in respiratory changes under heat stress.

Key words: heat stress, fever, hyperthermia, control of breathing

INTRODUCTION

Exposure to heat stress is accompanied by an increase in ventilation irrespective of a reason of elevated body temperature. Despite of low metabolic demands, hyperthermia and fever are accompanied by an increase in pulmonary ventilation (1). For this reason, a heat-mediated increase in ventilation must be caused by a change in sensitivity and/or activity of neural and/or chemical respiratory control and by its altered interaction with thermoregulation.

The aim of this review is to present and summarize the insights into the interaction between thermoregulation and control of breathing and to discuss recently suggested mechanisms in the light of current literature and the results of our experimental studies.

Thermoregulation and abnormal elevation of body temperature

Body temperature is closely regulated by homeostatic mechanisms that strike a balance between heat production and heat dissipation (2). Heat is a product of all metabolic processes and it is dissipated over the body surface. The skin accounts for about 90% of heat loss, with the lungs contributing most of the remaining 10%. In the basal state, about 70% of the body's thermal load is dissipated by conduction; 30% is removed by the evaporation of insensible perspiration. Radiation and convection are less important mechanisms of heat removal. When the ambient temperature rises or metabolic heat production increases, evaporation accounts for the major share of heat dissipation (3). In humans, controlling centre for thermoregulation is located in hypothalamus. Hypothalamus detects changes in the body's thermal state directly from changes in blood temperature and indirectly by peripheral inputs from thermosensitive receptors located in the skin and in the muscles (4). The preoptic nucleus of the anterior hypothalamus functions as the thermal control centre and acts to maintain the body temperature at a set value. Thus, it serves as so-called hypothalamic thermal set point (5).

Abnormal elevation of body temperature, or pyrexia, can occur as a result of hyperthermia or fever. In hyperthermia, thermal control mechanisms fail, so that heat production

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exceeds heat dissipation. The most important clinical causes of severe hyperthermia are heatstroke, neuroleptic syndrome, and malignant hyperthermia of anesthesia. Experimental hyperthermia is usually performed by body surface heating using a heating pad and radiant heat from an infrared lamp.

In contrast, in fever, the hypothalamic thermal set point rises, and intact thermal control mechanisms are involved to rise body temperature up to the new set point (3). In febrile response, body temperature is raised by both increased heat production and decreased heat loss (6). A classical model of fever pathogenesis implies a direct effect of various pyrogens on hypothalamus to elevate the thermal set point (7) (Fig.1). Experimentally, fever can be induced with a bolus injection of pyrogenic substance to laboratory animals. Administration of commonly used lipopolysaccharide (LPS) results in mono- or polyphasic body temperature rise and other inflammatory sequelae of infection (6). It is generally considered that LPS-induced fever is mediated by production of cytokines (interleukin 1 β and 6) and subsequent induction of prostaglandine E₂ as a central mediator of fever in hypothalamus. Features of experimental fever strongly depend on several critical factors: the serotype of lipopolysaccharide, the dose and the way of administration (7).

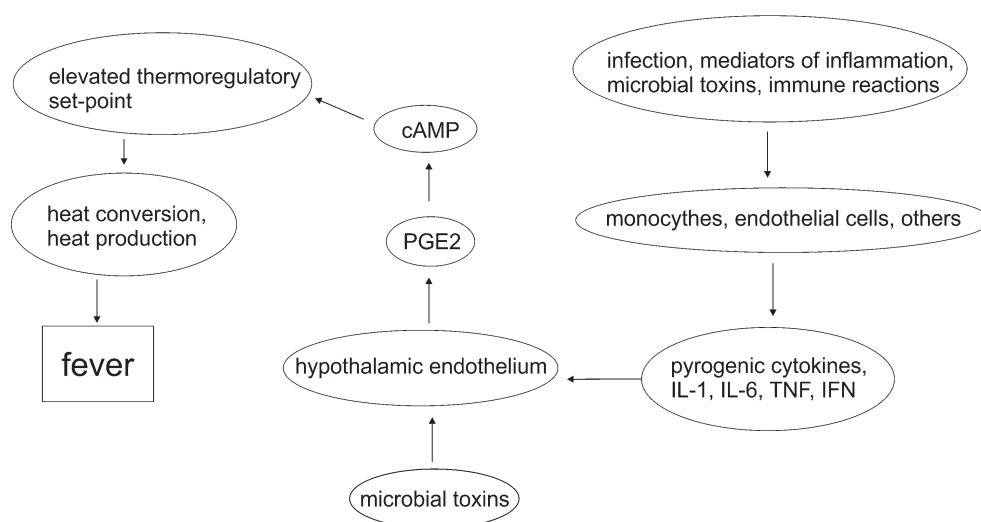


Fig. 1 Events in the course of fever induction

Breathing pattern and neural control in thermal stress

The body temperature changes are accompanied with marked alterations in breathing pattern. These changes are evoked by alterations in the peripheral temperature, or modified by shifts in the central body temperature, eventually by other mechanisms. Hyperpnoea associated with elevated core temperature was described for the first time by Haldane in 1905 (8) and since then numerous studies have been focused on investigation of breathing control in elevated-body temperature conditions. For many animals an elevation in body temperature enhances respiratory frequency but decreases inspiratory amplitude and its duration. The increase in respiration helps to cool the body and restore its temperature to a normal range. In fact, the temperature-dependent modulation of respiratory frequency (e.g. panting) is a major mechanism to dissipate heat and avoid heat-stroke.

What are the mechanisms that mediate the hyperthermic hyperventilation? The scale is extensive as hyperventilation was shown to be mediated by increase output from structures of central nervous system, through increase in peripheral output from skin recep-

tors, by increases in output of central or/and peripheral chemoreceptors or changes in their sensitivity and through changes in thermoregulatory mechanisms (9 - 12) (Fig. 2).

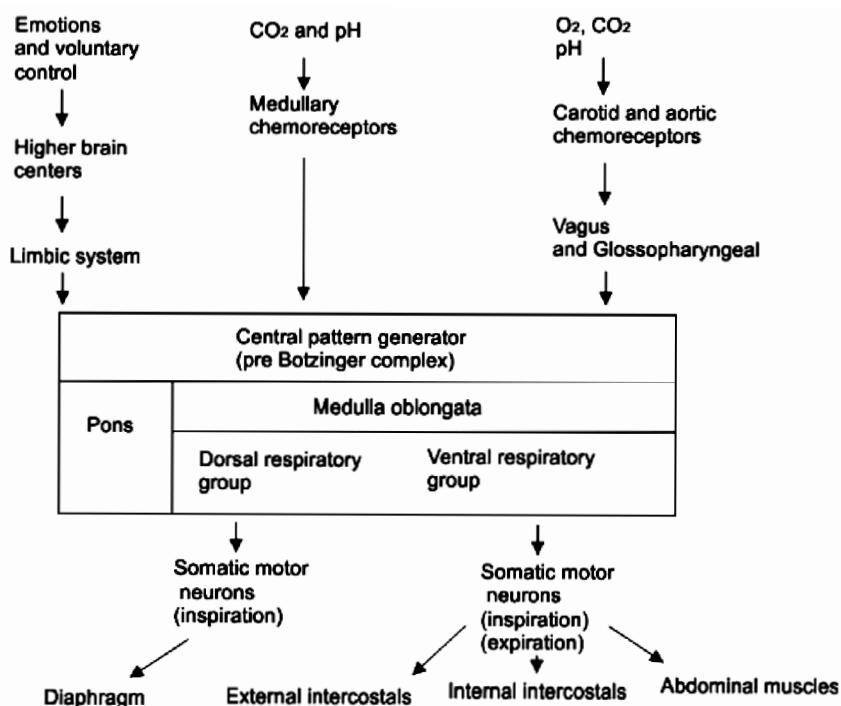


Fig. 2 Sensors, centres and effectors involved in control of breathing

Several studies confirmed a crucial role of hypothalamus in modulating respiratory frequency with changes in body temperature (13 - 15). In some species, a specialized breathing pattern known as panting is mediated by the thermoregulatory system in the preoptic area of the hypothalamus. However, temperature can also directly affect the activity of the respiratory neural network located in the medulla. For instance, area of pre-Bötzinger complex was suggested to play critical role in a change of breathing pattern during thermal stress (16). Direct effect of endotoxin on respiratory centres should also be considered. It was reported that endotoxin may induce alterations in respiratory motor output independently of fever response (17). Saxton (18) documented hyperventilation associated with a reduction in end-tidal PCO₂ in heat stressed subjects suggesting two major reasons of altered breathing pattern: first, a change in sensitivity of central chemoreceptors and second, a change in the threshold of that response.

Besides central mechanisms, elevated body temperature can evoke a change of breathing pattern also by peripheral feedback control or by a combination of both. Slowly adapting pulmonary stretch receptors and vagal afferent fibres from lungs play an important role in the regulation of respiration rate and tidal volume in mammals (19). Activation of pulmonary slowly adapting stretch receptors elicits Hering-Breuer reflex, that is defined by inhibition of inspiratory and prolongation of expiratory phases of respiratory cycle (20). Crucial importance of this reflex is to accomplish inspiratory to expiratory switching and to prevent overinflation of the lungs. Under conditions of thermal stress, a contribution of this control varies among species (10, 19, 21-23) as enhanced

sensitivity and increased response of Hering-Breuer reflex were found in rats exposed to progressive hyperthermia (24), but not in rabbits (10).

During fever, besides apparent thermal response, a direct effect of endotoxin on vagal sensory receptors of the airways and lungs must be considered. Our data have shown that both the strength and duration of Hering-Breuer reflex were reduced in rats during fever response (25). Endotoxemia is known to cause the local release of various chemical mediators in lung tissues and produces bronchoconstriction, increased lung stiffness, and tissue edema (26). It is possible that these endotoxin-induced consequences may serve as chemical and mechanical stimuli to activate lung vagal sensory receptors. Several investigators (27-32) have suggested that various local or reflex pulmonary responses to endotoxin are linked to the stimulation of lung vagal sensory receptors included C-fibres.

Chemical control of breathing in thermal stress

Chemical control of breathing involves a complex interaction of central and peripheral sensors, integrating centre and effector system (33). A direct effect of elevated temperature on the chemoreceptors can induce heat-hyperpnea. However, it is difficult to directly measure chemoreceptor output in response to thermal stress (9). Direct heating of central chemoreceptors led to decrease in minute ventilation in cats (34). Electrophysiological studies have revealed that carotid body activity increases with increasing temperature (35 - 39). This thermal sensitivity is high and hyperthermia, like hypoxia, increases carotid chemoreceptor CO₂ sensitivity (40). As an alternative approach, in most of studies a measurement and an analysis of ventilatory responses to hypoxia or/and hypercapnia have been applied.

Hypercapnic ventilatory response (HCVR) is mediated by both, central and peripheral chemoreceptors. While the model of central chemoreflex is expressed as a straight-line relation between the ventilatory response and the arterial level of carbon dioxide, the peripheral chemoreceptors are complex sensors and their sensitivity to carbon dioxide is controlled by hypoxia in a non-linear way. This physiological concept of chemical control mechanisms illustrates methodological complexity of chemoreflexes measurement. Investigation of HCVR mediated by central chemoreceptors has brought contradictory observations. Some authors have found increased central chemoreflex sensitivity (41-44), but other studies have not confirmed any change in sensitivity of central chemoreceptors under conditions of thermal stress (e.g. 45). In some experiments, a decrease of HCVR in a warm environment was observed (46). There are several possible reasons for contradictory observations, among them the different methods of investigation seem to be the most feasible. Recently, it has been proposed that hyperventilation accompanying heat stress is associated with an increase in chemoreceptor sensitivity (47, 48). Some authors suggest an interaction between thermal and central chemoreceptor drives to breathe (41). This relation is multiplicative rather than additive. There is also an additive ventilatory drive component due to thermal stimuli independent of carbon dioxide. The role of thermoregulatory structures as a possible place of interaction hypercapnia/hyperthermia is still questionable, and the results differ in dependence of animal species. While in cats an increased activity of central thermoreceptors during hypercapnia was found (49), in hamsters the activity of preoptic thermosensitive neurons decreased with a rise of CO₂ (50). Berquin et al. (51) identified also caudal hypothalamus as a site of central hypercapnic chemoreception that extends the possibilities where an interaction between an elevated body temperature and hypercapnia takes place.

Ventilatory responses to hypoxia (HVR) are mediated primarily by the carotid bodies (90 %) and partially by the aortic bodies (10 %) (52). Previous works on animals have shown a larger increase in ventilation to a given hypoxic stimulus during elevated body temperature (53, 54). However, Watanabe and co-authors (55) have shown that respiratory response mediated via peripheral chemoreceptors decreases in higher environmen-

tal temperature in kittens. It is probable that the metabolic rate reduction leads to lower amplitude of oscillation in PaCO_2 , and thus to lower intensity of respiratory response to hypoxia. Similar inhibition of isocapnic HVR was observed in hyperthermic rabbits (56). Only few studies have investigated HVR under conditions of thermal stress in man and data has shown augmented HVR sensitivity, but also large inter-individual variability associated with HVR test (57, 58). Therefore, recently recommended HVR method may allow more detailed assessment of ventilatory responses mediated by peripheral chemoreceptors as well as easier comparisons of results and help to avoid methodological discrepancies (59).

Conclusion

It becomes clear that changes in respiratory control and in thermoregulation affect each other. Heat stress strongly affects the ventilation as a result of additive and maybe multiplicative responses of neural and chemical control loops. Up to now, these interactions are not satisfactory clarified and a lot of work still must be done in this field. Theoretically, such effects could give rise to failure of the respiratory system and there is some circumstantial evidence to support the concept that on occasions such interactions may be of importance in sudden unexpected death in infancy.

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QUANTIFICATION OF NONLINEAR FEATURES IN CARDIOVASCULAR SIGNALS

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Abstract

The analysis of spontaneous heart rate and blood pressure oscillations under standardized conditions is a rapid, sensitive, noninvasive and reproducible tool for the assessment of cardiovascular autonomic dysfunction. Since the heart and vessels are controlled by a nonlinear deterministic system, measures derived from nonlinear systems theory are increasingly used in cardiovascular variability analysis. New nonlinear methods with applicability to real biological signals are continuously developed to quantify new aspects of cardiovascular signals with the potential to reveal subtle changes in the cardiovascular control system. Nonlinear measures provide complementary information about qualitative features of the analyzed signal. Since the analysis of variability magnitude and signal nonlinear characteristics (complexity, recurrences, time irreversibility, etc.) can provide independent information on cardiovascular system control, we suggest that simultaneous use of both groups of measures can increase the information value and thus improve the sensitivity and reliability of the detection and monitoring of the cardiovascular system dysregulation during various pathological conditions.

Key words: heart rate variability, blood pressure variability, nonlinear dynamics, complexity

1. CARDIOVASCULAR PARAMETERS VARIABILITY

Cardiovascular dysregulation (not only as a consequence of autonomic neuropathy) is accompanied by the markedly increased risk of severe cardiovascular complications including fatal arrhythmia and sudden cardiac death. The diagnosis of dysregulation was usually performed by evaluation of heart rate and blood pressure changes evoked by Ewing tests, including Valsalva manoeuvre, deep breathing test, orthostatic test and isometric handgrip test. However, these tests require active patient participation and cooperation, are time consuming, difficult to standardize, and carry a potential risk of adverse effects (1, 2).

It was shown that provoked changes as a tool to investigate the cardiovascular control could be substituted by the analysis of *spontaneous cardiovascular parameters oscillations*. Heart rate and blood pressure continuously fluctuate over time (Fig. 1) and are influenced by different control mechanisms maintaining cardiovascular homeostasis. The analysis of short-term (on the time scales of seconds to minutes) spontaneous heart rate and blood pressure oscillations (heart rate variability – HRV and blood pressure variability – BPV) provides *important information on the autonomic control of circulation* in normal and diseased conditions (4).

The analysis of HRV and BPV under standardized conditions (supine rest, orthostasis) is a rapid, sensitive, noninvasive and reproducible tool for the assessment of cardiovascular autonomic dysfunction (5). Reduction in HRV magnitude is the earliest indicator of cardiovascular dysregulation (e.g. in diabetes mellitus) (6). Since HRV originates predominantly from oscillations in parasympathetic nervous traffic and blood vessels are predominantly under sympathetic nervous system control, beat-to-beat analysis of simultaneously obtained heart rate and blood pressure signals can provide information about both components of cardiovascular control (7, 8).

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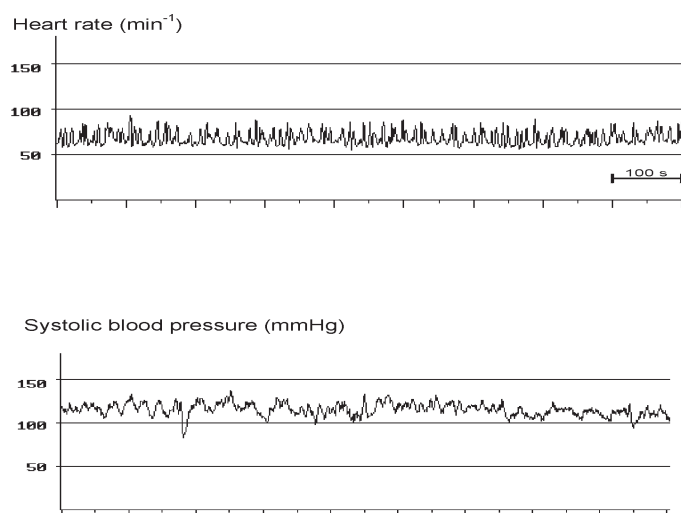


Fig. 1. Spontaneous short-term oscillations of heart rate and systolic blood pressure during supine rest. Analysis of these oscillations can provide important information on autonomic control of cardiovascular system (3)

HRV and BPV can be quantified in various ways. In 1996, the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology established a standard set of time and frequency domain measures. These numerical characteristics quantifying the magnitude of oscillations are also called *linear measures* because of the assumption of linear dynamics with the proposed output in the form of a set of more or less periodic oscillations (5). The linear measures are strongly mutually dependent and their values markedly overlap between healthy and pathological groups. Therefore, new parameters able to quantify additional information (independent of signal magnitude) hidden in the HRV and BPV dynamics are needed.

Importantly, HRV and BPV reflect the complex interactions of many different control loops of the cardiovascular control system. A detailed description and classification of dynamic changes using linear (time and frequency domain) measures is often insufficient. Given the complexity of the system modulating the sinus node activity, a predominantly *nonlinear behavior* where the output is not proportional to input has to be assumed. It was proposed that the measures from nonlinear dynamics could be important for the description of various phenomena involved in such *complex control system* (9, 10).

Since the heart and vessels are controlled by a nonlinear deterministic system, measures derived from nonlinear systems theory are increasingly used in cardiovascular variability analysis. However, the application of traditionally used nonlinear methods (developed for mathematical and physical applications mostly in 1980s-90s; e.g. correlation dimension, largest Lyapunov exponent) is limited only to long stationary signals – a condition that is only rarely met in biology (11) and their uncritical application to biological data can lead to serious pitfalls (12). New methods with applicability to real biological signals are continuously developed to quantify new aspects of short quasistationary HRV and BPV signals with the potential to reveal subtle changes in the cardiovascular control system (9).

The aim of this paper is to summarize novel nonlinear approaches developed to quantify various aspects of cardiovascular signal characteristics from short data re-

cordings (~ 5-15 min). In the following sections, the methods for assessment of complexity, recurrences in dynamics and time irreversibility are briefly introduced.

2 CARDIOVASCULAR COMPLEXITY

Integrated biological systems are characterized by various interacting subsystems and feedback loops processing internal and external inputs. This complex organization results in a *dynamical complexity* that can be revealed and quantified by the analysis of the time course of system variables under control (e.g. heart rate, systolic blood pressure). Cardiovascular regulation in the healthy human body is mediated by a variety of neural, hormonal, genetic and external interactions that operate across multiple time scales. Output variables of that system exhibit *complex fluctuations* - the measured output signal is characterized by great complexity. Human body is a complex adaptive system and the complexity in its behavior allows for broadest range of adaptive responses inherent to a healthy individual. A wide class of various disease states as well as aging appear to reduce this complexity hereby reducing the adaptive capacity of the individual. Therefore, the *loss of complexity* was proposed as a general feature of *pathological dynamics* (13-15).

Numerous studies have suggested that the quantification of complexity is of importance for the understanding as well as for the classification of heart rate oscillations (14, 16). In contrast, complexity analysis of blood pressure fluctuations have only been performed rarely (17).

2.1 QUANTIFICATION OF COMPLEXITY

2.1.1 Entropy measures

In the framework of Shannon's information theory (1946) (18), entropy is the measure of information of a given message. A message with a low entropy /information is characterized by repetition. For example, the message '123123123123' could be simply expressed by '4x123' without any loss of information. A message with high entropy/information on the other hand is characterized by little redundancy, e.g. '234365434463'.

In the case of HRV and BPV analysis, entropy measures are therefore used to quantify the complexity / regularity of heart rate / blood pressure fluctuations. Firstly, the complexity analysis of HRV was performed by calculation of *Approximate Entropy* (*ApEn*) introduced by Pincus in 1991. This measure was aimed to provide widely applicable data analysis measure enabling to classify data based on their regularity (19).

Sample entropy (*SampEn*) is an improved version of the approximate entropy. *SampEn* calculates the probability that NN interval sequences (NN denotes the time duration of normal RR interval) of length m that are similar within a tolerance r remain similar at the next point. *SampEn* is precisely the negative natural logarithm of the conditional probability that a dataset of length N , having repeated itself within a tolerance r for m points, will also repeat itself for $m + 1$ points, without allowing self-matches (20) (Fig. 2).

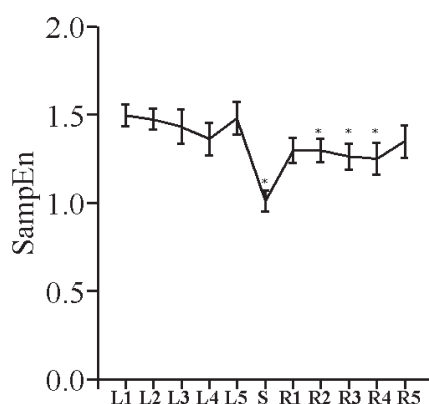


Fig. 2. Sample Entropy (SampEn) of heart rate oscillations and its changes in subject during supine rest (L1-L5), orthostasis (S), and recovery after physical exercise. The reduction of SampEn during orthostasis and recovery after exercise indicates the simplification of heart rate dynamics accompanying the shift of autonomic nervous system balance towards sympathetic predominance. The asterisks indicate the significant difference from supine rest values (21)

A different way to assess entropy is based on data compression technique. In information theory, the smallest algorithm that produces a string is at the same time the entropy of that string (Chaitin-Kolmogorov entropy). Although it is theoretically impossible to develop such an algorithm, data compression techniques might provide a good approximation. The modified version of the LZ77 algorithm for lossless data compression introduced by Lempel and Ziff (1977) (22) can be used to compress time series. The ratio of the uncompressed to the compressed time series named *compression entropy* (H_c) is used as a HRV and BPV complexity measure (13) (Fig. 3).

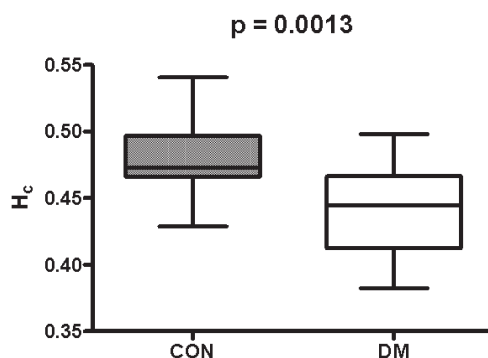


Fig. 3. Compression entropy (H_c) of heart rate is significantly lower in young patients with type 1 diabetes mellitus (DM) compared to control group (CON) according to the concept of complexity loss as an indicator of cardiac dysregulation (23)

2.1.2 Symbolic dynamics

The concept of *symbolic dynamics* goes back to Hadamard (1898) and allows a simplified description of the dynamics of a system with a limited amount of symbols. In HRV analysis from ECG, consecutive RR intervals or their changes, respectively, are encoded according to some transformation rules into a few symbols of a certain alphabet. Subsequently, the dynamics of that symbol string are quantified, providing some more global information about the heart rate / blood pressure dynamics. Several techniques are used to describe dynamics encoded in symbolic sequence (24, 25).

Approach according to Voss et al (1996) (24): The difference between each NN interval and the mean NN interval is transformed into an alphabet of 4 symbols {0, 1, 2, 3} according to the transformation rule described in (24). Symbols '0' and '2' reflect low deviation (decrease or increase) from the mean NN interval, and symbols '1' and '3' reflect a stronger deviation (decrease or increase over a predefined limit). Subsequently, the symbol string is transformed to words of three successive symbols, e.g. '023' or '221'. The distribution of word types reflects some nonlinear properties of HRV. From this symbolic dynamics the following measures of complexity can be calculated: *FORBWORD* – number of word types that occur seldom, i.e. with a probability less 0.001, *shannon* – Shannon entropy over all word types as defined by Voss et al. (24). In the pathological conditions *FORBWORD* increases while *shannon* decreases reflecting the simplification of system dynamics – i.e. the loss of complexity.

Approach according to Porta et al (2001) (25): NN interval sequences are uniformly spread on six levels, resulting in 6 different symbols {0, 1, 2, 3, 4, 5}. Two approaches are used for analysis of resulting symbolic time series. *Normalized complexity index (NCI)* is a measure of complexity and is computed as a minimum of normalized corrected conditional entropy (*NCCE*) as a function of *L* (length of pattern). *NCCE* is a measure of the amount of information (corrected for short-term time series) carried by the *L*-th sample when the previous *L*-1 samples are known. *NCCE* remains constant in case of white noise; decreases to zero in case of fully predictable signals; exhibits a minimum if repetitive pattern is embedded in noise. *NCI* is a measure of the complexity of pattern distribution. It ranges from zero (maximum regularity) to one (maximum complexity) – the larger the *NCI*, the more complex and less regular the time series. The second approach is focused on the pattern classification within dynamics (26): all the patterns (symbolic sequences) with *L* = 3 were grouped into 4 families according to number and types of variations from one symbol to the next one. The pattern families are: 1) patterns with no variation (*OV*, all three symbols are equal); 2) patterns with one variation (*1V*, two consecutive symbols are equal and the remaining one is different); 3) patterns with two like variations (*2LV*, three symbols form an ascending or descending ramp), 4) patterns with two unlike variations (*2UV*, three symbols form a peak or a valley). The rates of occurrence of these patterns are indicated as *OV%*, *1V%*, *2LV%* and *2UV%* (Fig. 4).

A

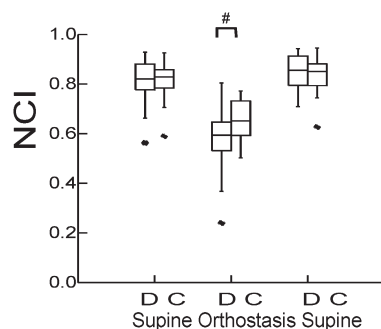


Fig. 4. Symbolic dynamics analysis of heart rate during orthostasis revealed significantly lower complexity (index NCI, i.e. nonlinear complexity index; # indicates the statistically significant difference) in girls with major depression (D) compared to control group (C). While no differences in NCI were found in both supine positions (before and after orthostasis) these results stress the importance of cardiovascular variability testing during various standardized conditions (27)

2.1.3 Multiscale entropy

From recently developed nonlinear methods, measures of short-term complexity (various entropy measures) have shown to be promising for analysis of both heart rate and blood pressure signals (13, 26, 28). However, since beat-to-beat RR interval and BP time series under healthy conditions have a complex temporal structure with correlations on multiple scales, single-scale based traditional entropy measures (e.g. *Sample Entropy*) may fail to account for the multiple time scales inherent in the complex physiological system dynamics. Therefore, a meaningful measure of complexity should take into account *multiple time scales*. Recently, Costa *et al* (2002) (29) introduced a new method to calculate entropy over multiple scales – *Multiscale Entropy analysis (MSE)*.

MSE is computed according to the procedure published by Costa (29). First step involves the construction of coarse-grained time series for each of the analyzed time scales determined by the scale factor τ . In other words, coarse-grained time series for scale τ are obtained by taking arithmetic mean of τ neighbouring original values without overlapping. For scale 1, the coarse grained time series is simply the original time series. In the next step, *SampEn* values are calculated for each one of the coarse-grained time series and plotted against the scale factor. In this way, *MSE* describes the entropy (complexity) for various time scales of fluctuations within the analysed signal (Fig. 5).

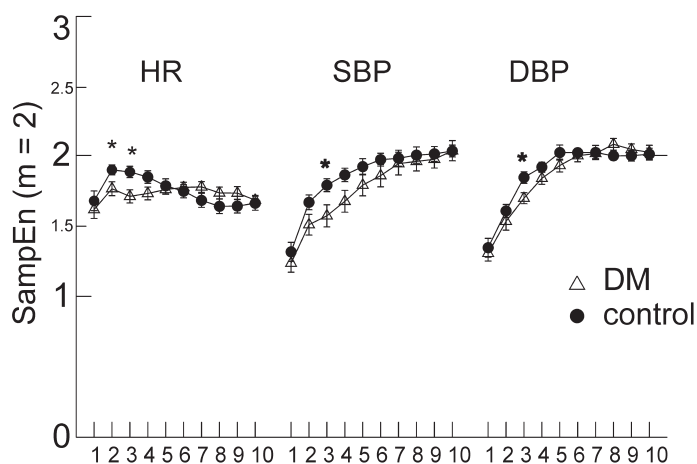


Fig. 5. Multiscale entropy analysis of heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) oscillations during supine rest in patients with type 1 diabetes mellitus (open triangles, DM) and age and gender matched control subjects (full circles, control). In multiscale entropy analysis, Sample Entropy (SampEn) values are plotted against the corresponding time scale (in this study, for time scales 1-10). While reduction in complexity in DM was not detectable on scale 1 (original time series), significant loss of complexity in all three signals was detected on higher scales (2-3) indicating the importance of multiscale approach for cardiovascular signals complexity analysis. MSE was the only method capable to reveal subtle differences between both groups of subjects in blood pressure variability signals (17)

2.2 Recurrences in cardiovascular dynamics

Recurrence is a basic feature of many dynamical systems – it is defined as the repeated occurrence of a given state of the system in time. *Recurrence plot* (RP) is a graphical representation of such recurrences in a dynamical system (30) (Fig. 6).

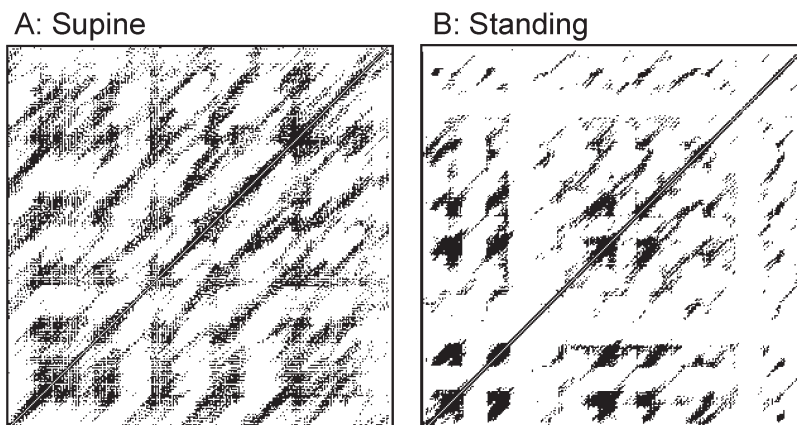


Fig. 6. A representative example of the recurrence plots (RP) constructed from heart rate oscillations during supine (A) and standing positions (B). RP was constructed as follows: any recurrence of state in time i (x -axis) with the state in time j (y -axis) is plotted as a black dot. In both presented RPs, the percentage of recurrence (%Rec; percentage of black points) was fixed to 5% - it means that the 5% of all points of RP were recurrent. The differences were found in the structure of patterns within RP: in standing position, higher proportion of points were grouped into diagonals (percentage of determinism - %Det: 41% in supine vs. 78% in standing position) and verticals (Laminarity - Lam: 0.593 in supine vs. 0.860 in standing position) (31)

As shown by analysis of model signals, RPs are sensitive to subtle qualitative changes in the system's dynamics (bifurcations) and could therefore be used for the characterization of shifts in basic cardiovascular parameters dynamics during both physiological and pathological conditions. The major advantage of recurrence plots is their applicability to short and nonstationary physiological time series. The power of RP also resides in its independence from constraining assumptions and limitations plaguing other analyses. Because recurrence structures are simply tallied within the signal, there is no need to pre-condition the data by filtering, linear detrending, or transforming the data to conform to any particular statistical distribution. For these reasons RPs has proven to be ideally suited for the study of numerous real-world systems (30).

The structures exhibited by RP reveal information on system properties and can be described quantitatively by *recurrence quantification analysis (RQA)* (32, 33). RQA was useful for detection of heart rate dysregulation in various pathological conditions including chronic fatigue syndrome, hypertension, ventricular arrhythmia (34, 35).

2.3 Time irreversibility

Recently, it was shown that heart rate and blood pressure signals show a feature of *time irreversibility*. This phenomenon is specific for nonequilibrium systems with inherent complexity (36) and can be described as the lack of signal statistical properties stability after the signal time reversal. In other words, the signal is irreversible if its statistical properties are changed after the reversal of time (when one looks on the signal from right to left) (37). Several methods to quantify time asymmetry and their robustness and independence are introduced in this issue of Acta Medica Martiniana (38).

3 Conclusion

Autonomic function testing is essential to diagnose cardiovascular dysregulation which might result in fatal endpoints such as sudden cardiac death. The magnitude as well as nonlinear features of HRV may be easily quantified on relatively short recordings in a routine clinical setting, requiring only ECG and automated computer software, being neither cost nor labour intensive. HRV and BPV analysis is a useful tool for the diagnosis and monitoring of autonomic dysregulation and should include nonlinear analysis along with traditional time and frequency indices. Assessing HRV and BPV might also be suited to monitor different pharmacological and non-pharmacological treatment strategies.

While linear variability measures provide information on the overall magnitude of oscillations (*quantitative* analysis), nonlinear measures provide complementary information about *qualitative* features of the analyzed signal. Since analysis of variability and nonlinear characteristics (complexity, recurrences, time irreversibility, etc.) can provide *independent information on cardiovascular system control*, it is beneficial to use both groups of measures simultaneously in the studies demanding the application of HRV and BPV analysis as a noninvasive tool for the assessment of cardiovascular autonomic nervous system function. We suggest that simultaneous use of both groups of measures can increase the information value and thus *improve the sensitivity and reliability* of the detection and monitoring of the cardiovascular system dysregulation during various pathological conditions. To verify this, other studies on the HRV/BPV complexity measures application during specific pathological conditions are needed.

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TIME IRREVERSIBILITY OF HEART RATE OSCILLATIONS DURING ORTHOSTASIS

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Abstract

Introduction: Time irreversibility is a characteristic feature of non-equilibrium, complex systems involving cardiovascular control mediated by autonomic nervous system. Its analysis in heart rate variability (HRV) signal represents a new approach to assess cardiovascular regulatory mechanisms. The purpose of this paper was to assess the changes in various time irreversibility indices during the orthostatic test (a balance of autonomic nervous system shifted towards sympathetic predominance). In addition, we tested the behaviour of the selected irreversibility indices in relation to different lengths of analysed data and their mutual dependence.

Methods: We examined the irreversibility of HRV time series obtained from 28 healthy young subjects recorded during 20 minutes in the supine position followed by 15 minutes in the standing position. We used three different time irreversibility indices – Porta's, Guzik's and Costa's indices (P%, G% and A, respectively). The proposed irreversibility indices were derived from data segments containing 300, 600 and 1000 beat-to-beat intervals.

Results: Two of three time irreversibility indices (P% and A) were sensitive to the shift in sympathovagal balance during the orthostatic challenge even when calculated from the shortest data length. Indices were relatively insensitive to data length – there were no significant differences in relation to data length. Despite the difference in algorithms for the calculation of indices P% and A, these are closely mathematically related and do not provide mutually independent information.

Conclusion: We conclude that heart rate irreversibility indices are sensitive to the changes in autonomic tone after the orthostatic challenge even when derived from the 300 interbeat intervals. Porta's index seems to be more sensitive to autonomic nervous balance shift compared to Guzik's index. Costa's index is closely related to P% and do not provide any additional information compared to other indices.

Keywords: heart rate variability, time irreversibility, orthostatic challenge, sympathovagal balance

INTRODUCTION

Heart rate variability (HRV) is a physiological phenomenon and its assessment can provide useful information about the mechanisms involved in the cardiovascular system control. HRV is traditionally quantified in time and frequency domain by linear analysis methods (1). However, healthy cardiovascular regulatory system comprises complex interactions of many control loops and manifest nonlinear behaviour (2, 3). Therefore nonlinear methods measuring complexity and other system dynamic features are more suitable for a detailed description of cardiovascular control system characteristics (3, 4).

From several nonlinear aspects of cardiovascular signal, time irreversibility has recently gained more attention. A signal is said to be time irreversible if its statistical properties change after its time reversal, i.e. it is characterized by a temporal asymmetry (3, 5, 6). The phenomenon of irreversibility is specific for nonequilibrium systems (6) and its presence in the heart rate results from the complexity of cardiovascular control system typical for the healthy human (7).

In previous papers time irreversibility indices have been applied to HRV analysis to detect the changes in cardiovascular regulation in the pathological groups of chronic heart failure patients (4), patients after acute myocardial infarction (8) and also in healthy subjects after physical activity (7, 8), during passive head-up tilt (2, 5) and active ortho-

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static challenge (2). However, there is still a lack of information describing the changes of time irreversibility in HRV during the shift in autonomic nervous system status.

The aim of this study was to determine the effect of the orthostatic test on the time irreversibility of the RR interval time series in healthy young subjects quantified by various time irreversibility indices: Porta's (P%), Guzik's (G%) and Costa's index (A). The postural change from supine to the standing position is a well-known manoeuvre characterized by a shifted balance of the autonomic nervous system towards sympathetic predominance. We also tested the behaviour of the selected irreversibility indices in relation to different lengths of analysed data (300, 600 and 1000 RR intervals).

METHODS

1.1 Subjects

We investigated recordings from 28 healthy young volunteers (21 female, 7 male) aged 20.4 ± 0.2 years (SEM) (range: 17.9 – 23.8 years). They were instructed to avoid substances influencing cardiovascular system activity (caffeine, alcohol) and smoking for 12 hours before examination. They were normotensive, non-obese (BMI: $21.7 \pm 0.5 \text{ kg m}^{-2}$) and were taking no medication for the duration of study. The study was approved by the Ethics Committee of Jessenius Faculty of Medicine, Comenius University and all participants gave their informed consent prior to examination.

1.2 Protocol

After the stabilization period (15 minutes resting in a supine position) the ECG signal was recorded for 20 minutes in a supine position. Subsequently, the subjects were asked to stand up slowly (in about 5 seconds) and the recording continued in the standing position for another 15 minutes. Complete study protocol can be found in (3).

1.3 Data recording and pre-processing

The ECG signal (bipolar thoracic ECG lead) from Cardiofax ECG-9620 (NihonKohden, Japan) was transferred into PC by analog-to-digital converter PCL-711S (Advantech, Taiwan) at a sampling frequency of 500 Hz. After the QRS complex detection, the RR interval was defined as the time interval between two consecutive R peaks. The results were carefully visually checked and rare ectopic beats were interpolated linearly.

1.4 Time irreversibility analysis

Irreversibility analysis was performed on three time series segments of different lengths (300, 600 and 1000 RR intervals) for each position (lying in a supine position – L, standing position – S) using custom-made software. Following indices were used to measure the time irreversibility of HRV series:

1. *Porta's index P%* (5, 6) based on the evaluation of the percentage of negative ΔRR (i.e. ΔRR) with respect to the total number of $\Delta RR \neq 0$. ΔRR is defined as a difference of two consecutive RR intervals length.

$$P\% = \frac{N(\Delta RR^-)}{N(\Delta RR \neq 0)} \cdot 100 \quad (1)$$

where $N(\Delta RR^-)$ denotes the number of negative ΔRR and $N(\Delta RR \neq 0)$ indicates the number of all non-zero ΔRR .

2. *Guzik's index G%* (5, 6, 9) based on the evaluation of the percentage of the cumulative square values of positive ΔRR (ΔRR^+) to the cumulative square values of all ΔRR s.

$$G\% = \frac{\sum_{i=1}^{N(\Delta RR^+)} \Delta RR^+(i)}{\sum_{i=1}^{N(\Delta RR)} \Delta RR^2(i)} \cdot 100 \quad (2)$$

Contrary to the P%, the calculation of G% considers also the magnitude of the difference between two RR intervals.

3. *Costa's index A* (10) based on the evaluation of the difference between the percentage of increments and decrements between consecutive RR intervals according to:

$$A = \frac{\sum_{i=1}^{N(\Delta RR)} H[-\Delta RR(i)] - \sum_{i=1}^{N(\Delta RR)} H[\Delta RR(i)]}{N(\Delta RR \neq 0)} \quad (3)$$

where H is the Heaviside function ($H(a) = 0$ if $a < 0$ and $H(a) = 1$ if $a \geq 0$). The larger departure of A from 0, the more irreversible the corresponding time series is.

Statistics

The differences between asymmetry (irreversibility) indices calculated during standing (S) compared to the supine (L) position were analyzed by the nonparametric Wilcoxon signed-rank test. A p value < 0.05 (two-tailed) was considered statistically significant. Wilcoxon signed-rank test was also used to analyze differences between the values of single irreversibility index derived from the different number of beats (300, 600 and 1000).

RESULTS

We calculated the values of P%, G% and A according to the Eqs. (1) – (3). All irreversibility indices (P%, G% and A) were derived from data segments containing 300, 600 and 1000 beat-to-beat intervals. Statistical analysis showed no significant differences in indices in relation to data length.

Fig. 1 shows the box plots of all investigated irreversibility indices in both experimental conditions – lying ($P_L\%$, $G_L\%$, A_L) and standing ($P_S\%$, $G_S\%$, A_S) and for all analyzed data lengths – 300 RR intervals (on the left), 600 RR intervals (in the middle) and 1000 RR intervals (on the right). Significantly higher values of P% and A were found during standing compared to the supine position for all analysed data lengths. HRV analysis revealed no significant differences in G% during standing compared to the supine position.

Despite the differences in algorithms, the behaviour of P% and A is very similar indicating an evidential mathematical relationship between P% and A (for a detailed description see Appendix).

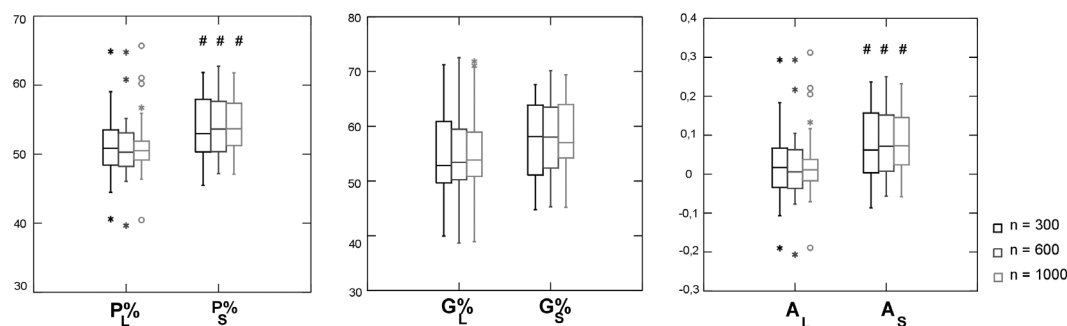


Fig. 1: Box-and-whisker plots representing irreversibility indices calculated from RR interval time series; in the lying position ($P_L\%$, $G_L\%$, A_L) and standing position ($P_S\%$, $G_S\%$, A_S). Indices are derived for both conditions from 300 data points (on the left), 600 data points (in the middle) and 1000 data points (on the right). Asterisks and circles correspond to outlier values, # corresponds to significant difference of the parameter in S with respect to L. #: $P_S\%$ ($p_{300} = 0.012$, $p_{600} = 0.002$, $p_{1000} = 0.007$) and A_S ($p_{300} = 0.012$, $p_{600} = 0.003$, $p_{1000} = 0.007$) are significantly larger than $P_L\%$, A_L for all data lengths.

DISCUSSION

The major finding of our study is that indices P% and A are sensitive to the increase in the sympathetic activity during orthostatic challenge. Interestingly, even very short data series (300 heart beats) provided the information useful to distinguish the time irreversibility of heart rate dynamics. We also demonstrated that all indices are relatively insensitive to data length, as there were no significant differences in relation to the number of RR intervals indicating no systematic differences in their values in relation to data length. Our results suggest that algorithm for calculation of indices P% and A is more sensitive to autonomic nervous balance shift compared to index G%. In addition, we showed in the present study that there is a close relationship between indices P% and A (see Appendix). Therefore they do not provide mutually independent information and their simultaneous quantification is redundant.

The increased presence of temporal asymmetries in the HRV signal after the orthostatic challenge might reflect changes in autonomic tone (2, 5). Results of previous studies suggest that simple two-dimensional irreversibility tests (Porta's, Guzik's and Ehler's index) are sensitive to the increase in the sympathetic modulation produced by head-up tilt at highest inclination angles (i.e. 75° and higher) (2, 5). However, there is a lack of information about the changes of time irreversibility indices during active change of posture from supine to standing. Casali et al. (2) performed the two- and five-dimensional irreversibility test during active orthostatic challenge using Ehler's index, which is utilizing the skewness of ΔRR distribution. But contrary to our results, Ehler's index reflected the increase in irreversible dynamics only in the five-dimensional space (2). Therefore our results suggest that Porta's and Costa's index might be more sensitive to the change in autonomic control during active orthostatic challenge.

Although temporal asymmetries are more likely over short time scales than over longer ones (5), it is well-known that the physiological system is characterized by complex behaviour over multiple scales. Therefore, it should be more suitable to adjust the irreversibility analysis for different time scales in order to extract more information about the cardiovascular control system (2, 6, 8, 10). In consequence, further investigations need to focus on time irreversibility methods that are mathematically independent and are able to quantify irreversibility of HRV series over multiple time scales to improve the performance of the technique.

CONCLUSION

Heart rate irreversibility indices are sensitive to the shift in sympathovagal balance induced by postural change even when derived from 300 interbeat intervals and on a single scale. Porta's index seems to be more sensitive to autonomic nervous balance shift compared to Guzik's index. Costa's index is closely related to the Porta's index and therefore does not provide additional information. We suggest that irreversibility indices have the potential to provide additional information about cardiovascular control mechanisms, but further investigation on properties, robustness and behaviour of these indices is needed to improve the performance and interpretation of the method.

APPENDIX

According to the original definition of Costa's index (10), Eq. (3) can be expressed as:

$$A = \frac{N(\Delta RR^-) - N(\Delta RR^+)}{N(\Delta RR \neq 0)} \quad (4)$$

By applying the equation $N(\Delta RR \neq 0) = N(\Delta RR^-) + N(\Delta RR^+)$ to Eq. (4), we obtain the relation of A and P:

$$A = \frac{N(\Delta RR^-) - N(\Delta RR \neq 0) + N(\Delta RR^-)}{N(\Delta RR \neq 0)} = 2 \frac{N(\Delta RR^-)}{N(\Delta RR \neq 0)} - 1 = 2P - 1 \quad (5)$$

$$\text{where } P = \frac{P\%}{100}.$$

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CARDIAC VAGAL CONTROL IN DEPRESSION AND ATTENTION DEFICIT/HYPERACTIVITY DISORDER

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Abstract

The importance of the vagus nerve in the two way communication between the brain and the heart has been known for over 100 years. Recently, integrative theories that link central nervous system structures to cardiac vagal regulation, such as the polyvagal theory, have of late emerged. Based on the polyvagal theory, the respiratory sinus arrhythmia (as an index of cardiac vagal control) is considered as a psychophysiological marker of many aspects of behavioural functioning and emotion regulation in both children and adults. Although a lack of sensitive heart rate autonomic control likely reflects impaired cardiac nervous system regulation, the sophisticated brain-heart interactions are incompletely understood. Importantly, cardiac vagal dysregulation is associated with the increased risk of cardiovascular morbidity reflecting various pathophysiological states. Thus, we believe that the identifying of cardiac vagal control changes in mental disorders should represent an initial step towards the understanding of a potential pathomechanisms leading to later cardiac adverse outcomes; especially in children and adolescents.

Key words: cardiac vagal regulation, central nervous system, respiratory sinus arrhythmia, heart rate variability, mental disorders

INTRODUCTION

Autonomic nervous system plays a key role in a wide range of somatic and mental disorders. The ANS is generally conceived to have two major branches – the sympathetic system, associated with energy mobilization, and the parasympathetic system, associated with vegetative and restorative functions. The activity of the ANS is in dynamic balance; thus, the autonomic imbalance implying sympathetic overactivity and parasympathetic underactivity is associated with various pathological conditions indicating a lack of dynamic flexibility and health (1).

Psychological states and processes are known to profoundly influence the autonomic nervous control of the cardiovascular system with likely contribution to the increased cardiovascular morbidity in patients with mental disorders (2,3). During this decade the pathomechanisms by which central nervous system (CNS) modulates changes in autonomic nervous system (ANS) in various mental disorders as well the potential links between cognitive/emotional processes and cardiac autonomic regulation have drawn increasing interest (4,5).

1. NEURAL CORRELATES OF THE CARDIAC VAGAL REGULATION

Cardiac function is extremely sensitive to autonomic influences. In healthy systems both branches of the ANS are tonically active with sympathetic activity associated with heart rate acceleration and parasympathetic activity associated with heart rate deceleration (6). The studies concerning the brain-heart connection emphasize the modulation of the cardiac activity by the cortex; thus, an extensive research has been directed at identifying the pathway by which this neurocardiac control is achieved (1, 7). Benarroch (8)

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has described the central autonomic network (CAN) as an integrated component of an internal regulation system through which the brain controls visceromotor, neuroendocrine, and behavioural responses that are critical for goal-directed behaviour and adaptability. Structurally, the CAN includes the anterior cingulate, insular, and ventromedial prefrontal cortices, the central nucleus of the amygdala, the paraventricular and related nuclei of hypothalamus, the periaqueductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract, the nucleus ambiguus, the ventrolateral and ventromedial medulla, and the medullary tegmental field. The primary output of the CAN is mediated through the sympathetic/parasympathetic neurons innervated the heart. Moreover, the interplay of sympathetic and parasympathetic (vagal) outputs of the central autonomic network at the sinoatrial node producing the complex beat-to-beat heart rate variability is characteristic of a healthy and adaptive organism (9). Neurochemically, tonic inhibition of the CAN is achieved by γ -aminobutyric acid (GABA) activity within the nucleus tractus solitarii, and by GABAergic frontal cortex inhibition of subcortical sympatho-excitatory defensive circuits (9, 1). Hence, the importance of the inhibition of cardiac activity by prefrontal cortex is emphasized.

Specifically, a series of studies using neuroimaging have provided evidence that activity of the prefrontal cortex is associated with vagal function (10, 11). Therefore, it appears that the modulation of cardiac activity by the cortex is vagally mediated. Interestingly, integrative theories that link CNS structures to cardiac vagal regulation, such as the polyvagal theory (12), have of late emerged. A central feature of this theory is the existence of separate brainstem centers involved in parasympathetic cardiac control, with distinct evolutionary origins and significance and with divergent influences on cardiac vagal tone related to psychological and behavioral processes (Table 1).

Table 1. Three phylogenetic stages of the neural control of the heart proposed by the polyvagal theory

Phylogenetic stage	Autonomic nervous system component	Behavioural function	Lower motor neurons stage
III	Myelinated vagus	Social communication Self-soothing and calming, inhibit sympathetic-adrenal influences	Nucleus ambiguus
II	Sympathetic-adrenal system	Mobilization (active avoidance)	Spinal cord
I	Unmyelinated vagus	Immobilization (passive avoidance)	Dorsal motor nucleus of the vagus

2. RESPIRATORY SINUS ARRHYTHMIA AS AN INDEX OF CARDIAC VAGAL CONTROL AND EMOTIONAL REGULATION

Respiratory sinus arrhythmia (RSA) refers to rhythmic fluctuation in the heart rate associated with respiration, which results from activity of a branch of vagal fibers from the medullary nucleus ambiguus terminating at the sinoatrial node and it is mediated through physiological mechanisms by which the heart rate increases during inspiration and decreases during expiration (13, 14). As interpreted by Porges in his polyvagal theory (12, 15), the nucleus ambiguus is considered as an origin of the more recently developed „smart“ vagus to facilitate the complex emotion responses/social behaviour. Two sources of structural evidence link RSA to emotion. Efferent fibers from the nucleus ambiguus innervate the larynx, an important structure for communication of emotional state through vocalization (12). Also, afferent fibers of the nucleus ambiguus are believed to terminate in the source nuclei of the facial and trigeminal nerves, which facilitate the emotion behaviours of facial expression and vocalization. Recently, along with struc-

tural evidence, empirical studies relating RSA to emotion in humans have accumulated (16, 11). Then, the polyvagal theory posits that the myelinated vagus, originating in the nucleus ambiguus, is a dynamic contributor to the processes of the emotion and social interactions (12, 15). Therefore, the respiratory sinus arrhythmia should be considered as an index of both cardiac vagal and emotional regulation.

One way to index the central control of the heart via the vagus nerve is the use of the short-term **heart rate variability**, *i.e.* the amount of the heart rate fluctuations around the mean heart rate (17). In regard to RSA evaluation, the HRV spectral analysis allows to isolate the faster high frequency (HF) respiratory-coupled influences on the HRV (0.15-0.4 Hz) from slower sources of the HRV reflecting mainly sympathetic and parasympathetic activity via baroreflex. Thus, a spectrally derived index of vagally-mediated heart rate variability (HF-HRV) provides information about the cardiorespiratory oscillations reflecting in the respiratory sinus arrhythmia. Moreover, several studies have documented correlation between HF-HRV and cerebral blood flow in certain cortical (prefrontal, insular cortex) and subcortical regions (10). As such, vagally-mediated HRV serves to index central-autonomic nervous system integration, and consequently, is a psychophysiological marker for adaptive environmental engagement (12).

3. CARDIAC VAGAL CONTROL IN PSYCHIATRIC CONDITIONS

Depression

Generally, impaired cardiac vagal function is likely important contributor to the cardiac adverse outcomes associated with internalizing disorders, *e.g.* major depression (18). Since reduced cardiac vagal modulation is a common finding in adult patients with depression (19), other studies reported unchanged parasympathetic cardiac activity (20). Moreover, depressive disorder is often overlaps with anxiety. Trait anxiety may moderate the relationship between cardiac vagal regulation and depression, with which anxiety is often associated. Low vagal heart rate control was found only in high-anxious subset of a depressed sample (21). In this regard, the reduced vagal modulation of the heart in depression could represent a chronic, consolidated anxiety-related response to everyday hassles and aggravations (22).

In contrast to adults, the studies relating the cardiac vagal control and adolescent major depression are rare. The adolescence could be a critical and vulnerable age-period to a potential depression-induced cardiac autonomic dysregulation due to developmental and brain maturational changes (23). In our studies, the HRV linear (spectral) and nonlinear (symbolic dynamics) analysis revealed reduced cardiac vagal control in adolescent girls with major depression prior to pharmacological treatment (Fig. 1, 2). These results were in agreement with other studies related to adult depression and extend them into the adolescent age. Since cardiac vagal dysregulation is associated with increased risk of cardiovascular morbidity, this finding underscores the importance of impaired cardiac vagal control in adolescent major depression (24, 25).

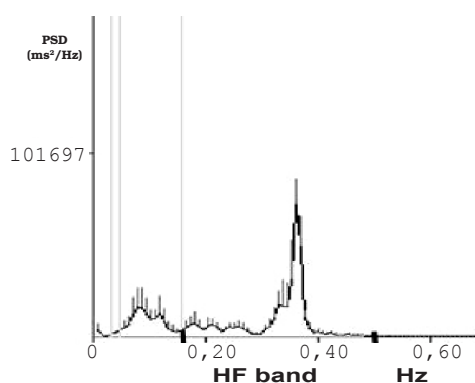


Fig. 1 Graphic protocol of the heart rate variability spectral analysis in 17-year-old healthy subject. HF band reflects predominantly the respiratory sinus arrhythmia as an index of cardiac vagal control. HF – high frequency.

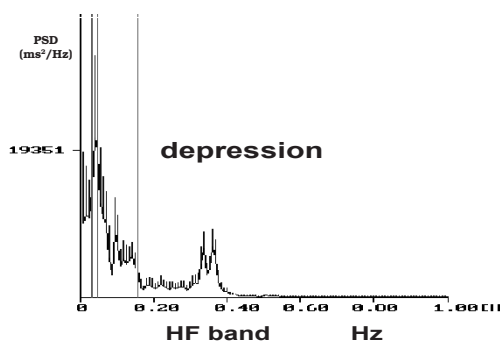


Fig. 2 Graphic protocol of the heart rate variability spectral analysis in 17-year-old patient with major depression. Reduced HF band refers lower respiratory sinus arrhythmia indicating impaired cardiac vagal control in adolescent patient with major depression. HF – high frequency.

Attention deficit/hyperactivity disorder (ADHD)

ADHD as one of the most commonly diagnosed externalizing mental disorders among children characterized by distractibility, hyperactivity, impulsive behaviours and the inability to remain focused on tasks or activities. Some studies found deficient respiratory sinus arrhythmia in children with ADHD. For example, Crowell et al. (26) compared autonomic profiles of preschool children (aged from 4 to 6 yrs) with ADHD and oppositional defiant disorder (ODD) with controls. Children with ADHD and ODD were not significantly different in baseline RSA, but authors referred to a potential impaired cardiac vagal regulation in later age-period related to emotion dysregulation and lability.

We studied the cardiac vagal control using the HRV spectral analysis under rest and physiological stress stimulus (orthostasis) in children with ADHD. Our results indicated decreased cardiac vagal modulation in a supine position and in response to standing (27). Beauchaine (4) referred to reduced respiratory sinus arrhythmia, indicating altered cardiac vagal control, as a potential marker of the dysregulated emotional states. Additionally, the excessive vagal reactivity could characterize emotional lability. In our study, the vagal reactivity during orthostasis was significantly higher in ADHD group compared to controls (-36% vs. -23%). Thus, it is questionable whether our findings of lower cardiac vagal control combined with higher vagal reactivity are related to the feature of the ADHD (i.e. emotional lability due to emotional immaturity) or it is reflection

of subclinical abnormal dynamic activation of the ANS in response to posture change in children with ADHD (27). Moreover, it seems that emotional maturation should represent an important factor connected to parasympathetic-linked cardiac activity.

4. THE POTENTIAL PATHOMECHANISMS LEADING TO CARDIAC VAGAL DYSREGULATION IN MENTAL DISORDERS

Their understanding is complicated by the large number of cortical, subcortical and brainstem structures coordinating cardiac vagal control (28). Firstly, some authors emphasize the neural correlates of vagal function and the role of brain, particularly the inhibitory role of the prefrontal cortex. Thus, the prefrontal cortex and its abnormalities (e.g. prefrontal hypoactivity) could play a critical role in cardiac autonomic dysregulation associated with depression because of the failure of the prefrontal cortex to inhibit amygdala as a region that mediates cardiovascular and autonomic responses (7). Similarly, in ADHD children, the deficit in frontal functioning connected to limbic system and consequent alteration of baroreflex function as well as the modifications in a network of brain region are supposed (29). Secondly, behavioural/lifestyle factors should be associated with impaired cardiac vagal function in mental disorders. Rottenberg (30) revealed that the diagnosis of depression exerts a small-to-medium effect on cardiac vagal control and referred to other factors influencing cardiac function (e.g. lack of physical activity). Thus, it seems that the question related to predominant role of the neurobiological basis, including neurotransmitter's changes/ hypothalamo-hypophysis-adrenal axis dysfunction, or behavioural/psychological effects on the vagally mediated heart rate control, remains unclear.

5. CONCLUSION

The deficiency of the cardiac vagal control indicating impaired autonomic neurocardiac integrity in mental disorders is associated with increased risk of cardiovascular morbidity. We suggest that an interdisciplinary approach, including the integration of basic physiological research and pedopsychiatry, may contribute to the elucidation of the pathomechanisms and to the early diagnosis of the heart rate dysregulation in young people with mental disorders.

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