Chapter 18
Repetitive Measurements of Intrarenal Oxygenation *In Vivo* Using L Band Electron Paramagnetic Resonance

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Abstract Intrarenal oxygenation is heterogeneous with oxygen levels normally being highest in the superficial cortex and lowest in the inner medulla. Reduced intrarenal oxygenation has been implied in the pathology of several kidney diseases. However, there is currently no method available to repetitively monitor regional renal oxygenation using minimally invasive procedures. We therefore evaluated implantable lithium phthalocyanine (LiPc) probes, which display a close correlation between EPR line width and oxygen availability.

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LiPc probes were implanted in the kidney cortex and medulla in the same mouse and EPR spectra were acquired using a L band scanner during inhalation of air (21 % oxygen) or a mixture of air and nitrogen (10 % oxygen). In order to separate the signals from the two probes, a 1 G/cm gradient was applied and the signals were derived from 40 consecutive sweeps. Peak-to-peak comparison of the EPR line was used to convert the signal to an approximate oxygen tension in MATLAB. Kidney cortex as well as medullary oxygenation was stable over the 45 day period (cortex 56 ± 7 mmHg and medulla 43 ± 6 mmHg). However, 10 % oxygen inhalation significantly reduced oxygenation in both cortex (56 ± 6 to 34 ± 2 mmHg $n = 15$ $p < 0.05$) and medulla (42 ± 5 to 29 ± 3 mmHg $n = 7$ $p < 0.05$).

In conclusion, L band EPR using LiPc probes implanted in discrete intrarenal structures can be used to repetitively monitor regional renal oxygenation. This minimally invasive method is especially well suited for conditions of reduced intrarenal oxygenation since this increases the signal intensity which facilitates the quantification of the EPR signal to absolute oxygenation values.

Keywords Kidney • LiPc • L-Band EPR • NMRI mice • Oxygenation

1 Introduction

Intrarenal oxygenation is heterogeneous with oxygen levels normally being highest in the superficial cortex and lowest in the inner medulla [1]. Arterial and venous vessels are closely aligned causing oxygen to diffuse from the well-oxygenated arterial blood to the less oxygenated venous blood, constituting a functional arterial-venous shunting mechanism [2, 3]. A consequence of this arrangement is poor oxygenation of the inner parts of the renal medulla, making this structure work at the brink of hypoxia already during normal physiology.

Reduced intrarenal oxygenation has been proposed as common pathway to chronic kidney disease [4]. However, there is currently no method available to repetitively monitor regional renal oxygenation using minimally invasive procedures. A methodological problem has been to monitor changes in intrarenal oxygenation over time due to the lack of suitable methods. However, implantable lithium phthalocyanine (LiPc) oxygen probes have been used in tumor research to monitor such changes [5–7] and we therefore evaluated if this technique also can be used to study functionally relevant changes in intrarenal oxygenation. Longitudinal measurements were performed to determine long-term stability and acute interventions using reduced oxygen content in the inhaled air were used to demonstrate that rapid and reversible changes in intrarenal oxygenation could be detected.

2 Methods

All measurements were performed using a L band Elexsys II E540 (Bruker BioSpin GmbH, Rheinstetten, Germany).

2.1 Animals

Eight-week-old male NMRI mice were purchased from Taconic (Ryd, Denmark) and were housed two per cage at Linköping University's animal facility and all procedures were approved by the local Animal Use and Care Committee. Food and water were given *ad libitum*.

2.2 LiPc Probe Preparations

LiPc (Clin-EPR, NH, USA) oximetry probes were manufactured by loading aggregates of LiPc crystals into 23 G needles as previously described [6].

2.3 Insertion of Probes into Renal Tissue

Mice were anaesthetized with 2 % Isoflurane (Florene, Apoteket AB, Sweden) in air and 10–15 mm vertical incisions were made on both sides below the diaphragm. A 4 mm horizontal injection was made in the left kidney for placement of the cortical probe and 4 mm transversal injection in the right kidney for placement of the medullary probe. The incision was closed (6.0 Vicryl, AgnTho's AB, Lidingö, Sweden) and 5 mg/kg/24 h of Carprofen (Rimadyl Bovin, Apoteket, Sweden) was administered subcutaneously.

2.4 In Vivo Measurements with L Band EPR

Mice were measured at day 9, 13, 17, 21 and 45. Before measurements, mice were anaesthetized (2 % Isoflurane in air) and placed inside the resonator. For normal physiology, mice were allowed to breathe normal air (21 % oxygen) and for acute hypoxic measurements mice were breathing a mixture of equal amounts of air and nitrogen during 5 min, resulting in a final concentration of 10 % oxygen. Acute hypoxic measurements were performed after normal air. EPR measurements were performed using a Bruker Elexsys E540 L band EPR spectrometer equipped with an E540 GCL Triple axis coil set (gradient field strength up to 40 G/cm) and an E540 R36 L band Resonator (36 mm sample access) connected to an EPR 066L-AMC L band Microwave Bridge. The spectrometer settings were: 36 mW applied microwave power, 0.2 G modulation amplitude, 20 ms time constant, 5 s sweep time, 256 measurement points, 3 G sweep width and 40 sweep added together for each measurement. The EPR signals from the two probes in each mouse were then separated with 1 G/cm (gradient angle: $\Phi = 0$, $\theta = 0$ along B_0). No EPR signal could be detected for the empty resonator. The recorded EPR spectra were imported into MATLAB and peak-to-peak line width were analyzed using an in-house developed MATLAB

script. Oxygen tensions were calculated by comparison of the EPR line width for the two LiPc probes with spectra obtained from a calibration probe made from the same batch and measured at different oxygen tensions.

2.5 Statistical Analysis

All statistics were performed with Graphpad Prism 6.0. Student's t-test was used to compare cortical vs. medullary and 21 % oxygen vs. 10 % oxygen and one way ANOVA was used to analyze cortex and medulla over time. All data are displayed as mean ± SEM and $p < 0.05$ was considered significant.

3 Results

Two crystals placed in distinctively different intrarenal structures in the same mouse could easily be detected as two separate signals (Fig. 18.1) that can be converted into absolute oxygen tension values.

Rapid alterations in intrarenal oxygenation could easily be detected by the two crystals when altering the oxygen content in the inhaled air (Fig. 18.2). Estimation of cortical tissue measured from 56 ± 6 to 34 ± 2 mmHg (n = 15) and medullary tissue from 42 ± 5 to 29 ± 3 mmHg (n = 7).

Oxygenation monitored using LiPc crystals in both kidney cortex and medulla was stable over the 45 days at cortex 56 ± 7 mmHg and medulla 43 ± 6 mmHg, respectively. The optimal monitoring window seems to be around day 15–20 after installation of probes (Fig. 18.3).

4 Conclusions

Renal physiology is dependent on adequate oxygenation in order to fulfill the requirements to reabsorb and to secrete electrolytes and waste products. Insufficient oxygenation has been proposed as a common pathway to kidney disease [4]. In order to fully understand the role of deranged kidney oxygenation, we need methods allowing for repetitive and continuous monitoring of oxygen levels with the different anatomical structures of the kidney. The possibility to simultaneously measure cortical and medullary tissue improves our understanding of diminished oxygenation in different diseases. The results from the present study demonstrate that EPR oximetry using LiPc crystals is able to distinguish oxygenation in different parts of the kidney and also detect rapid and physiological relevant changes in intrarenal oxygenation. Thus, this method would be suitable to finally establish the role of deranged oxygenation as a pathway to kidney disease. In order to solidify such a

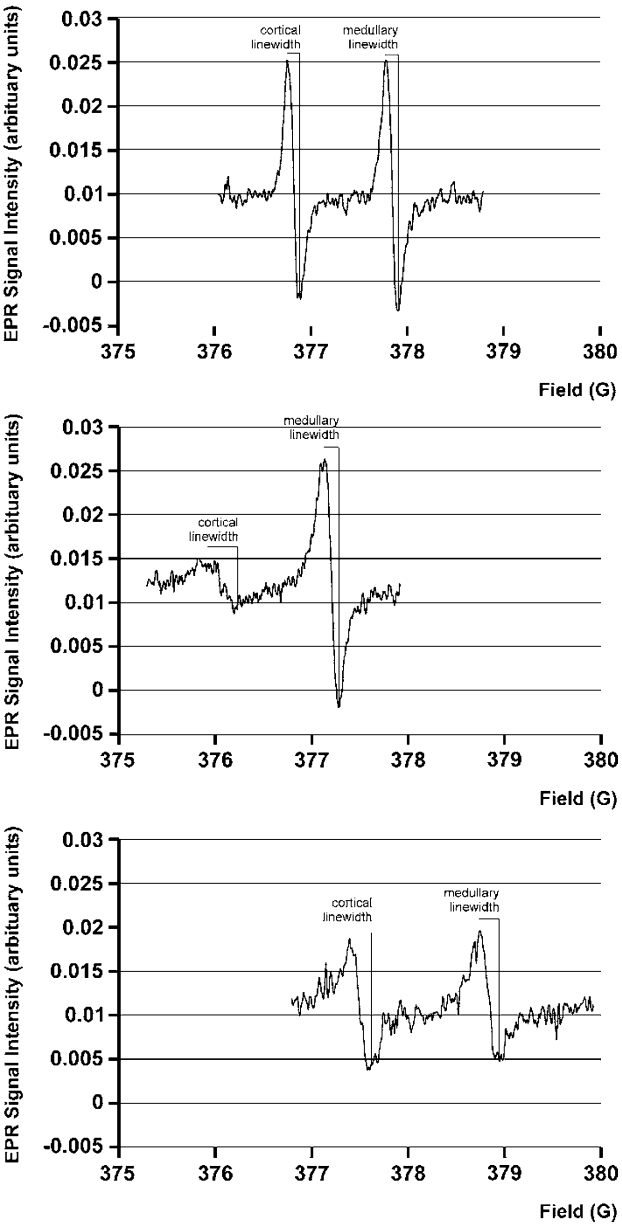

Fig. 18.1 *Upper panel*, spectra of two LiPc implanted in the kidney cortex (*left peak*) and medulla (*right peak*) of a euthanized mouse measured by L band EPR. *Middle* and *bottom panel*, representative L band EPR spectra of two LiPc implanted in the kidney cortex (*left peak*) and medulla (*right peak*) of a mouse during inhalation of air (21 % oxygen; *middle panel*) and a mixture of air and nitrogen (10 % oxygen; *bottom panel*). Kidney oxygen tensions during inhalation of air were 72 mmHg in cortex and 29 mmHg in medulla. Inhalation of 10 % oxygen resulted in reduced oxygen tensions in both cortex and medulla

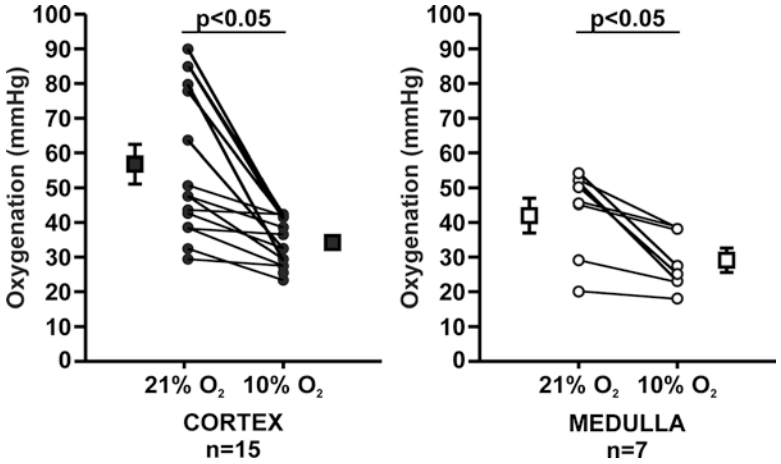

Fig. 18.2 Cortical and medullary oxygenation during inhalation of air (21 % oxygen) or a mixture of air and nitrogen (10 % oxygen). *p < 0.05 versus corresponding air inhalation

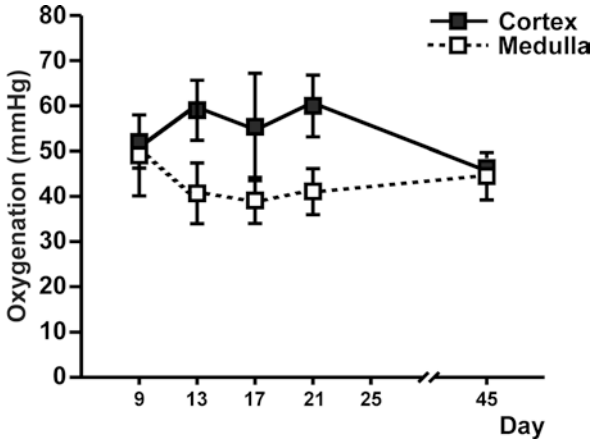

Fig. 18.3 Cortical and medullary (n = 5–10) oxygenation over time

common pathway, it is necessary to demonstrate that reduced oxygenation occurs before the onset of kidney disease and that interventions to correct pathologically low oxygenations are successful in preventing disease development. An obvious advantage of this technique is the increasing sensitivity as oxygenation decreases.

There are several limitations using EPR oximetry and LiPc probes to detect intrarenal oxygenation: the measurements are restricted to where the probes are placed. Fibrosis development may occur around the probes, which potentially could affect oxygen diffusion and we are currently restricted to using mice due to the limited space inside the resonator.

In conclusion, L band EPR using LiPc probes implanted in discrete intrarenal structures can be used to repetitively monitor regional renal oxygenation. This minimally invasive method is especially well suited for conditions of reduced intrarenal oxygenation since low oxygen increases the signal intensity.

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