GENERAL REVIEW

UDC: 612.1.062:612.8.067]::616.1-07
DOI: 10.2298/VSP110707019B

Methodology of monitoring cardiovascular regulation

Metodologija praćenja kardiovaskularne regulacije

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Key words:
cardiovascular system; monitoring, physiologic; methods; blood pressure; heart rate; hemodynamics.

Ključne reči:
kardiovaskularni sistem; fiziološke funkcije, praćenje; metode; krvni pritisak; srce, frekvencija; hemodinamika.

Introduction

Adaptation of an organism to changes in external and internal environments is regulated by the autonomic nervous system (ANS) [1–4]. The ANS is structurally and functionally positioned to interface between the internal and external milieu, coordinating bodily functions to ensure normal homeostasis and adaptive responses to environmental changes [4]. The neural control of cardiovascular (CV) system plays a major role in such adaptations, even if different humoral mechanisms also participate in this control. In fact, dynamic environmental changes contrasting basic functional needs of the organism dramatically challenge the CV adaptive mechanisms. The fact that "cardiovascular diseases are the leading cause of death in the world today and will remain so by the year 2020" (The WHO MONICA Project [5]) strongly supports the need for new insights into CV regulatory mechanisms. This review considers recent studies which focus on the understanding of CV regulation and the methodology for monitoring CV regulation.

Cardiovascular regulation

CV neural regulation occurs through both sympathetic and parasympathetic (vagal) outflow to the heart and vessels. Central autonomic drives act directly from the central nervous system (CNS) on the heart and vessels, while peripheral drives are relayed to the heart and vessels through, among others, the baroreflex function. These drives are relayed to the heart through sympathetic and parasympathetic outflows and to blood vessels through sympathetic outflow only (Figure 1). Classically, central integration modifies the performance of individual reflexes according to the prevailing behavioural needs (*ie* exercise) [6]. Thus, heart period (HP) is modified together with other controlled hemodynamic variables, such as vascular resistance and, consequently, arterial blood pressure (BP) [7]. Numerous techniques for studying autonomic control of CV system are based on HP and arterial BP analysis (Figure 2). These techniques can be divided in two groups: techniques based on induced fluctuations of arterial BP and techniques based on analysis of spontaneous fluctuations of BP (Figure 2). Here we put emphasis on the techniques of HP and BP spontaneous fluctuations due to their numerous advantages with respect to the techniques based on induced fluctuations of arterial BP. The resulting HP and arterial BP values obtained by this technique reflect the overall interaction between central and peripheral mechanisms of CV regulation, without providing straightforward information on separate central and peripheral contributions being the result of rather complex interplay [8]. As a result of the activity of different mechanisms involved, HP and arterial BP fluctuations are commonly observed in physiological conditions. These fluctuations are present even in the absence of motor behaviour, like in paralysed animals [9]. Such fluctuations in hemodynamic parameters reflect both the presence of a variety of naturally occurring physiological perturbations to CV homeostasis (i.e. respiration, postural shifts, thermoregulation) and the dynamic response

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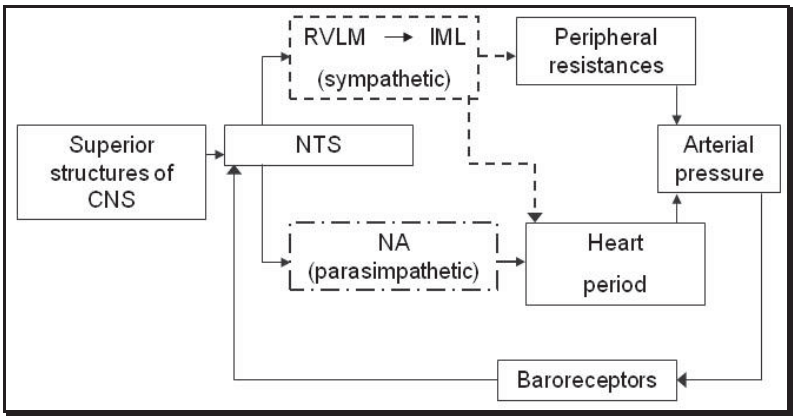

Fig. 1 – Mechanisms of peripheral (baroreflex) and central regulation of cardiovascular system
NTS – *nucleus tractus solitarii;* RVLM – rostral ventrolateral medulla; IML – intermediolateral column; NA – *nucleus ambiguus*.
Long dash spotted line-parasympathetic nervous system drive, dashed line-sympathetic nervous system drive

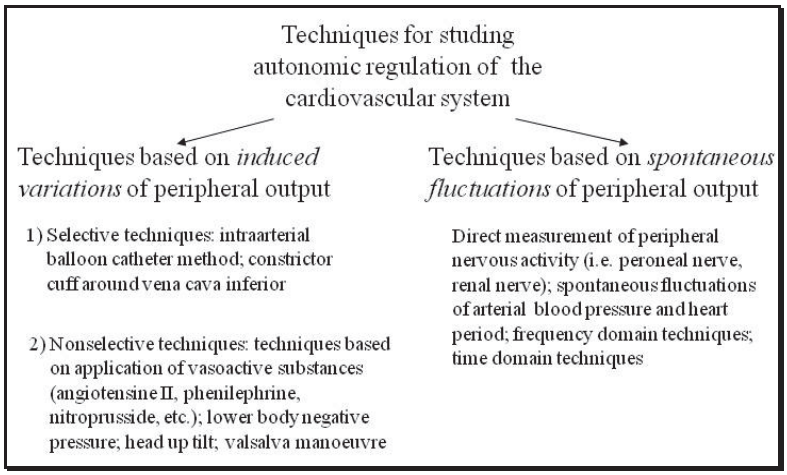

Fig. 2 – Scheme of techniques for studying the autonomic regulation of cardiovascular system

of the CV control systems to these perturbations [10]. Increase of arterial BP and HP variability after sinoaortic baroreceptor deafferentation and their decrease after following pharmacological sympathetic and parasympathetic ganglionic block [9, 11] suggest that a significant part of spontaneous, steady-state arterial BP and HP variability is due to the interaction in central and peripheral mechanisms of CV control.

Central control

There is a lack of data on the central pathways subserving "central command" responses (Figure 1). The idea of a "central command" signal originated from the observations that heart rate, ventilation and BP increase almost immediately at the onset of voluntary exercise [6]. "Central command", in the case of BP and heart rate increase at the beginning of the exercise has a clear functional implication in matching blood flow to increased metabolic rate of an organism [12].

A dramatic demonstration of "central command" as a feed-forward regulation in the absence of muscle activity is shown in the study of Gandevia et al. [13], in which a paralysed, artificially ventilated human subject attempted to perform isometric contractions. It resulted in marked concomitant increases in arterial BP and heart rate, which were graded according to the degree of the attempted force. Concomitant hypotensive and bradycardic changes central by origin are found in sleep [14] and opioid anesthesia [15]. According to our findings central command has different impact on the organism with respect to the age [16].

A number of cerebral areas appear to be involved in central control of CV function. These areas are mostly located in the frontal cortex and include parts of the cingulate and insular cortex [17], orbitofrontal cortex [18], amygdala [19], dorsomedial hypothalamic nucleus [20] and midbrain [21, 22].

Baroreflex control

Baroreflexes represent classic negative feed-back mechanisms (Figure 1). Changes in baroreceptor input to the brain provoke changes in neural output in two branches of the ANS – sympathetic and parasympathetic branches. The parasympathetic (vagal) system controls about 75% of the fastest baroreflex effector loop – heart rate, up to 100 beats/min. The sympathetic system controls the remaining 25% of this effector, and further controls conductance and contractility of the heart, and total peripheral resistance [1].

The parasympathetic system acts fast and powerfully, and can change heart rate within one beat [1]. Due to the different dynamics of neurotransmitter release, different intra-

cellular effector molecular mechanisms and different mechanisms of neurotransmitter removal from neuromuscular synaptic cleft, sympathetic nervous system act slower with respect to parasympathetic system [23].

Blood pressure set point

Baroreceptor reflex performance is modified by various mechanisms of the CNS. Though criticised for erroneous associations it might provoke between biological systems and the servo control system, the term "set point" is in use for description of the "desired level" of BP and baroreflex mechanism [24].

The set point (level around which arterial pressure is regulated) varies under different physiological (*ie* exercise) [6] and pathophysiological (*ie* hypertension) [25] conditions. A natural selection appears to favour a control system that regulates arterial pressure around a set point that varies according to an animal's behaviour [24].

Baroreflex sensitivity

Baroreflex sensitivity (BRS), or baroreflex gain is defined as a transfer function between a primary (input) change in BP and reflex (output) compensatory change in BP or heart rate [24]. From the classical studies of baroreflex functioning [26] till the recent investigations [9, 11 27–29] the open loop baroreflex studies were done in anesthetised or pharmacologically treated animals. In conscious animals and humans, it is very difficult to perform an open-loop analysis of baroreflex gain.

Methods for studing mechanisms of cardiovascular regulation-techniques

Techniques for analysis of HP and BP induced fluctuations

These methods apply external stimulus for the evaluation of baroreflex loop and perform so-called "spot" analysis of BRS [30].

Many basic laboratory techniques are applicable to experimental evaluation of selective carotide or aortic arch baroreceptors (intravascular occlusion of corresponding artery [31]. Selective unloading of cardiopulmonary baroreceptors is performed by an inflating cuff placed around *vena cava inferior*. All these methods are invasive, demanding anesthesia and applicable only to laboratory animals.

Non-selective tests of baroreceptor function stimulate whole groups of baroreceptors, without any care for their regional and functional differences.

Orthostatic tests like stand test, tilt test and lower body negative pressure (LBNP) test are widely used for clinical and scientific purposes of investigating CV regulation [32]. The head-up tilt test is the method used for investigation of syncope, presyncope, dizziness and palpitations related to dysautonomia symptoms [33]. Lately, the test has been criticized due to great variation in sensitivity and specificity rates in different studies, as well as for its limited accuracy and reproducibility [34]. In this technique baroreflex stimulus is physiological, but the specificity is limited, due to unloading of cardiopulmonary baroreceptors and stimulation of vestibular centers [35].

LBNP induces, with the depression below iliac crest, fluid shift (blood and interstitial fluids) towards the lower part of the body. LBNP stimulates CV system, in a particular a baroreflex regulation loop by unselective unload of these receptors. The LBNP test can cause syncope and progressive fluid shift can cause CV changes that are not stationary, not providing this important condition for further mathematical analysis of the signal [36].

A method for application of vasoactive substances (Oxford method) was founded by Smyth et al., [37] in 1969. It is based on intravascular injection of vasoactive substances, like angiotensin II, phenylephrine or nitroprusside. It is used as the gold standard method for BRS measuring. It is methodologically simple, more specific, but it quantifies only arterial BP-HP baroreflex loop. Vasoactive substances also act directly on the CNS structures, cardiopulmonary receptors, as well as on sinoatrial node [38].

Valsalva manoeuvre is based on tachicardic or bradicardic response on the initial decrease or increase of arterial BP appearing during constant expiratory pressure (40 mmHg) lasting for 15–20 s. It is a noninvasive, simple method, but its disadvantages are the involvement of chemoreceptors, cardiopulmonary baroreceptors, muscle receptors and it also requires active collaboration of a patient [39].

Techniques for analysis of HP and BP spontaneous fluctuations

A basic methodological advantage of these techniques is continuous measurement of BRS and higher level of sensitivity on baroreflex disfunction as compared to classical methods [30, 40, 41].

Direct measurement of sympathetic nerve activity in peroneal nerve or in renal nerve allows measurement of BRS as the responsiveness of sympathetic nervous activity to the changes of BP [42]. The sympathetic bursts are synchronized with transient reductions of BP and are silenced during increased pressure [43].

Spontaneous fluctuations in HP and arterial BP have been explored both in frequency and time domains during the last two decades. Spontaneous sequences of HP and arterial BP beat-to-beat values have been used to study different aspects of CV regulation in physiological and pathophysiological states. An important step in evaluation of CV control came from the recognition that oscillations in HP and BP result both from the operation of feed-back regulatory loops [41, 44] and from "central commands" [45–47]. In clinical use, there are different software packages, like Nevrokard®, compatible with Finapress®, Portapress®, Colin® and BIOPAC® monitors.

Frequency domain techniques

Studies on the frequency domain [10, 40, 48, 49] have provided a novel insight into the interplay of sympathetic and vagal CV modulations, leading to new tools for studying CV control. The frequency components of these fluctuations can

be assessed by spectral analysis [50] and reflect major changes in autonomic control of heart and vessels. HP power spectra depict the modulation of autonomic control on sinoatrial node, not its absolute value [51]. In many conditions, the modulation amplitude is proportional to its absolute value [48, 52]. In HP power spectra, the low-frequency band (less than 0.15 Hz in humans [50], 0.45 Hz in rats [53], 0.6 Hz in mice [54]), has been associated with the modulation of both sympathetic and parasympathetic outflow, while the high frequency band (greater than 0.15 Hz in humans [50], 1.04 Hz in rats [53], 1.0 Hz in mice [54]) has been associated with the modulation of parasympathetic outflow [55]. The contribution of sympathetic and parasympathetic efferent activity to low frequency and high frequency HP and BP power spectra, respectively, has been confirmed during wakefulness [56] and sleep [57] by experiments using selective pharmacological blockade (propranolol, atropine).

Time domain techniques

Analysis of the continuous relationship of beat-by-beat changes in arterial pressure and HP revealed that spontaneous increases or decreases in systolic arterial pressure ("ramps") induce directionally similar reflex changes in HP [58]. On this basis, a novel technique called "spontaneous baroreflex analysis" was developed for dynamic studying of the arterial baroreflex control of the sinus node [44]. This widely accepted method [45, 59–62] is based on a computer scanning of BP and HP time series to identify sequences of spontaneously occurring consecutive beats in which BP and HP change in the same direction, (named "baroreflex sequences") i.e. hypertensive/bradycardic and hypotensive / tachycardic sequences [44].

Measuring BRS from spontaneous variations in BP and heart rate [44] has several advantages over methods that artificially induce changes in BP. This method excludes administration of vasoactive compounds or external appliances that could influence the baroreceptor reflex by a direct action on receptor or effector sites [63]. BRS is measured within physiological BP ranges, allowing computation of the gain at BP close to the operating set point value, with minimal non-specific effects from other efferent nerves [40]. The baroreceptor gain thus obtained is closest to the physiological one. These methods do not arouse subjects or animals, thereby reducing stress-induced effects. In contrast to pharmacological or mechanical methods, they are suitable to assess BRS over prolonged periods of time [30, 41].

Methods that evaluate BRS from spontaneous changes in BP and HP make use of linear regression analysis of HP *vs* spontaneously occurring ramps in BP [44, 58, 64], and of spectral analysis [65] or other statistical relationships between BP and pulse interval changes [66].

BRS calculated as a slope of HP *vs* BP linear regression in spontaneously occurring pressure ramps [44] shows the best correlation to reference pharmacological methods and gives zero value following interruption of the baroreflex arch [41, 44, 60].

Apart from "baroreflex sequences", beat-to-beat analysis of the continuous relationship between spontaneous fluctuations in BP and HP also reveals the occurrence of sequences of consecutive beats in which BP and HP change in the opposite direction (*ie* hypertensive/tachycardic and hypotensive/bradycardic sequences). These sequences have been defined as "non-baroreflex" [44].

The physiological meaning and thus the possible role of non-baroreflex sequences in evaluation of central command of the CV regulation is still controversial. Oosting et al. [41,] include in BRS index calculation all BP sequences, non-baroreflex sequences as well, regardless the direction of HP changes with respect to pressure changes. The idea behind this approach is that the relationship between HP and arterial BP includes both baroreflex and random influences; if baroreflex – mediated effects on HP are present, they should appear as such when averaging over ramps is performed [41]. In addition, this technique included 49.8 ± 4.1% of all the recorded beats in BRS index calculation. Calculation also included a significant number of sequences that corresponded to non-baroreflex ones.

The main limitation of this approach is that the BRS index is mainly a measure of parasympathetic reaction, being calculated on short sequences (9.7 ± 1.6 beats, mean ± SEM) and with a delay of HP *vs* arterial BP (3, 4 and 5 beats) that is too short to take account a full sympathetic reaction to an arterial pressure change [46]. HP changes induced by vagal reactions would superimpose upon slow sympathetically induced ones [41].

Furthermore, Legramante et al. demonstrated in anesthetised rabbits [62] and humans [45] that spontaneously occurring non-baroreflex sequences can be considered an expression of autonomic regulatory mechanisms operating with feed-forward features, as it is the case of "central command". They have calculated a baroreflex gain on sequences where heart rate and BP changed in the same direction [44], while the gain of feed-forward mechanisms was calculated on non-baroreflex sequences. The same authors demonstrated that both branches of the ANS take part in feed-forward mechanisms of short-term CV neural regulation [45, 62]. Recent investigation on conscious freely moving rats [47] have provided further evidence that non-baroreflex sequences reflect mechanisms feed forward in origin. A complete autonomic pharmacological blockade reduced the number of non-baroreflex sequences, as did sympathetic blockade, selective alpha-receptor blockade did not induce changes, while beta-receptor blockade induced a significant decrease in non-baroreflex sequences occurrence. Moreover, parasympathetic blockade induced increase in non-baroreflex sequences. The results of Pagani et al. [48] demonstrate that physiological role of non-baroreflex sequences is an expression of feed-forward type of short term CV regulation being in dynamic interaction with feed-back mechanisms of baroreflex origin.

Still, the intrinsic limitation of this method in evaluating feed-forward mechanisms is a small number of beats (in animals ≈5% [62] and in humans ≈7% [45]) organised in sequences characterised by a non-baroreflex pattern. This finding is in contrast with the fact that feed-forward mechanisms can be engaged for a prominent fraction of time [6].

Zoccoli et al. [46] suggest that parallel analysis of both the BRS index of Bertinieri et al. [44] and the BRS index of Oosting et al. [41], and a novel index in time domain sensitive to slow sympathetic fluctuations would overcome: the limitations of the method of Oosting et al. [41] in estimating the relative contribution of feed-back control, and the limitations of the method of Legramante et al. [47] in estimating feed-forward control over HP and offer a more complete picture of the interrelation between peripheral and central mechanisms in HP control. The index [46] b_{HPMAP} is calculated as an index of linear regression of arterial BP *vs* HP 30s sequences. It correlates well with indexes of Bertinieri et al. [44] and Oosting et al. [41] in quiet wakefulness of the conscious rats, while in active sleep correlates significantly with the sympathovagal index. We have reported that the index b_{HPMAP} can reflect sympathetic changes in the time domain as well [46]. This data suggest that the overall picture of baroreflex-central command interaction can be achieved by comparative analysis of more than one method for calculation of BRS and feed-forward gain proposed in the literature.

Short-comment and conclusion

It is well-known that phasic and tonic increases in central drive to the heart both as impaired baroreflex regulation might increase the incidence and severity of cardiac arrhythmias [67]. An increased central drive is also present in acute stress (classic "defense" or "alerting" response [68], chronic psychological stress [69], acute physical stress [70] as well as during arousal [71], acoustic stimulation [72]). In all circumstances, the central drive and impaired baroreflex both were positively correlated to the incidence of cardiac arrhythmia in susceptible subjects. On the basis of these results, the techniques of CV monitoring keep an important place in studying pathophysiological mechanisms of arrhythmogenesis.

Acknowledgement

This work was supported by grants No. 173033 (to Esma R. Isenović) and III 41028 (Dragan Alavantić) from the Ministry of Science, Republic of Serbia. We would like to thank Prof. Carlo Franzini for his contribution in writing this text.

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Received on July 7, 2011.
Revised on September 5, 2011.
Accepted on October 11, 2011.
OnLine-first, June, 2012.