Arteriovenous oscillations of the redox potential: Is the redox state influencing blood flow?

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Objective: Studies on the regulation of human blood flow revealed several modes of oscillations with frequencies ranging from 0.005 to 1 Hz. Several mechanisms were proposed that might influence these oscillations, such as the activity of vascular endothelium, the neurogenic activity of vessel wall, the intrinsic activity of vascular smooth muscle, respiration, and heartbeat. These studies relied typically on non-invasive techniques, for example, laser Doppler flowmetry. Oscillations of biochemical markers were rarely coupled to blood flow.

Methods: The redox potential difference between the artery and the vein was measured by platinum electrodes placed in the parallel homonymous femoral artery and the femoral vein of ventilated anesthetized pigs.

Results: Continuous measurement at 5 Hz sampling rate using a digital nanovoltmeter revealed fluctuating signals with three basic modes of oscillations: ~ 1, ~ 0.1 and ~ 0.01 Hz. These signals clearly overlap with reported modes of oscillations in blood flow, suggesting coupling of the redox potential and blood flow.

Discussion: The amplitude of the oscillations associated with heart action was significantly smaller than for the other two modes, despite the fact that heart action has the greatest influence on blood flow. This finding suggests that redox potential in blood might be not a derivative but either a mediator or an effector of the blood flow control system.

Keywords: blood flow, arteriovenous potential, EMF, electromotive force, oscillations, redox state

Introduction

The blood flow control system manifests itself in rhythmic activities [1] that have been frequently studied to understand the mechanisms of cardiovascular control. Several distinct frequencies have been identified, such as metabolic (0.008–0.02 Hz), neurogenic (0.02–0.05 Hz), myogenic (0.05–0.15 Hz), respiratory (0.15–0.4 Hz), and cardiac (0.4–2.0 Hz) [2–4]. The wavelet-based method of analysis of blood flow oscillations has been adopted for further research on cardiovascular control under various conditions [5–8]. However, we still do not understand the explicit physiologic meanings of these characteristic frequencies, and the underlying mechanisms require further validation at a biochemical level. The main issue is that oscillations in blood apply not only to blood flow or respiratory gases but also to blood pressure (so called Mayer waves), pH [9], or blood volume [10], not to mention the various oscillations that appear in pathological states.

The Fick principles stem from an idea that arteriovenous concentration differences can be used to determine the state of metabolism [11]. As the electrochemical potential measured between blood in the artery and vein was assumed to be an effect of pH and the partial pressures of O_2 and H_2, platinum electrodes with the aid of injection or inhalation of hydrogen were used to detect intravascular shunts [12]. As the arteriovenous concentrations of metabolites were extensively studied [13–15], the researchers' attention shifted to measurements of certain blood components (such as pH or pCO_2) with specialized electrodes. In particular, the arteriovenous CO_2 difference was frequently measured – it was assessed as a marker of, among others, hemodynamic status [16], septic shock [17], regional ischemic or hypoxic hypoxia [18]. Recently, arteriovenous differences of blood gases were supplemented by markers of anaerobic metabolism (such as lactate) and outperformed other methods in the assessment of the hemodynamic state of patients with circulatory failure [19]. Only recently, Ivanisevic and coworkers systematically assessed arteriovenous blood metabolomics via targeted and untargeted mass spectrometry, revealing a wide spectrum of changes and concluding that this type of analysis might provide a new means of identifying possible metabolic pathway disruptions [20].

While it is common to consider redox pairs as a major driving force regulating biological events [21–23], biochemical constraints related to enzyme availability often make them

indicators, not regulatory forces [24,25]. All of the factors mentioned above (hemodynamic status, septic shock, and hypoxia) as well as many others are collectively represented by the state of blood redox components. A direct influence of certain redox pairs has been shown for blood metabolism [26] and for blood flow [27]. All of the above suggests that arteriovenous redox potential difference must be coupled to blood flow, at least at the non-neurogenic level. The difference in redox potential between arterial and venous blood was assessed several times. The redox potential was either measured directly [28,29] or assessed using concentrations of lactate/pyruvate couple [30]. In addition, the redox potential of arterial and venous blood was measured simultaneously with the same reference electrode [31]. In all cases, the measured values were in the range of +/- 200 mV. However, given that the electrode preparation protocol strongly influences the results of redox potential measurements of blood [32], it is likely that all of these values bear a significant error. None of the studies mentioned above used the arteriovenous redox potential difference to assess blood flow.

In this study, we monitored the physiological and induced changes in the arteriovenous potential difference on the timescales of a second to dozens of minutes using 1-5 Hz sampling rates. Oscillations of different frequencies were identified in the measured signal, with three clear major modes most probably stemming from heart action, breathing and possibly metabolism – similar to studies on blood flow oscillations. The amplitudes of the oscillations with the highest frequency (cardiac) were much lower than the other oscillations. This observation suggests a presence of complex relationships between the redox status of the blood and the blood flow; as such, it opens a new avenue for research on cardiovascular control mechanisms.

Materials and Methods

Animal preparation and placement of electrodes

The study protocol was approved by the Local Ethics Committee at Warsaw University of Life Sciences in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The studies were performed on six polish landrace sows (60-95 kg body weight). During the entire study, the sows were healthy. Intramuscular injection with Azaperone (Stresnil, 3 mg/kg b.wt., i.m., Janssen Pharmaceutica, Belgium) was administered as the sedative management at the beginning. Afterwards, the combination of Medetomidine (2 μg/kg ScanVet, Poland), Butorphanol (Butomidor 0.025 mg/kg b.wt., i.m.,

Richter Pharma AG) and Ketamine (Ketamina 10%, 20 mg/kg b.wt., i.v., Pharmanovo GmbH, Germany) was used as the preanesthetic. The induction of general anesthesia was performed with intravenous injection with propofol (3 mg/kg b.wt., i.v., Fresenius Kabi, Germany). The endotracheal intubation was made, allowing for the maintenance of the general anesthesia with isoflurane (Aerrane 5-2 ‰, Baxter) under the control ventilation (IPPV). The animals were sacrificed with pentobarbital (Morbital 0.5 ml/kg Biowet Pulawy, Poland) at the end of the research procedure.

Arterial and femoral sheaths (4F) were placed bilaterally into the external iliac artery and the vein. Two standard diagnostic catheters (4F fixed curve, 4 – pole diagnostic catheters with 2,2,2 or 5,5,5 electrode spacing, St. Jude Medical, MN, USA) were placed in the parallel femoral artery and the femoral vein with x-ray control (Fig. 1).

The maintenance of general anesthesia was performed with isoflurane (1,5 vol. %). The signal was stabilized typically after 2 minutes from the probes via intra-arterial insertion. The data were collected 5 times per second and 1 time per second, while the respiration rate was maintained at either 8 times per minute or 12 times per minute. Due to the limitations of data acquisition equipment, a lower sampling rate was required for simultaneous monitoring of two or more voltaic channels.. The blood oxygen saturation level was 100% throughout all the experiments to rule out the possibility that we measure saturation oscillations caused by hypoxic conditions [33,34].

Signal acquisition

The dedicated system was set up on the basis of Agilent 34970A Data Acquisition/Switch unit equipped with 34901A 20-channel multiplexer module, using either Agilent BenchLink Data Logger 3 software (tested by us to be sufficient for 4 galvanically separated channels with a 1 Hz sampling rate) or a built in-house application that enabled the sampling rate of 5 Hz for a single channel. Both programs enabled an on-line graphical interface. Agilent company guaranties 6½ resolution with 0.004% accuracy and a thermal drift smaller than 3 μV/deg outside the reference temperature range of 18-28 ºC (291-301 K). Altogether, the above indicates that eventual biases should not exceed 50 μV. The partial oxygen concentration was in parallel monitored with the aid of a MOX-4 Gas Sensor (MediceL®) attached to the subsequent channel of the multiplexer.

The differences in the arteriovenous potential were monitored using pairs of sensors located in parallel (see Fig. 1A). In all the setups, the terminal pairs were found not applicable due to the resulting extremely high noise level, probably due to vibrations from the terminal parts of the probes caused by irregular blood flow.

Data analysis

The signals were analyzed using R language and its procedures for signal analysis (package *stats*), including cross-correlation analyses. First of all, linear trends were removed from the data prior to analysis using the "detrend" function from the package *pracma*. Analysis of oscillations were done using two methods, Lomb-Scargle periodogram and wavelet power spectrum.

In the first case, we have proceeded under the assumption that data are not sampled evenly (standard deviation during 5-Hz sampling was 0.0005 s). Therefore the assessment of statistical significance of observed oscillations was performed using the Lomb-Scargle periodogram for unevenly sampled data implemented in the *lomb* package. Oscillations were judged to be statistically significant if their p-value was lower than 0.01.

In addition, we analyzed the data using the *WaveletComp* package to obtain the wavelet spectrum of the signal. Data were spread evenly using "approx." function implemented in R *stats* library. Computations of wavelet spectrum were done using 100 simulations (default of *WaveletComp* package) with surrogate time series generated with while noise model. On all wavelet plots contour lines delineate areas for which p-value was lower than 0.1. Coherence analysis was done using the same package. Arrows indicate areas with p-value lower than 0.05.

Results

Single channel monitoring

Electrochemical potential difference between arterial and venous blood was initially monitored for 30 minutes at a 5-Hz sampling rate but was restricted to a single channel. During the experiment, the respiration rate was precisely controlled at 8 breaths per minute in two 10-minute blocks. During the central 10-minute period (10-20), the respiration was increased up to 12 breaths per minute. Oscillations of the same frequency representing the actual respiratory rate were observed in both cases (see Fig. 2, panels A and B). The increase

in the respiratory rate resulted in a decrease in the amplitude of the EMF oscillations: from ca. 1 mV at 8 bpm to ca. 0.5 mV at 12 bpm. Cross-correlation analyses of the breathing signal and the measured potential revealed a significant correlation of the two, with breathing leading by ca. 1.2 s to the redox potential in blood. In addition, the interference of respiratory-related oscillations with higher frequency oscillations (~ 1 Hz, 50 μV amplitude) could have been easily resolved. The latter effect should be clearly related to cardiac actions (observed at 60-80 beats per minute).

Wavelet analysis confirmed the existence of the dominant EMF oscillation of the frequency consistent with the respiration rate (Fig. 2C). The observed high-frequency modulations could also be identified as a band of signals located at periods 0.3-1, although these were not judged as statistically significant using this method. These values are consistent with the heart beat rates monitored during the experiment. Variations in the EMF induced by the heart beat were assessed by correction for respiratory oscillations by removing from the data the regular sinusoidal signal. The resulting variability in amplitude and frequency is shown in blue in Fig. 3. The unambiguous evidence of fast low-amplitude oscillations (attributed to the effect of cardiac action) was supported by statistical analysis using the Lomb-Scargle periodogram method.

The third putative region revealed slow oscillations, the frequency of which could be estimated in the range 0.5-1 min^{-1} (Fig. 2C), which was also judged to be statistically significant in the periodogram analysis. However, often, the frequency of these oscillations was not sharply defined.

Multiple channel monitoring

In the succeeding experiment, for a comparison, we in parallel monitored four signals at a 1-Hz sampling rate. Respiration-induced oscillations were again observed at exactly the same frequency as the respiratory rate, and the amplitudes were approximately 1 mV, as observed earlier. The phase of these oscillations was clearly synchronized with respiratory action: the rise of the signal indicated exhalation, while the drop indicated inhalation. The signal appeared to be aligned with the O_2 oscillations in breathing air (Fig. 4A). Using cross-correlation analysis, we found that in all four pairs, the measured signal had a similar course, although it appears that either the electrodes had a different sensitivity or the location (e.g., touching the vessel wall) influenced the signal because there were substantial differences in its absolute values (Fig. 1 A). Wavelet coherence analysis of signals from electrodes and from

oxygen sensor indicate high coherence between the two, with the measured signal almost consistently lagging across the timeframe of the measurement by roughly one-fourth of the period (Fig. 4B). This value is consistent with the results from the cross-correlation analyses. A presence of areas of statistically significant coherence between the breathing and the redox potential at higher periods (Fig. 4B) requires further investigation, since these frequencies are not always identified in the wavelet power spectrum of the oxygenation signal.

Spectral density analyses again clearly evidenced respiration-related oscillations albeit slow changes of the frequency 0.3-0.4 min^{-1}, and their first harmonics at 0.7 min^{-1} were also found to be statistically significant. Lomb-Scargle periodogram analyses again clearly evidenced cardiac oscillations, respiration-related oscillations, and their putative harmonics (Fig. 3 B).

Signal variability

Placement of the electrodes and electrode exhaustion played a substantial role in the quality of the acquired signal. As long as the signal was still in the range of tens of mV, the oscillatory properties were clearly visible in all the animals. Small shifts (on the order of millimeters) in the placement could and indeed did dramatically worsen the quality of the signal. In addition, electrodes were exhausted to the point that almost no signal was acquired on the order of hours. Due to these issues, comparative analysis of the data was not possible because we did not acquire data in the same physiological stage for any animal.

Discussion

Here, we report the analysis of oscillatory modes in the arteriovenous redox potential of blood. The three modes of observed oscillations agree quite well with the three major peak oscillations of blood flow (see [35] and references therein) – respiratory frequency, heart beat frequency and several floating slow modes of approximately 0.05 Hz. The same frequencies were found in blood pressure, heart-rate variability, or peripheral blood flow (although the latter exhibits the most complex low-frequency component).

Cardiac action and respiratory action result in changes in the flow of the blood. Periodic changes in the velocity of blood flowing around the electrodes in arteries can reach 10-fold but typically are approximately 5-fold [36]. Such changes are reflected in the measurement, as reactions on the electrodes will be influenced by oscillatory changes of the environment

around the electrode. No biochemical process in the blood has sufficient speed to be responsible for the changes in the signal.

Respiratory oscillations of the measured signal are supplemented by the large arterial oscillations of oxygen partial pressure that naturally occur in blood due to breathing. The characteristics of the response of the signal to the ventilation period is similar to previously recorded measurements of pH oscillations caused by breathing in arterial blood [37,38] and in medullary extracellular fluid [39]. Coherence between the measured signal and the oxygenation levels observed in our experiments does not seem to be surprising, given the location of the electrodes. The coherence between skin blood flow and oxygen saturation in limbs has been already observed [40]. However that study failed to observe the coherence where the measurements were from deeper tissues of arms and legs. The most plausible explanation is the complexity of the tissue structure and, resulting from that, very different power spectra of oxygen saturation. In this study, where isolated vessels were studied, the signal was less prone to surrounding noise of the tissues. As for the source at biochemical level, the putative candidate for this redox reaction is any pH-dependent one, such as in the NAD^+-NADH couple.

The oscillations with the longest period (ca. 100 s, frequency of ~ 0.01 Hz) are difficult to interpret directly from metabolic processes or from oxygen partial pressure. It has been already suggested that these are metabolic-related frequencies [2]. Further studies on the influence of vasodilators on blood flow pointed to an endothelium-mediated mechanism [3]. However, these studies were focused on peripheral blood flow, where endothelium-based vasodilation has a substantial effect on blood flow. In major vessels, endothelium-based vasodilation is not likely to have significant effect on blood flow. In the search of the mechanisms, we reviewed the knowledge regarding erythrocyte metabolism. Hald and coworkers have built a model of metabolic entrainment of erythrocytes based on oscillatory changes in the partial pressures of oxygen and carbon dioxide due to travel between arterial and venous blood [41]. This model predicts an oscillatory overshoot of the central metabolites of erythrocyte's glycolysis, with a period comparable to the circulation time. Spikes in the lactate flux that presumably stem from the overshooting phenomena of erythrocyte glycolysis have a period that is in agreement with the observed oscillations. The lack of a well-defined, narrow signal is easily explainable by different circulatory travel times of red blood cells. Lactate oscillations of a similar period (few minutes) have been shown for cerebral spinal fluid [42]; unfortunately, no experimental confirmation for blood exists yet. Further support for

this hypothesis stems from results of Ivanisevic and coworkers [20], as they have shown that lactate levels are significantly different between arterial and venous blood. However, further studies on dynamics of the lactate metabolism should be performed.

As shown, the oscillations in the redox potential differences between arterial and venous blood are explainable by known processes in the circulatory system. Given the larger data collection times, we would probably observe other oscillations, such as insulin spikes or circadian clock parameters. However, such observation would require a novel experimental setup, as our experiments clearly have shown the limits of standard platinum catheters.

Obviously, our work again raises the question of whether the absolute values of that potential are also meaningful and interpretable. Despite numerous attempts at such an interpretation [13,16,28,29,31], at this point, we support the general premise of the Flohe essay on the GSSG-GSH couple [24] that redox potential is more of an analytical tool, i.e., an indicator, and not the direct cause of the changes in the system. To have a regulatory role, several redox couples should be allowed to reach equilibrium, which is often not the case. However, further studies are required to clarify the putative link between blood flow and oscillations of biochemical markers in blood.

Acknowledgements

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Figure captions

Fig. 1. The placement of electrodes and alignment of their signal. A: Arrows indicate which pair of electrodes were used for measurement in the majority of experiments. Four pairs were used for multiple channel monitoring. B: Alignment of the signal for the experiments with multiple channel monitoring.

Fig. 2. Agreement between oscillations in redox potential and breathing. Fragment of the oscillations for two breathing frequencies, 8 per minute (panel A) and 12 per minute (panel B). The scale is preserved – faster breathing resulted in visibly lower amplitude of oscillations. Panel C shows the wavelet analysis of the measured signal. The change in period of the observed oscillations due to change in the breathing frequency is clearly visible. Heart beat oscillations are seen as a wide band in the period range of 0.3-1. Slow oscillations are visible, albeit not statistically significant, in this measured window.

Fig. 3. A: Lomb-Scargle periodogram – dashed line denotes threshold of statistical significance (p-value of 0.05). Oscillations with normalized power above dashed line are statistically significant. B: Observed signal (red), dominating the sinusoid contribution (black) and the difference between these two (blue) that represents oscillations induced by the cardiac action.

Fig. 4. A: Alignment between oxygen levels in breathing air (upper panel) and the redox potential difference between arterial and venous blood (lower panel). B: Wavelet coherence analysis of oxygenation signal and redox potential. Areas delineated by white contour are areas with significance level of 0.1. Arrows indicate phase difference and are placed in areas of p-value of 0.05 or lower.