ABSTRACT

The purpose of this article is to review the physiologic mechanisms causing heart rate variability. In addition, the rationale for using low (LF) and high frequency (HF) components of heart rate variability to study cardiac sympatho-vagal balance is discussed based on these physiologic mechanisms. Heart rate variability is almost exclusively mediated by the autonomic nervous system, as indicated by the finding that heart rate is virtually constant in patients following heart transplantation. Sympathetic actions on the sinus node are mediated by the intracellular second messenger cAMP. Thus, sympathetic-mediated heart rate fluctuations require likewise fluctuations in intracellular cAMP concentration, which is dependent on the activities of adenylyl cyclase and phosphodiesterase. In contrast, parasympathetic modulation of sinus node function does not depend on intracellular second messengers and can be mediated by a direct action of M2-muscarinic receptors on acetylcholine-operated potassium channels \( K_{\text{ACH}} \)-channels. Due to these different intracellular mechanisms, heart rate can be altered faster by the parasympathetic than by the sympathetic nervous system. Thus, HF heart rate variability is exclusively mediated by the cardiac parasympathetic nervous system, while LF heart rate variability can be mediated by both branches of the autonomic nervous system. Support for this concept comes from studies employing acute perturbations of the autonomic nervous system or utilizing autonomic receptor blockers.

KEY WORDS

Sinus node, power spectral analysis, sympathetic nervous system, parasympathetic nervous system, CAMP.

INTRODUCTION

Tachycardia and bradycardia are important clinical signs of a variety of diseases, such as hyper- and hypothyroidism, heart failure, fever and hypothermia, anemia, preexcitation syndromes and others. In addition to alterations in the frequency of the heartbeats, variations in the time interval between individual heartbeats can occur in conditions such as atrial fibrillation, bradycardia-tachycardia syndrome, extrasystoles, or atrio-ventricular blocks. In contrast to the aforementioned forms of cardiac arrhythmias, heart rate variability refers to variations in the time intervals between “normal heartbeats” that originate from the sinus node\(^1\). The more variable these time intervals are, the higher the heart rate variability and the more constant these time intervals are, the lower the heart rate variability. The clinical importance of heart rate variability is documented in a continuously increasing number of studies demonstrating that reduced heart rate variability coincides with increased cardiovascular risk\(^2\), hypertension\(^3\), heart failure\(^4\), diabetes\(^5\)-\(^7\), hypercholesterolemia\(^8\) and other conditions\(^9\)-\(^12\).

Instead of focusing on clinical applications of heart rate variability, the purpose of this article is to review physiologic mechanisms that contribute to heart rate variability in healthy subjects. Knowledge of these physiologic mechanisms can provide a better understanding of the alterations in heart rate variability associated with cardiovascular diseases. Specifically, the following aspects will be addressed: (1) effects of autonomic innervation on sinus node function; (2) origin of major periodi-
cities in heart rate variability; (3) importance of time delays in autonomic signal transduction; and (4) heart rate spectral analysis as indicator of cardiac autonomic balance.

**EFFECTS OF AUTONOMIC INNERVATION ON SINUS NODE FUNCTION**

In the absence of autonomic innervation, such as after cardiac transplantation, cardiac contractions occur with the precision of a “Swiss watch” and heart rate is almost constant without fluctuations in the time intervals between successive heartbeats. To understand how autonomic innervation affects heart rate variability, one needs to consider the mechanisms that cause a constant heart rate in the absence of autonomic innervation. Figure 1 illustrates the major ionic currents contributing to the periodic generation of slow action potentials of the sino-atrial node. The inward current I_t (funny current, mainly sodium) and the decline in potassium permeability (I_K) contribute to the slow diastolic depolarization that characterizes phase 4 of the action potential. As the slow diastolic depolarization approaches a threshold potential of approximately −40 mV, voltage-operated Ca++-channels open. The resulting inward Ca^{++}-current (I_{Ca}) contributes to the last third of the slow diastolic depolarization and, finally, initiates phase 0 of the next action potential. The depolarization at the end of phase 4 and during phase 0 activates potassium channels, which allow for the outward potassium current (I_K) that contributes to repolarization following phase 0. Spontaneous inactivation of the potassium channels, causing a decline in I_K, contributes to the slow diastolic depolarization during phase 4.

More than 50 years ago, Hutter and Trautwein studied the effects of sympathetic and parasympathetic innervation on slow action potentials in the sinus node of frog hearts. Figure 2 demonstrates the major effects of electrical stimulation of cardiac parasympathetic (top) and sympathetic (bottom) efferent nerve fibers. Cardiac parasympathetic nerve stimulation (Figure 2, top) decelerated the slow diastolic depolarization (phase 4) and reduced the membrane potential at the end of the repolarization phase of the slow action potentials. In contrast, sympathetic nerve stimulation (Figure 2, bottom) accelerated the slow diastolic depolarization (phase 4) and increased the amplitude of the action potentials. The cellular mechanisms, causing these responses to cardiac sympathetic and parasympathetic nerve stimulation are illustrated in Figure 3. Norepinephrine, released from varicosities of cardiac sympathetic nerve fibers, binds to ß-adrenergic receptors that increase the activity of adenylyl cyclase via a stimulating G protein. This leads to formation of cAMP from ATP. An increase in intracellular cAMP levels increases the funny current I_t and the Ca^{++}-current I_{Ca}. The greater funny current I_t accelerates the slow diastolic depolarization during phase 4 of the slow action potential and the greater Ca^{++}-current I_{Ca} increases the amplitude. Both of these effects of the sympathetic nervous system on the slow action potentials of the sinus node cells were nicely demonstrated in the classic experiments by Hutter and Trautwein (Figure 2, bottom). Vice versa, acetylcholine, released from cardiac vagal efferent nerve terminals, binds to M2-muscarinic receptors and inhibits adenylyl cyclase via an inhibitory G protein. This decreases cAMP formation and reduces the funny current I_t and the Ca^{++}-current I_{Ca}. In addition to the inhibition of adenylyl cyclase, M2-muscarinic receptors also increase the open probability of acetylcholine-operated potassium channels (K_{ACh}-channels). This increases the outward potassium current I_K, leading to a greater hyperpolarization during the repolarization phase of the slow action potentials of the sinus node cells. Thus, the slow diastolic depolarization during phase 4 starts at a more hyperpolarized (more negative) membrane potential and it takes longer until the threshold for the next action potential is reached. Again, the experiments by Hutter and Trautwein (Figure 2, top) nicely demonstrate these actions of parasympathetic innervation of the sinus node.
The mechanical theory is based on the consideration that ventilatory fluctuations in intrathoracic pressure cause likewise fluctuations in venous return to the heart, cardiac filling, and stroke volume. This elicits similar oscillations in cardiac output and arterial blood pressure. The respiratory fluctuations in blood pressure would elicit the baroreceptor reflex that generates respiratory fluctuations in heart rate by modulating cardiac sympathetic nervous system activity, respectively. A similar circadian rhythm is likely to exist for cardiac sympathetic nerve activity and may therefore contribute to circadian fluctuations in heart rate.

Respiratory sinus arrhythmia: Another obvious periodicity in heart rate is respiratory sinus arrhythmia that is characterized by tachycardia during inspiration and bradycardia during expiration (Figure 4). It has been suggested that respiratory sinus arrhythmia is mainly mediated mechanically by periodic fluctuations in cardiac filling secondary to ventilatory changes in venous return to the heart. In contrast, it has also been suggested that respiratory sinus arrhythmia is the result of entrainment of central nervous system oscillators driving respiration and autonomic nervous system activity, respectively.

The intracellular mechanisms of autonomic control of sinus node function require synthesis of the second messenger cAMP via the sympathetic nervous system. Norepinephrine (NE) binds to $\beta_1$-adrenergic receptors, which increases the activity of adenyl-cyclase via a stimulating G protein (GS). This causes synthesis of cAMP, which increases the funny current $I_f$ and the calcium current $I_{Ca}$. Acetylcholine (Ach) binds to M2-muscarinic receptors. This activates a G protein, which increases to open probability of acetylcholine-dependent potassium channels (KACh), leading to an increase in the hyperpolarizing potassium current $I_K$. M2-muscarinic receptors also inhibit adenyl-cyclase via an inhibitory G protein (Gi) and, therefore, antagonize sympathetic, $\beta_1$-adrenergic receptor-mediated effects on $I_f$ and $I_{Ca}$. It is important to note that sympathetic actions on the sinus node require the synthesis of the 2nd messenger cAMP; whereas parasympathetic control of sinus node function does not require synthesis of 2nd messengers.
Thus, even...

In his review, he points...

...via the Bainbridge reflex. Fluctuations in cardiac filling can also modulate cardiac autonomic and parasympathetic nerve activity. In addition, ventilation-related fluctuations in cardiac filling can also modulate cardiac autonomic nervous system activity via the Bainbridge reflex.

The central oscillator theory goes back to the work of Barman and Gebber, who demonstrated in anesthetized, vagotomized, paralyzed, and artificially ventilated cats that cardiac sympathetic nerve activity continues to discharge at a frequency similar to the respiratory frequency even when central respiratory activity was completely blocked by hyperventilation. Mechanical effects of ventilation on venous return to the heart and on cardiac filling were prevented in these experiments by pneumothoracotomy. Thus, it was concluded that a central nervous system oscillator driving cardiac sympathetic nerve activity exists, that is independent from the respiratory oscillator. The intrinsic frequency of this oscillator is close to the respiratory frequency. However, under physiologic conditions, the central sympathetic oscillator is entrained to the central respiratory oscillator. The central respiratory oscillator also modulates cardiac parasympathetic nerve activity. Rentero et al. demonstrated in anesthetized, paralyzed, and artificially ventilated rats that cardiac vagal motoneurons in the nucleus ambiguous exhibit a respiratory-related discharge pattern, that persists even if the ventilator is switched off. These experiments demonstrated that the central respiratory oscillator modulates the activity of cardiac vagal motoneurons in the nucleus ambiguous. Thus, under physiologic conditions, the central respiratory oscillator can modulate cardiac sympathetic and parasympathetic nerve activity independent from afferent respiratory signals originating in the periphery. This conclusion is in line with the observation that respiratory sinus arrhythmia persists during breath hold.

**Figure 4.** Strong respiratory sinus arrhythmia (high frequency heart rate variability) in a young healthy female subject. Top: ECG, middle: respiration (Resp), bottom: heart rate (HR). Note the increase in heart rate during inspiration and the decrease in heart rate during expiration.

...and parasympathetic nerve activity. In addition, ventilation-related fluctuations in cardiac filling can also modulate cardiac autonomic nervous system activity via the Bainbridge reflex.

Physiologic mechanisms of heart rate variability

**LOW FREQUENCY PERIODICITIES**

In addition to periodicities that are directly related to obvious physiologic phenomena, such as respiration, heart rate variability also contains low frequency periodic fluctuations that are not related to any obvious physiologic phenomenon. The most prominent low frequency oscillation in heart rate is characterized by a wavelength (period duration) of 10 s in humans. An example is shown in Figure 5. It has been postulated that these oscillations in heart rate originate from a pacemaker located in the central nervous system that generates sympathetic nerve activity patterns with a periodicity of 10 s. This postulate is derived from studies demonstrating that low frequency oscillations in sympathetic nerve activity can be observed in the absence of sensory inputs from the periphery by and by other studies, showing that low frequency oscillations can be present in the heart rate signal even if no low frequency oscillations are present in the blood pressure signal. This pacemaker theory has been reviewed recently by Claude Julien. In his review, he points out that the frequencies of central nervous system oscillators identified in most studies are not consistent with the frequency range of low frequency blood pressure variability. Thus, even so the pacemaker theory can explain some of the phenomena related to low frequency heart rate variability, it should still be considered a matter of debate. Another hypothesis that may explain the occurrence of low frequency cardiovascular variability is the so-called “baroreflex theory”. This theory implies that the arterial baroreceptor reflex exhibits a resonance frequency within the low frequency range of blood pressure variability. This low frequency blood pressure variability would then be introduced to heart rate via the autonomic nervous system that gets synchronized with this resonance frequency via afferent nerve fibers originating from the baroreceptors. This theory is indeed well-supported by experimental data in rats, demonstrating that the frequency response of arterial blood pressure to periodic stimulations of the aortic depressor nerve exhibits a resonance frequency within the frequency range of low frequency blood pressure variability.

**OTHER FLUCTUATIONS**

Since afferent nerve fibers originating from the baroreceptors modulate autonomic nervous system activity, all fluctuations...
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in arterial blood pressure may cause likewise fluctuations in heart rate. Based on these considerations, it is obvious, that all sources of blood pressure variability can also be sources of heart rate variability. Different blood pressure control systems that can elicit blood pressure variability have been reviewed recently\(^4\). Although it has been demonstrated in rats that the renin-angiotensin system can elicit very low frequency (< 0.2 Hz) blood pressure variability\(^3\), its impact on blood pressure variability in humans is still unknown\(^5\). In rats, blood flow autoregulation by myogenic vascular function can cause very low frequency (<0.2 Hz) blood pressure variability\(^6\). Studies on the dynamic properties of cerebral blood flow autoregulation in humans\(^7\) suggest that the frequency range of blood pressure variability that is affected by myogenic vascular function in humans is located below ~0.15 Hz and thus, includes the low frequency and very low frequency components. Especially, the study by O’Leary et al\(^7\) is of importance, because these investigators performed transfer function analysis between mean blood pressure and total peripheral resistance. The gain of this transfer function can be seen as an index of myogenic vascular function of the whole circulation. At frequencies below 0.2 Hz, changes in mean blood pressure were followed by a directionally similar change in total peripheral resistance as would be expected from myogenic vascular function\(^7\). Finally, in rats and mice, the endothelial-derived nitric oxide system affects very low frequency (0.02-0.2 Hz) and low frequency (0.2-0.6 Hz) blood pressure variability\(^8\). However, it has been questioned if blood pressure variability is affected by endothelial-derived nitric oxide in humans\(^9,10\). Because blood pressure variability can be translated into heart rate variability via the baroreceptor reflex, one may hypothesize that the blood pressure regulating systems discussed above also affect heart rate variability. However, systematic studies investigating this possibility are currently lacking.

**IMPORTANCE OF TIME DELAYS IN AUTONOMIC SINGAL TRANSDUCTION**

Because heart rate in patients with cardiac transplantation is almost constant and heart rate variability is practically lost, one can conclude that the autonomic nervous system is the single most important factor mediating heart rate variability. Figure 3 demonstrates that a major difference in sympathetic and parasympathetic modulation of sinus node function is that sympathetic effects depend on formation of the second messenger cAMP, whereas parasympathetic effects do not require formation of any second messenger. M2-muscarinic receptors can directly change the hyperpolarizing potassium current \(I_{\text{K,HP}}\) by acting on acetylcholine-dependent potassium channels (\(K_{\text{AcCh}}\)). Thus, sympathetic-mediated heart rate fluctuations can only occur, if intracellular cAMP concentrations fluctuate at the same rate. This in turn, depends on formation of cAMP by adenylyl cyclase and its breakdown by phosphodiesterase. Adenylyl cyclase and phosphodiesterase impose considerable time delays on the initiation and termination of sympathetic actions on the sinus node. As a result, sympathetic-mediated fluctuations in heart rate are slow, whereas parasympathetic-mediated heart rate variability can occur at a much faster rate. Experimental evidence for this assumption comes from a study, in which the hypothalamic paraventricular nucleus, from which direct pathways project to the nucleus ambiguous (cardiac parasympathetic origin) and to the intermediolateral cell column of the spinal cord (origin of the peripheral sympathetic nervous system), was electrically stimulated at different frequencies in conscious rats\(^11\). This stimulation procedure elicited simultaneous sympathetic and parasympathetic activation. During \(\alpha\)-adrenergic receptor blockade (sympathetic signal transduction blocked, parasympathetic signal transduction intact), all stimulation frequencies (up to 2.0 Hz) were translated into corresponding heart rate oscillations. However, during muscarinic receptor blockade (parasympathetic signal transduction blocked, sympathetic signal transduction intact) stimulation-induced heart rate oscillations were markedly reduced at stimulation frequencies above 0.2 Hz\(^12\). These findings are in line with the assumption that sympathetic modulation of sinus node function is slower than parasympathetic modulation because sympathetic modulation of sinus node function is delayed by synthesis and breakdown of cAMP.

![Figure 5. Pronounced low frequency heart rate variability in a middle-age male subject. From top to bottom: ECG, respiration (Resp), mean arterial blood pressure (MAP), and heart rate (HR). Note the prominent heart rate fluctuation with a period duration of 10-15 seconds. These heart rate fluctuations are not related to respiration (shown in the second panel).](image-url)
HEART RATE SPECTRAL ANALYSIS AS INDICATOR OF CARDIAC AUTONOMIC BALANCE

Due to the different response times for sympathetic and parasympathetic control of sinus node function, it is not surprising, that attempts have been made to use heart rate variability to assess “cardiac sympathetic and parasympathetic nerve activity”. The idea is that high frequency (respiration related) heart rate variability reflects “cardiac parasympathetic nerve activity” and low frequency heart rate variability represents “cardiac sympathetic and parasympathetic nerve activity”. However, absolute changes in heart rate, resulting from autonomic activity directed to the sinus node, also depend on cardiac autonomic responsiveness. This includes receptor density, density of ion channels regulating the membrane potential of the sinus node cells, intracellular Ca\(^{++}\) handling, and others. Therefore, it is generally accepted that heart rate variability cannot serve as a surrogate measure for “cardiac autonomic nerve activity”. However, heart rate variability can be very useful to study acute perturbations of cardiac autonomic activity, such as during orthostatic tilt\(^{52}\). The technique usually employed with this regard is called power spectral analysis. Mathematical algorithms (fast Fourier transform, autoregressive modeling etc.) can be used to generate 2-dimensional diagrams (power spectra) with the frequency of the oscillatory components of heart rate variability on the x-axis and a measure for the amplitude of the oscillatory components (so-called spectral power) plotted on the y-axis. Using this technique, it is possible to determine the spectral power (a measure for the amplitude) of the low frequency and high frequency components of heart rate variability. An acute increase in cardiac sympathetic nerve activity, such as in response to mental stress\(^{59}\), is likely to increase low frequency spectral power, but may not affect high frequency spectral power, because the heart rate response to sympathetic activity is too sluggish to respond at high frequencies. High frequency spectral power may however increase during cardiac parasympathetic activation, such as postprandial after a meal\(^{64}\), because parasympathetic control of heart rate can occur relatively fast.

Heart rate responses to autonomic inputs depend on cardiac autonomic responsiveness, which can change in response to chronically altered cardiac autonomic tone. Therefore, heart rate spectral analysis cannot be used to assess chronic alterations in sympathetic or parasympathetic nervous system activity. For example, reduced heart rate variability in patients with heart failure\(^{67}\) may not be due to alterations in cardiac autonomic activity but to reduced autonomic responsiveness of the failing heart. Thus, the current view is that heart rate spectral analysis can assess cardiac autonomic balance\(^{52,53,55}\), rather than “cardiac autonomic tone”\(^{56}\). Evidence for this view comes from experiments were autonomic balance was disturbed by interventions, such as orthostatic tilt\(^{57}\), nitroglycerin-induced hypotension\(^{57}\), coronary artery occlusion\(^{58}\), mental arithmetic stress\(^{58}\), or exercise\(^{65}\). All these acute interventions, that are known to increase sympathetic nerve activity, also increase low frequency heart rate variability. Thus, acute perturbations of sympathetic nervous system activity are indeed reflected in alterations in low frequency heart rate variability.

This concept is further supported by experiments using pharmacological interventions\(^{59}\). Ganglionic blockade (trimethaphan), that eliminates sympathetic and parasympathetic outflow to the periphery, has been demonstrated to significantly reduce low frequency and high frequency heart rate variability in humans\(^{59}\). Similarly, muscarinic receptor blockade (atropine) almost abolishes low frequency and high frequency spectral power of heart rate\(^{59,60,61}\), indicating that cardiac parasympathetic nerve activity plays a major role in modulating low and high frequency components of heart rate variability in humans.

However, it is important to point out, that the effects of autonomic receptor blockers depend on the baseline level of autonomic nervous system activity. For example, \(\alpha\)-adrenergic receptor blockade (propranolol) did not elicit significant effects on low or high frequency spectral power of heart rate in subjects resting in the supine position\(^{61}\), because baseline sympathetic nerve activity is low under these conditions. However, if the beta blocker was administered in the standing position, when sympathetic activity is elevated in response to the orthostatic challenge, a significant reduction in low frequency, but no change in high frequency spectral power of heart rate was observed\(^{61}\). Again, this finding indicates that sympathetic control of sinus node function is slow and only affects the low frequency components of heart rate variability.

Exercise training can have pronounced effects on autonomic nervous system activity. Endurance-trained athletes typically have lower resting heart rates owing to increased cardiac parasympathetic and reduced sympathetic nervous system activity. The effect of these exercise-induced alterations in autonomic nervous system activity has been studied in marathon runners, living and training in the Andeans at an altitude of 4220 m\(^{62}\). Resting heart rate in these athletes was in the range of 55-60 bpm compared to 70-75 bpm in a sedentary control group living at the same altitude, indicating high resting parasympathetic tone in the trained subjects. While low frequency heart rate variability was not different between sedentary and trained subjects, high frequency spectral power of heart rate was almost 5 times greater in trained subjects than in the sedentary control group. As a result, resting total heart rate variability (expressed as variance of the heart rate time series) was doubled in the trained subjects. Six to eight hours after a marathon competition,
absolute spectral power of low and high frequency heart rate variability were not different than before the race. However, total heart rate variability was markedly reduced 6-8 h after the marathon. Therefore, it may be more insightful to express spectral powers relative to total heart rate variability instead of absolute units. If expressed in relative units low frequency heart rate variability was significantly elevated 6-8 h after the marathon competition and returned to baseline levels 20-24 h after the race. Similarly, 6-8 h after the marathon the ratio of low frequency to high frequency spectral power (LF/HF ratio) was 6 times higher than before the race and returned to baseline levels within 20-24 h. The authors interpreted these results as a temporary “sympathetic predominance” following the marathon competition62. However, it needs to be pointed out, that the correct interpretation of parameters indirectly derived from heart rate variability, such as relative spectral powers and LF/HF ratio, is still a matter of debate63,65.

CONCLUSION

Heart rate variability is defined as variations in normal RR-intervals, defined as time intervals between adjacent QRS complexes resulting from sinus node depolarizations1 and should not be confused with cardiac arrhythmias. These variations in normal RR-intervals are almost exclusively due to modulation of sinus node function by cardiac autonomic nervous system activity. Due to the cellular mechanisms involved in sympathetic and parasympathetic signal transduction, the sinus node responds faster to parasympathetic than to sympathetic inputs. Therefore, fast fluctuations in heart rate (at the frequency of respiration) are exclusively mediated by the parasympathetic nervous system, whereas both branches of the autonomic nervous system can elicit slower fluctuations (~0.1 Hz) in heart rate. Based on these concepts, low frequency (LF) and high frequency (HF) components of heart rate variability as well as the LF/HF ratio are frequently used to assess cardiac “sympatho-vagal balance”. It is important to consider that parameters derived from heart rate variability depend on (1) central nervous system processing of afferent inputs from the periphery; (2) autonomic outflow from the central nervous system; (3) peripheral nerve conduction; (4) autonomic receptors; (5) intracellular signal transduction; and (6) end-organ responsiveness. Thus, alterations in heart rate variability in disease states, with ageing, in response to exercise training, or in other conditions can be mediated by any combination of these factors.

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