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Functional EPR imaging of ischemic rat heart: monitoring of tissue oxygenation and pH

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Abstract

Purpose—EPR imaging in spectral-spatial domain with application of soluble paramagnetic probes provides an opportunity for spatially-resolved functional measurements on living objects. The main purpose of this work was development of EPR methods for visualization of oxygenation and acidosis of ischemic myocardium.

Methods—EPR oxygen measurements were performed using isotopically substituted ²H, ¹⁵N-dicarboxyproxyl. The radical has EPR linewidth of 320 mG and oxygen-induced line broadening of 0.53 mG/mmHg providing oxygen sensitivity down to 5 μM. pH measurements were performed using previously developed pH-sensitive imidazoline nitroxide. The radical has EPR spectrum with pH-dependable hyperfine splitting, pK = 6.6, providing pH sensitivity of about 0.05 units in physiological range.

Results—EPR imaging of isolated and perfused rat hearts were performed in 2D + spectral domain. Spatial resolution of the measurements was about 1.4 mm. Marked tissue hypoxia was observed in ischemic area of the hearts after occlusion of left anterior descending coronary artery. Tissue oxygenation was partly restored upon reperfusion. EPR mapping of myocardial pH indicated acidosis of ischemic area down to pH 6.7 – 6.8.

Conclusion—This work demonstrates capability of low-field EPR and the nitroxide spin probes for mapping of myocardial oxygenation and pH. The developed approaches might be used for noninvasive investigation of microenvironment on living objects.

Keywords

EPR imaging; EPR oximetry; spin probes; nitroxide radicals; myocardial ischemia; myocardial acidosis

Introduction

Methods of magnetic resonance represent a powerful instrument for noninvasive investigation of biological objects. The technique of magnetic resonance in the presence of external field gradients opens a possibility to perform spatially-resolved spectroscopic measurements. MRI of water protons became an invaluable tool in medical practice providing information about structural peculiarities of living organs. However for the variety of biomedical studies it is also important to know functional information about tissue microenvironment.

Pathological conditions are usually accompanied with certain metabolic changes. As an example, proliferation of tumor tissues proceeds under hypoxic conditions because of underdeveloped vasculature (1–3). Low oxygen concentration and high energy demand of fast growing tissue results in acidification of extracellular medium (4–5). Myocardial ischemia is another example. Occlusion of a coronary artery results in fast depletion of oxygen in the ischemic area. Anaerobic metabolism causes acidosis of the tissue and transsarcolemmal ion transport malfunction eventually leading to persistent damage to cardiomyocytes (6–7). Restoration of blood supply to the ischemic region is necessary for tissue revival. However reintroduction of oxygen on reperfusion results in a burst of reactive oxygen species and causes an additional damage to the tissue (8–9). Noninvasive investigation of tissue microenvironment could provide important information about the pathology and its dynamics and become a useful tool for development of new therapeutic strategies.

Several NMR-based methods were developed for functional measurements in vivo. Blood oxygen level dependent (BOLD) contrast imaging is an actively developing method for noninvasive mapping of brain oxygenation (10–11). The method suffers from low functional signal to noise ratio and its application is mostly based on thorough statistical analysis of experimental data (12). In order to improve functional sensitivity some studies suggest using fluorocarbons as contrast agents for in vivo oxygen mapping by means of ^{19}F -NMR (13). ^{31}P -NMR spectroscopy of inorganic phosphate or exogenous probes is widely employed for pH measurements in living tissues (14–16). However the NMR methods of pH measurements are hardly applicable for the imaging due to overlapping of multiple ^{31}P resonances from different biological molecules. A novel contrast mechanism based on chemical exchange saturation transfer (CEST) was recently introduced for pH mapping by MRI (17–18).

EPR imaging technique represents an alternative modality capable for noninvasive spatially resolved functional measurements (19–21). In contrast to NMR, EPR possesses much higher intrinsic sensitivity due to the higher magnetic moment of electron. On the other hand EPR signal of a probe does not overlap with other resonances due to the lack of endogenous paramagnetic compounds that substantially simplifies data processing of spectral-spatial images. EPR imaging has been applied for oxygen measurements in ischemic myocardium (20,22–23) and for visualization of tumor oxygenation (24–26).

Application of EPR for functional measurements in vivo requires exogenous paramagnetic probes. Among the other paramagnetic compounds soluble triarylmethyl and nitroxide radicals are the most interesting probes for functional EPR measurements (27). Triarylmethyl radicals are promising spin probes for functional EPR imaging since they have very narrow EPR lines and demonstrate good stability in biological milieu. For in vivo applications highly hydrophilic and nontoxic spin probes are required. Although a significant progress has been made recent years in development of new trityl-based functional spin probes chemical synthesis of hydrophilic triarylmethyl radicals is still challenging and only few probes are currently available (28–31). In contrast to trityl radicals synthetic chemistry of nitroxides is well developed allowing for design and synthesis of the probes with desired properties (32–34).

In this work we employed specially developed nitroxide radicals for visualization of oxygenation and pH of ischemic myocardium by EPR imaging technique. The model of isolated and Langendorff perfused rat hearts was used for the experiments.

Materials and Methods

Spin probes

Oxygen sensitive spin probe (3R(S),4R(S))-3,4-dicarboxy-2,2,5,5-tetra(²H₃)methylpyrrolidin-(3,4-²H₂)-(1-¹⁵N)-1-oxyl (²H, ¹⁵N-DCP) was synthesized according to the scheme presented in Fig. 1. The starting compound **1** (Fig. 1) was prepared by the procedures described earlier in (35). Detailed description on the synthesis and spectroscopic data are provided in Supporting Information (available online). The pH-sensitive nitroxide radical linked to glutathione molecule (RSG, fig. 1) was synthesized as described previously (36).

Experimental setup

EPR experiments were performed using Bruker Elexsys E540 EPR spectrometer/imager. Isolated rat hearts were perfused directly inside 23 mm reentrant L-band EPR resonator (E540R23, Bruker). Schematic illustration of the experimental setup for heart perfusion is presented in Fig. 2. Isolated hearts were supplied with perfusate buffer at 37 °C via water-jacketed tubing. Perfusion was performed in constant flow mode using a peristaltic pump (BT100-2J, LongerPump). Perfusion pressure was continuously monitored using MPVZ5050GW pressure transducer (Freescall Semiconductor). Contractile function of the hearts was monitored using a water-filled latex balloon inserted into the left ventricular. The balloon was connected to the second pressure transducer via hydraulic line. Analog signals from the transducers were digitized using Olimexino STM32 board and recorded on a computer. At the beginning of the experiment the balloon volume was adjusted to end diastolic pressure of 10 mmHg. Due to the presence of left ventricular balloon the hearts contracted in isovolumetric mode with no significant movements allowing for spatially resolved EPR measurements. A double-walled cell thermostated with perfluorooctane was inserted into the resonator to maintain the hearts at 37 °C. Perfluorooctane was chosen as a heat transfer fluid because it is chemically inert and possesses low dielectric loss tangent (i.e.

does not interfere with EPR measurements). Circulation of perfluorooctane in the system was performed using a small centrifugal pump (NJ 1200, Aquarium systems).

Isolated hearts preparation and ischemia model

Wistar rats (250 – 300 g) were used for the experiments. The animals were handled in compliance with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The animals were narcotized with intraperitoneal injection of sodium thiopental, 50 mg/kg. After onset of complete anesthesia trachea was cannulated to provide mechanical ventilation of lungs with room air. Sodium heparin, 1000 U/kg, was administered by bolus injection into jugular vein. Midsternal thoracotomy was performed to expose the heart. Ascending aorta was cannulated and the heart was infused with 1 ml cardioplegic solution (120 mM NaCl, KCl 30 mM). The heart was excised and placed on ice. Silk suture thread, 7/0, was pulled under left anterior descending (LAD) coronary artery and knotted (but not tightened) on a small piece of soft plastic material (Fig. 1, right). After that the heart was transferred to the perfusion setup. Retrograde perfusion was initiated at constant flow of 12 ml/min which corresponded to the perfusion pressure of 40 – 70 mmHg. The perfusion was performed with modified Krebs-Henseleit buffer: 11 mM glucose, 125 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4 , 15 mM $NaHCO_3$, 1.25 mM $CaCl_2$. Perfusate buffer was continuously bubbled with gas mixture of 95% O_2 and 5% CO_2 . Before the experiments the buffer was routinely filtered through 0.2 μm polystyrene membrane filter. Isolated hearts were perfused for 20 minutes to achieve stabilization of contractile function then a spin probe was added to the perfusate buffer. Further perfusion was performed in recirculating mode in order to minimize usage of the spin probe. Regional ischemia was initiated after 10 minutes of perfusion with the spin probe by tightening the LAD ligature. EPR data acquisition was started after 15 minutes of regional ischemia. For reperfusion after ischemia the LAD ligature was released and after additional 15 minutes EPR data were collected once again. At the end of EPR experiments the hearts were removed from perfusion setup and LAD ligature was tied. Methylene blue solution was infused through cannulated aorta and the hearts were sliced in axial plane to confirm and visually localize the ischemic area.

Image acquisition, reconstruction and spectral data fitting

EPR imaging experiments were performed in 2D + spectral domain with spatial field of view $20 \times 20 \text{ mm}^2$. For pO_2 mapping isolated hearts were perfused in the presence of 0.5 mM 2H , ^{15}N -DCP spin probe. Low-field component of the probe EPR spectrum was centered at the 5 G spectral window. EPR projections were acquired at 15 polar and 10 pseudo angles with maximum magnetic field gradient of 4.33 G/cm (which corresponds to pseudo angle, $\phi = 60^\circ$). Acquisition time of a single projection was set to 5.12 s which resulted in total image acquisition time of 19 min. EPR spectrometer settings were as follows: modulation amplitude, 0.2 G; modulation frequency, 100 kHz; microwave power, 1.8 mW. pH imaging experiments were performed in the presence of 1 mM RSG spin probe. Spectral window of EPR images was set to 50 G. EPR projections were acquired at 31 polar and 12 pseudo angles with maximum field gradient of 17.4 G/cm ($\phi = 34.84^\circ$), sweep time, 5.12 s; total image acquisition time, 39 min. EPR spectrometer settings were as follows: modulation amplitude, 2.5 G; modulation frequency, 100 kHz; microwave power, 3.6 mW.

Image reconstruction was performed by iterative backprojection algorithm using specially developed MATLAB code. First EPR projections were scaled (divided by cosine of pseudo angle, $\cos \phi$) and downsampled. In order to avoid artifacts on edges of resulting image the projections were appended both sides with zeros. The resulted projections were directly (i.e. without application of any filter) backprojected on a $192 \times 192 \times 192$ matrix. On the next step the matrix was projected and the result was compared to the initial projections. The residual was backprojected again. The operation was repeated 5 – 8 times to attain a consistent result and a good convergence with the initial projections. After reconstruction the resulted matrix was cropped to $128 \times 128 \times 128$ points which correspond to field of view of $20 \times 20 \text{ mm}^2$.

In order to calculate oxygen concentration spectral data of EPR images were fitted with convolution of Lorentzian and Gaussian functions (Voigt function). Rational approximation of complex error function was used to calculate Voigt profile (37). The fitting was performed by Levenberg-Marquardt algorithm using laboratory developed MATLAB code. Lorentzian component of the EPR linewidth was used to calculate oxygen concentration using the calibration curve (Fig. 3). Spectral data of pH images were fitted with triplet and hyperfine splitting constant was measured as half of the distance between low- and high-field component of the probe EPR spectrum. The pH value was calculated using the following

equation: $\text{pH} = \text{pK} + \lg \frac{a_N - a_N(\text{RH}^+)}{a_N(\text{R}) - a_N}$; where $a_N(\text{RH}^+) = 14.24 \text{ G}$, $a_N(\text{R}) = 15.25 \text{ G}$ and $\text{pK} = 6.6$ (38).

Calibration of the $^2\text{H}, ^{15}\text{N}$ -DCP probe

Calibration of the probe was performed at room temperature. The radical (0.2 mM) was dissolved in phosphate buffer (50 mM, pH 7.4). A test tube with 3 ml of radical solution was placed in the resonator of L-band EPR spectrometer. The solution was continuously bubbled with a mixture of argon and oxygen gases in various proportions. The gas mixtures were prepared using a latex balloon inflated with required volumes of pure gases. EPR spectra were recorded using the following spectrometer settings: modulation amplitude, 0.08 G; modulation frequency, 100 kHz; microwave power, 3.6 mW. For each concentration of oxygen the experiment was repeated three times by preparing a new gas mixture. Oxygen concentration in solutions was calculated assuming $k_H = 1.28 \text{ mM/atm}$ at room temperature and $k_H = 1.08 \text{ mM/atm}$ at 37°C (39).

Results

For EPR measurements of myocardial oxygenation we have synthesized isotopically substituted $^2\text{H}, ^{15}\text{N}$ pyrrolidine nitroxide radical, $^2\text{H}, ^{15}\text{N}$ -DCP (see fig. 1 for the structure). Isotopic substitution of hydrogen atoms by deuterium reduces hyperfine interaction constant of unresolved EPR structure and substantially decreases linewidth of the probe. Substitution of ^{14}N atom of nitroxide fragment by ^{15}N reduces the number of spectral components and increases their intensity by 50%, and causes an additional decrease in EPR linewidth due to elimination of dipole-quadrupole relaxation mechanism (40). EPR spectrum of the radical is represented by two narrow lines with peak to peak linewidth of low-field component of 320

mG. Spin exchange interaction of the probe with paramagnetic oxygen molecules shortens its relaxation time T_2 resulting in broadening of EPR spectral line. The dependence of the probe Lorentzian linewidth on concentration of oxygen in solution is presented in figure 3. The observed oxygen-induced line broadening is 0.53 mG/mmHg providing a possibility to measure oxygen concentration down to 5 μ M (corresponds to $\sim$ 3 mmHg at room temperature).

It has to be noted that spin exchange interaction between nitroxide molecules can also result in self-broadening of spectral lines. At high concentrations of the probe this self-induced broadening may interfere with oxygen measurements. In water solutions at neutral pH both carboxyl groups of the $^2\text{H}, ^{15}\text{N}$ -DCP radical are deprotonated. Electrostatic repulsion impedes interaction between the molecules and allows for usage of sufficiently high concentrations of the probe for a better signal to noise ratio without interference with oxygen measurements. As a demonstration of the effect figure 4 shows concentration dependences of EPR linewidth for $^2\text{H}, ^{15}\text{N}$ -DCP radical and pyrroline nitroxide with a single carboxyl group. As can be seen from the figure concentration broadening for $^2\text{H}, ^{15}\text{N}$ -DCP is considerably less steep than that for the radical with single carboxyl group and allows using probe concentrations up to 2 mM without noticeable self-induced broadening.

In order to evaluate the utility of EPR imaging using $^2\text{H}, ^{15}\text{N}$ -DCP spin probe for spatially-resolved oxygen measurements we have recorded spectral-spatial EPR image of a phantom sample. The sample was a 15 ml centrifugal tube filled with air-saturated solution of 0.5 mM $^2\text{H}, ^{15}\text{N}$ -DCP. Inside the tube there was a NMR tube filled with oxygen-free solution of the radical. Spectral data of the EPR image were fitted with a Voigt profile and the map of Lorentzian linewidth, $1/T_2$, was constructed (Fig. 5). As can be seen from the map the solutions are clearly distinguishable. Lorentzian linewidth of EPR signal from central tube was 170 mG and about 250 mG for outer tube. These values adequately reflect concentration of oxygen in the sample and just a little bigger than can be expected from the calibration curve shown in figure 3.

We have applied $^2\text{H}, ^{15}\text{N}$ -DCP spin probe for visualization of heart oxygenation by EPR imaging technique. Figure 6 shows 2D + spectral images of an isolated and perfused rat heart during normal perfusion, regional ischemia and reperfusion. The heart was oriented in coronal plane so that on the images left ventricular balloon can be seen at the bottom right part of the myocardium. The observed concentration of oxygen in the myocardial tissue during normal perfusion was 0.24 mM – 0.36 mM (180 – 270 mmHg). After tightening the LAD ligature concentration of oxygen in the expected ischemic area lowered down to 0.06 mM (45 mmHg). Simultaneously oxygenation of non-ischemic tissue was significantly increased. This could be explained taking into account that the perfusion was performed in a constant flow mode and the occlusion of LAD resulted in an increased perfusate flow through the rest parts of the myocardium. Reperfusion after release of the LAD ligature resulted in partial reoxygenation of postischemic tissue to about 0.12 mM (90 mmHg). The experiment was reproduced with three different hearts yielding the similar results.

EPR measurements of pH was conducted using pH-sensitive imidazoline nitroxide radical RSG. EPR spectrum of the radical demonstrates pH-dependable change in nitrogen

hyperfine splitting, a_N (38). The dependence of a_N on pH is described by standard titration curve with $pK = 6.6$ (at 37 °C) providing functional sensitivity to pH of about 0.05 units in physiological range (see Materials and Methods). To demonstrate the capability of EPR for pH mapping we performed spectral-spatial EPR imaging of a phantom sample. The sample consisted of three tubes filled with 1 mM RSG solution at different pH. As can be seen from figure 7 the calculated pH map accurately reflects pH of the solutions.

We performed EPR pH mapping of myocardial acidosis during ischemia in isolated rat hearts. The hearts were perfused in the presence of 1 mM RSG spin probe. Regional ischemia was induced by LAD ligation and EPR data acquisition was initiated after 15 minutes of ischemia. Figure 8 shows distribution of the radical and calculated map of pH of the heart subjected to regional ischemia. The heart was oriented in coronal plane and expected ischemic area is indicated by dashed line. The observed pH of ischemic tissue was 6.7 – 6.8 while pH of normally perfused tissue was 7.3 – 7.4 corresponding to the pH of perfusate buffer.

Discussion

Application of EPR for measurements of oxygen concentration requires a spin probe with narrow EPR line. Previously we have employed deuterated Finland trityl radical (41) to measure heart oxygenation during global ischemia by means of EPR spectroscopy (38). Although we have successfully measured the kinetics of myocardial oxygen depletion during global no-flow ischemia the prolonged perfusion of hearts in the presence of the trityl radical required for EPR imaging experiments resulted in gradual decline in contractile function and led to cardiac death in about 30 minutes of perfusion (data not shown). The observed cardiotoxicity of the Finland trityl radical apparently is the result of its lipophilic properties (41).

Nitroxide radicals are known to be significantly less toxic. One of the major drawbacks for application of nitroxides as in vivo probes is their susceptibility toward bioreduction into diamagnetic hydroxylamines. Dicarboxyproxyl nitroxide is known to demonstrate relatively high stability (42) and its acetoxymethyl ester derivative was previously considered as a pO_2 -sensitive probe for EPR imaging (43). Isotopically substituted nitroxides can provide higher EPR signal intensity and much better functional sensitivity compared to conventional nitroxides (44). In this work we used the new $^2H, ^{15}N$ -isotopically substituted dicarboxyproxyl nitroxide as oxygen sensitive spin probe. The $^2H, ^{15}N$ -DCP nitroxide is present in aqueous solutions at neutral pH in the form of dianion, therefore demonstrating several advanced properties. First, $^2H, ^{15}N$ -DCP shows comparatively low self-induced EPR line broadening allowing to neglect the effect at concentrations below 2 mM. Second, dianion structure causes high hydrophilicity of the probe with octanol-water partition coefficient of $(1.8 \pm 0.2) \times 10^{-3}$ at pH 7.4. Thus it is expected that $^2H, ^{15}N$ -DCP has very low diffusion rate through cellular membranes and can hardly penetrate into cytosol with high nitroxide reduction capacity. Indeed, the probe demonstrated excellent stability in myocardial tissue losing only ~ 10% of its initial EPR intensity during 30 minutes of global no-flow ischemia (data not shown). Moreover, cell-impermeable nitroxide radical is

expected to be less toxic. In accordance with that we did not observe any influence of the radical on heart contractile function.

The obtained data on myocardial oxygenation demonstrate strong oxygen depletion in the ischemic region of the heart. Interestingly that reperfusion resulted only in partial restoration of tissue oxygenation as compared to preischemic level. The depressed oxygenation on reperfusion is most likely caused by hampered perfusate flow through the compressed vasculature of ischemia damaged myocardium (45). These data are in a good agreement with those we have measured earlier by EPR spectroscopic method using deuterated trityl radical as a spin probe during global no-flow ischemia (38). The depressed oxygenation of post-ischemic myocardium was also observed by Ilango et al. (46) and Angelos et al. (47). These studies used the model of ischemia/reperfusion in Langendorff perfused hearts. However there are numerous in vivo studies which report an elevated oxygenation of reperfused myocardial tissue (48–52).

The pH-sensitive nitroxide radical RSG was recently used in several EPR studies and has proven itself as a useful probe for in vivo pH measurements (36,38,53–54). The radical has ionization constant $pK = 6.6$ which is perfectly suited for the measurements in physiologically relevant pH range. Imidazoline fragment of the radical is bound with glutathione molecule which enhances aqueous solubility of the probe and prevents its diffusion through cellular membranes. Radical center of the probe is protected with bulky ethyl groups. Extracellular localization and sterical protection of the nitroxide fragment contribute to the high stability of the probe in biological samples.

Previously we have successfully applied the RSG radical to monitor acidosis of ischemic myocardium by EPR spectroscopic method. The radical has demonstrated good functional sensitivity and high stability in myocardial tissue losing about 10 % of its initial EPR intensity during 30 minutes of global no-flow ischemia (38). Application of EPR imaging approach in the present work allowed us to visualize regional acidosis of myocardial tissue during ischemia. As a cell impermeable nitroxide radical RSG reports extracellular pH value. In the ischemic region of myocardium perfusate flow is ceased and transmembrane proton movement results in equilibration of intra- and extracellular pH (15). The observed pH of ischemic tissue was 6.7 – 6.8 that is in a reasonable agreement with data measured by pH electrodes (55–56) or by ^{31}P -NMR spectroscopy (57–58). Meanwhile pH of normally perfused tissue corresponds to the pH of perfusate buffer, 7.4.

In this work we used custom designed iterative backprojection algorithm for reconstruction of the spectral-spatial EPR images (see Materials and Methods for details). The iterative approach although requires much computational time considerably improved the quality of EPR images. Filtered backprojection algorithm is the most commonly used method for image reconstruction. However this method produces a good result only when number of projections is sufficiently large. Another problem is that the method requires convolution of the experimental projections with a ramp filter. That could drastically increase high frequency noise therefore requiring utilization of an additional low-pass filter (e.g. Shepp-Logan filter). Application of a low-pass filter may introduce unwanted distortions to spectral information and interfere with functional measurements. That is particularly important for

oxygen measurements where precise lineshape analysis is required to extract Lorentzian linewidth of EPR signal. The iterative backprojection algorithm developed in this work produces good results even for a relatively small number of projections and ensures virgin lineshape of EPR signals. Another important advantage of this approach is that straightforward summation of raw data during backprojecting averages high frequency noise and eventually leads to fair images even for EPR projections with poor signal-to-noise ratio. Spatial resolution of EPR images is determined by two major factors: the total number of projections and the ratio between spin probe linewidth and maximum field gradient. For 2D spatial images the resolution can be estimated as $\frac{FOV}{N} \frac{\pi}{4}$, where FOV (field of view) is the spatial window and N is the number of projections (59). In this work EPR mapping of oxygen concentration was performed at 15 orientations of magnetic field gradient. Thus the maximum achievable resolution for the measurements can be estimated as 1 mm. In practice spatially resolved measurements are affected by several other factors such as signal to noise ratio, stability of microwave frequency and linearity of magnetic field sweep and the gradients. In order to determine the real resolution of oxygen measurements we performed fitting of the intensity data on the edges of the phantom sample from fig. 5 with error function in analogy to references (60–61). Based on the results of the fitting spatial resolution of oxygen measurements was determined as 1.4 mm. EPR measurements of pH were performed at high modulation amplitude of 2.5 G in order to increase spectral sensitivity. Therefore spatial resolution of the pH measurements was limited by the ratio of spectral linewidth and gradient strength and expected to be 1.4 mm.

It has to be mentioned that EPR projections were acquired in 2D + spectral domain. This means that pO₂ and pH maps presented in figs. 6 and 8 are not the absolute values in a particular spatial location but integral averages of pO₂ and pH along the third unresolved spatial axis. In order to obtain the complete spectral-spatial information about an object it is necessary to perform 3D + spectral imaging. This requires a set of projections acquired at different polar and azimuthal angles at different gradient strengths (i.e. pseudo-angles). Feasibility of 3D + spectral EPR imaging has been demonstrated on isolated rat heart infused with glucose char during global ischemia (23) and for visualization of tumor oxygenation (24,26). However 3D + spectral EPR imaging is still challenging using commercially available continuous wave EPR equipment and stepped field gradients because it requires large time for data acquisition: from an hour for a poor resolution image and much longer for a better one. We hope that 3D functional EPR imaging will become available with the development of rapid-scan techniques, fast magnetic field gradients and pulsed EPR methods.

In summary, this work demonstrates great capability of low-field EPR imaging technique for visualization of myocardial oxygenation and pH. The proposed nitroxide spin probes demonstrate high stability in myocardial tissue, good functional sensitivity and revealed no cardiotoxicity. The developed approaches might be used for noninvasive investigation of microenvironment on living objects in a variety of biomedical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Left: preparation scheme of oxygen sensitive nitroxide spin probe, $^2\text{H}, ^{15}\text{N}\text{-DCP}$. Right: chemical structure of pH-sensitive nitroxide radical, RSG.

FIG. 2.

Left: schematic illustration of the experimental setup for heart perfusion (see text for details). Abbreviations are: PT – pressure transducer, ADC – analog to digital converter, PC – personal computer. Right: the picture of isolated and perfused rat heart inside EPR magnet. The ligature is placed on left anterior descending coronary artery.

FIG. 3.

Dependence of Lorentzian linewidth of 2H , ^{15}N -DCP low-field EPR spectral line on oxygen partial pressure. Insert: L-band EPR spectrum of the probe (0.2 mM) in oxygen free phosphate buffer (50 mM, pH 7.4). EPR spectrometer settings were as follows: modulation amplitude, 0.08 G; modulation frequency, 100 kHz; microwave power, 3.6 mW.

FIG. 4. Dependence of peak-to-peak EPR linewidth on concentration for $^2\text{H}, ^{15}\text{N}$ -DCP (●) and pyrroline nitroxide radical (●) with two and one carboxyl groups in their structures, correspondingly. EPR spectrometer settings are as indicated in Fig. 3 captions.

FIG. 5. 2D +spectral EPR image of a phantom sample. The sample consisted of two tubes filled with oxygen-free and air saturated solution of 0.5 mM ^2H , ^{15}N -DCP radical in phosphate buffer (50 mM, pH 7.4). Lorentzian linewidth of EPR signal is presented. EPR imaging settings: 12 field gradients at 15 polar angles, maximum gradient, 7.69 G/cm; total image acquisition time, 22.5 min.

FIG. 6.

Coronal plane 2D + spectral EPR images of an isolated and perfused rat heart during normal perfusion, regional ischemia and reperfusion. The heart was perfused in the presence of 0.5 mM ^2H , ^{15}N -DCP spin probe. Upper row: integral intensities of EPR signal. The area indicated by dashed line is expected to be ischemic. Bottom row: maps of myocardium oxygenation calculated from spectral data of the images. EPR imaging settings: 10 field gradients at 15 polar angles, maximum gradient, 4.33 G/cm; total acquisition time of a single image, 19 min.

FIG. 7.

2D +spectral EPR image of a phantom sample. The sample consisted of three tubes filled with solution of 1 mM RSG in phosphate buffer at pH 7.6, 6.6 and 5.6. The pH map was calculated from the observed hyperfine splitting constant of the probe EPR spectrum (see Materials and Methods for details). The calculated pH values in the tubes are 7.66 ± 0.10 , 6.57 ± 0.05 and 5.57 ± 0.12 , correspondingly. EPR imaging settings: 14 field gradients at 28 polar angles, maximum gradient, 30 G/cm; total image acquisition time, 48.5 min.

FIG. 8.

Coronal plane 2D + spectral EPR image of isolated and perfused rat heart during regional ischemia. The heart was perfused in the presence of 1 mM RSG spin probe. Left: integral intensity of EPR signal. The area indicated by dashed line is expected to be ischemic. Right: pH map calculated from spectral data of the image. EPR imaging settings: 12 field gradients at 31 polar angles, maximum field gradient, 17.4 G/cm; total image acquisition time, 39 min.