Telemetry-based oxygen sensor for continuous monitoring of kidney oxygenation in conscious rats

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1Department of Physiology, Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand; 2Nephrology, University Medical Centre Utrecht, Utrecht, Netherlands; 3Department of Physiology, Monash University, Melbourne, Australia; 4Millar Instruments, Auckland, New Zealand; and 5Department of Surgery, University of Melbourne, Melbourne, Australia

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Koeners MP, Ow CP, Russell DM, Abdelkader A, Eppel GA, Ludbrook J, Malpas SC, Evans RG. Telemetry-based oxygen sensor for continuous monitoring of kidney oxygenation in conscious rats. Am J Physiol Renal Physiol 304: F1471–F1480, 2013. First published April 10, 2013; doi:10.1152/ajprenal.00662.2012.—The precise roles of hypoxia in the initiation and progression of kidney disease remain unresolved. A major technical limitation has been the absence of methods allowing long-term measurement of kidney tissue oxygen tension (PO2) in unrestrained animals. We developed a telemetry method for the measurement of kidney tissue PO2 in unrestrained rats, using carbon paste electrodes (CPEs). After acute implantation in anesthetized rats, tissue PO2 measured by CPE-telemetry in the inner cortex and medulla was in close agreement with that provided by the “gold standard” Clark electrode. The CPE-telemetry system could detect small changes in renal tissue PO2 evoked by mild hypoxemia. In unanesthetized rats, CPE-telemetry provided stable measurements of medullary tissue PO2 over days 5–19 after implantation. It also provided reproducible responses to systemic hypoxia and hyperoxia over this time period. There was little evidence of fibrosis or scarring after 3 wk of electrode implantation. However, because medullary PO2 measured by CPE-telemetry was greater than that documented from previous studies in anesthetized animals, this method is presently best suited for monitoring relative changes rather than absolute values. Nevertheless, this new technology provides, for the first time, the opportunity to examine the temporal relationships between tissue hypoxia and the progression of renal disease.

Much of our current understanding of kidney oxygenation comes from studies using electrochemical oxygen electrodes or fiber-optic probes in acutely anesthetized animals (11). Other techniques, such as immunohistochemistry for pimonidazole adducts (36), electron paramagnetic resonance oximetry (1), blood-oxygen-level-dependent MRI, and dual-wavelength phosphorimetry (18, 35), are invasive and/or require anesthesia or sedation when applied to experimental animals. Consequently, when applied to experimental animals they can only provide information at a single point or relatively brief window of time (hours), precluding continuous temporal resolution. Consequently, the temporal relationships between tissue hypoxia and the progression of renal disease have not been examined in detail. Without knowledge of these relationships, we cannot move beyond demonstration of association of renal hypoxia and renal disease to demonstration (or refutation) of causation.

Carbon-paste electrodes (CPEs), which measure tissue PO2 by electrochemical reduction, are less prone to surface poisoning than other noble metal electrodes such as the platinum electrodes used in the “gold-standard” Clark-type electrode (5). CPEs are therefore excellent candidates for long-term measurement of tissue hypoxia and renal disease. Russell et al. (38) have recently developed the first fully implantable telemetry system, utilizing an implantable CPE, for the chronic measurement of brain tissue PO2 in freely moving rats. An important feature of this technology is that the batteries that power the telemeter are recharged noninvasively in vivo, using inductive power transfer. Consequently, the duration of experimental protocols is not limited by battery life.

Herein we report a series of studies designed to assess the validity of CPE-telemetry for long-term measurement of kidney tissue PO2 in unrestrained rats. We first assessed the performance of the CPE-telemetry system after acute implantation in the renal cortex and medulla by comparing its performance with the gold standard method, the Clark electrode. We then assessed the performance of the CPE-telemetry system after chronic implantation of the CPE in the renal medulla, a region of the kidney particularly susceptible to hypoxia (10, 33). We tested whether the CPE-telemetry system could provide stable measurements of tissue PO2 and reproducible re-
responses to systemic hypoxemia and hyperoxemia. Furthermore, the stability of telemeter calibration was assessed both in vitro and in vivo. The potential for PO₂ measurements to be confounded by tissue fibrosis and scarring was also investigated.

**METHODS**

**System overview.** The main system components have been previously described by Russell et al. (38). Briefly, these include an implantable telemeter containing a potentiostat circuit to maintain a constant −650 mV potential on the CPE with respect to the reference electrode. The nano-ampere current resulting from the reduction of oxygen in the carbon paste is converted by a trans-impedance amplifier to a voltage suitable for data acquisition. The telemeter (TR57Y, Telemetry Research; Millar Instruments, Houston, TX) weighs 11 g and is encapsulated in biocompatible silicon and designed to be placed within the abdomen of the rat. The telemeter samples the current between the CPE and the reference electrode at 2,000 Hz and transmits data wirelessly on a dedicated frequency in the 2.4-GHz band to a remote receiving station. The system offers continuous operation enabled by battery recharging through inductive power transfer through the use of a pad placed under the floor of the rat cage (SmartPad TR180, Telemetry Research; Millar Instruments). The main adaption of the system previously used in neuroscience applications (38) relates to the fixing of the electrodes in kidney tissue and the connection of the specialized CPE. Each telemeter had three leads to which the CPE, an auxiliary, and a reference electrode were attached. The specific attachment procedures are described in detail in an online document (www.millar.com/support). The electrodes were attached by exposing 5 mm of bare wire and inserting this into the stainless steel coiled wire of the telemeter. Silver conductive epoxy adhesive (8331–14G; MG-Chemicals, Ontario, Canada) was used to join the wires to provide mechanical strength and maintain electrical conductivity between the wires in the joint. The joint was then sealed with a layer of lacquer after which a layer of two-part epoxy was applied to confer mechanical strength.

The CPEs were supplied by Blue Box Sensors (Dublin, Ireland). The electrodes have a 200-µm diameter working surface area and a diameter of 256 µm after they are coated with polytetrafluoroethylene (24). The auxiliary and reference electrodes were made from polytetrafluoroethylene-coated silver wire (AG549511; Advent Research Materials, Suffolk, UK) with a bare wire diameter of 200 µm and a length of 50 mm. The tips of the auxiliary and reference electrodes were prepared by exposing 10 cm and 1 mm of bare silver wire, respectively. The auxiliary electrode was fashioned into a coil 10 mm in diameter before implantation.

**Animals.** All procedures were approved by the Animal Ethics Committee of University of Auckland (AEC R955) and the Animal Ethics Committee of the School of Biomedical Sciences, Monash University (SOBSPA/2010/30) and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 6 male Wistar rats (327 ± 19 g) and 28 male Sprague-Dawley rats (365 ± 9 g) were used.

**Acute studies in anesthetized rats.** Initially, PO₂ measurements obtained acutely by the CPE-telemetry system were compared with those provided by a Clark electrode. Clark electrodes have been extensively used to study tissue oxygenation, including renal tissue oxygenation (27) and, therefore, represent a well-accepted standard for comparison (11). Thus PO₂ in the kidneys of seven anesthetized Sprague-Dawley rats (351 ± 12 g) was recorded simultaneously using a Clark electrode and the CPE-telemetry system, while both the depth of implantation of the electrodes and inspired oxygen concentration were systematically altered.

The Clark electrode (OX-10: Unisense, Aarhus, Denmark) was connected to a picocammeter (PA-2000; Unisense), and the CPE was connected to the telemeter. Both electrodes were calibrated within a chamber containing saline (154 mM NaCl) held at 37°C by a water bath. Gases (100% N₂ and room air) corresponding to PO₂ of 0 and 151 mmHg, respectively, were bubbled through the saline solution. During bubbling, the Clark electrode was removed to prevent damage to the fragile 10-µm tip. After the solution had been bubbled for at least 5 min, bubbling was stopped, the Clark electrode was placed back into the solution, and the measurement was taken from both systems over a period of at least 5 min. Ordinary least squares regression analysis was performed to generate linear relationships between PO₂ and current for each electrode (28).

Rats were anesthetized with thiobutabarbital (150 mg/kg ip Inactin; Sigma-Aldrich). The trachea was cannulated to facilitate later artificial ventilation. Catheters were placed in the carotid artery for measurement of arterial pressure and in the jugular vein for infusion of drugs and maintenance solutions. The left kidney was then exposed through a midline incision and held in place using a cup designed for micropuncture. The renal capsule was pierced at the location of electrode entry, care being taken to prevent damage to the kidney tissue. The CPE and the Clark electrode were positioned with micromanipulators at opposite poles of the kidney. The reference and auxiliary electrodes were inserted into the kidney within 5 mm of the CPE. Once the surgical procedures were complete, pancuronium bromide (2 mg/kg plus 0.1 mg·kg⁻¹·h⁻¹ iv; Sigma-Aldrich) was administered to prevent spontaneous breathing movements and artificial ventilation commenced. Throughout the surgery and subsequent experiment, rats received an infusion of 2% wt/vol bovine serum albumin in saline to maintain fluid volume. Body temperature was maintained between 37 and 38°C by use of a heated operating table and infrared lamp.

The Clark electrode and CPE electrode were advanced so that their tips were either 2, 4, or 6 mm below the surface of the kidney. Recordings were made at all three depths in each rat, but the order in which they were tested was randomized. At each depth, kidney PO₂ was measured while the rats were ventilated for 10-min periods with 10, 18, 40, and 100% oxygen, bracketed in each case by 10-min periods of ventilation with room air (21% oxygen). The order of presentation of the gases was randomized between rats.

Kidney tissue PO₂ during the last 60 s of stable measurement at each concentration of inspired oxygen was used for the analysis. Absolute changes in kidney PO₂ from both systems were compared using the ordinary least products regression method, using a loss function to obtain 95% confidence intervals. This method for comparing methods of measurement has the benefit of distinguishing between fixed and proportional bias (28). ANOVA was also used to test for systematic bias, between the two methods of measurement, across the five levels of inspired oxygen (10, 18, 21, 40, and 80%). Confidence intervals (95%) were generated to determine whether each method was able to detect statistically significant changes in kidney PO₂ when the rat was subjected to mild hypoxia (18% inspired oxygen). All statistical analyses were performed using SPSS Statistics 19 (IBM, New York, NY).

**Chronic studies: CPE-telemetry system calibration.** The CPE-telemetry system was calibrated both before implantation and after explantation in six Sprague-Dawley rats (375 ± 8 g). The telemeters were subjected to a four-point calibration before implantation (pre-implantation) and after explantation (postexplantation). Gases (100% N₂ and 10, 21, and 40% oxygen) corresponding to PO₂ of 0, 75.7, 151.4, and 302.8 mmHg, respectively, were bubbled through the saline solution. After the respective gases were bubbled for 20 min, electrode current was measured during a quiescent 10-min period. Electrode current measured during the last 60 s of stable measurement at each oxygen concentration was used for the analysis. Comparison of precalibration values with those supplied by the manufacturers allowed us to establish quality control criteria before probe implantation. Comparison of the preimplantation and postexplantation values allowed us to assess the stability of the telemeter across the implantation period.
The surgical approach for placement of the CPE into the medulla required prebending of the electrode tip. For implantation in the medulla, the ordinary straight CPEs were bent at a 90° angle, 5 mm from the electrode tip. This has the potential to alter the exposed carbon paste surface area and thus the calibration of the telemeter unit. Therefore, separate bench-top experiments were performed to investigate the effects of bending the electrodes ($n = 8$) on the calibration values. In addition, we also assessed the effects of increasing the osmolarity of the PBS solution, from 300 to 1,000 mosmol/l, in duplicate in each of 4 telemeter units. The medullary interstitium is a hyperosmotic environment, and its osmolarity can change under physiological conditions (20). Thus this experiment allowed us to determine whether the hyperosmotic nature of the medullary interstitium, or changes in medullary interstitial osmolarity induced by physiological or pathophysiological processes, might confound measurement of tissue PO$_2$. Before and after bending or increasing osmolarity, the telemeters were subjected to a five-point calibration at steps of 15.7 mmHg. Electrodes were placed into a sealed glass cell containing a ministirring bead and 20 ml of PBS saturated with N$_2$. Aliquots of 100% oxygen saturated solution were added to the cell ($+416, +425,+434, +443,$ and $+452 \mu l$) equating to PO$_2$ of 15.8, 31.5, 47.3, 63.1, and 78.9 mmHg. After addition of each individual aliquot, the solution was stirred for ~10 s, and after 3–4 min of equilibration, PO$_2$ was measured for 60 s to generate the calibration relationships. Calibration relationships were also generated, for five implants, in the absence and presence of epinephrine (1 $\mu M$) and norepinephrine (1 $\mu M$) to determine whether the presence of these catecholamines might confound measurements of medullary tissue PO$_2$.

At the conclusion of the chronic monitoring period (see below), the zero-offset of the CPE-telemeter system was assessed in vivo. Before explantation of the telemeter, each rat received an intraperitoneal injection of 60 mg/kg of pentobarbital to induce a surgical level of anesthesia. The rats then received a bolus of either 2 ml 0.25 g/ml of potassium chloride (by cardiac puncture, $n = 8$ Sprague Dawley) or an overdose of pentobarbital (intraperitoneal, $n = 6$ Wistar) to induce cardiac arrest. Renal tissue PO$_2$ rapidly approaches zero within 30 s of death (32). Thus we used the PO$_2$ measured after cardiac arrest as a measure of the offset value from the system. This offset value was subsequently subtracted from data collected in vivo before subsequent data analysis.

**Chronic studies: stability of tissue PO$_2$ and the reproducibility of responses to altered inspired oxygen content in conscious rats.** The aim of this series of experiments was to assess the stability of the measurement of PO$_2$ by the CPE-telemetry system in conscious rats over an extended period of time and to determine the reproducibility of responses to acute hypoxia and hyperoxia. Anesthesia was induced in Wistar rats ($n = 6, 354 \pm 14$ g) or Sprague-Dawley rats ($n = 8, 376 \pm 7$ g) with 5% vol/vol isoflurane administered via a nose cone and maintained at 2–2.5% vol/vol. Body temperature was maintained between 37 and 38°C by use of a heated operating table. Before implantation, the telemeters with electrodes attached were sterilized in a 2% wt/vol solution of glutaraldehyde for a minimum of 1 h, after which the glutaraldehyde solution was replaced with sterile saline. Adopting a sterile approach, the left kidney was exposed by laparotomy and pre-punctured at two locations (~5 mm apart) with a 30-gauge needle (until a depth of 5 mm was reached). The CPE and reference electrodes were then advanced so that their tips lay 5 mm below the surface of the cortex (Fig. 1A). The electrodes were secured onto the kidney by application of tissue glue (Histoacryl, 1050044; B. Braun) onto the surgical mesh (Textile Development Associates) covering the electrodes at the point of insertion (Fig. 1B). The coiled auxiliary electrode was also secured onto the kidney away from the CPE and reference electrode by application of

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**Fig. 1.** Schematic diagram of the fully implantable carbon paste electrode (CPE)-telemetry system for measurement of PO$_2$. **A:** carbon paste and reference electrodes were inserted in the renal medulla, while the auxiliary electrode was coiled and placed on top of the kidney. **B:** electrodes were held in place by a piece of surgical mesh glued to the renal capsule and the leads were sutured on the aorta. **C:** electrodes were attached to a telemeter that was implanted in the abdomen. **D:** each rat was placed on a SmartPad that enabled transcutaneous recharging and continuous recording.
Innovative Methodology

TELEMETRY METHOD FOR MONITORING OF KIDNEY OXYGENATION

Thick tissue glue on the surgical mesh covering it. The electrode wires to which the telemeter were attached were sutured to the adventitia of the abdominal aorta (Fig. 1A and B). The battery/transmitter unit of the telemeter was sutured to the muscular layer of the rat and secured in place (Fig. 1C). The incision was closed using silk sutures for the muscle and skin layer and either silk sutures or staples for the skin layer. The rats were placed on a heating pad for up to 12 h after surgery to aid recovery. Body weight, fluid intake, and general health were monitored regularly throughout this period and for the duration of the experimental protocol.

Renal tissue PO2 was then measured continuously while the rats were moving freely in their home cages. Recharging of the telemeter occurs via inductive power transfer, allowing continuous operation of the telemeter (Fig. 1D). Measurements of tissue PO2 were acquired using a data acquisition system (PowerLab 4/35, PL3504/P; ADInstruments) and analysis software (LabChart, MLU607; ADInstruments). Measurements of kidney PO2 were filtered with a 25-Hz low-pass filter, and artifacts and outliers were removed by applying a threshold of 3–190 mmHg/s (depending on the amplitude of the artifacts and outliers) for the first order derivative with a window width of 71 points of the raw signal.

After a period of 5 days to allow recovery from surgery, responses to altered inspired oxygen were assessed every 3–4 days for 2 wk (4 times in total). The rat’s home cage was placed within a Perspex chamber through which gases were passed at a rate of 5 l/min. Rats were subjected to 30-min periods in which they inspired hypoxic (10% O2-90% N2) and hyperoxic (100% O2) gas, corresponding to an PO2 of 76 and 757 mmHg, respectively. The hypoxic and hyperoxic challenges were presented in an order randomized between rats, and each challenge was preceded and followed by a 30-min period in which the rat was exposed to room air (151 mmHg). The O2 concentration in the sealed box was measured using a gas analyzer (ADInstruments). Kidney tissue PO2 was recorded throughout the experiment. In some animals (n = 4), video recordings of the rat’s locomotor activity were captured for 2.5 h during responses to altered inspired oxygen, using a webcam and video capture extension in LabChart. The aim of these video recordings was to test the possibility of coupling between locomotor activity and alterations in PO2.

Histology. Implantation of electrodes into the kidney has the potential to induce fibrotic responses, which could confound measurement of kidney tissue PO2. To assess the potential for this to occur, electrodes alone (i.e., without the telemeter unit, two in each kidney) were implanted into a subset of rats (n = 4) and after 3 wk, the kidneys were perfusion fixed with 3% wt/vol paraformaldehyde in phosphate-buffered saline (pH 7.4) and postfixed in 3% paraformaldehyde for 2 days. Tissue blocks were processed in paraffin, sectioned at 5 μm, stained with Masson’s trichrome, and viewed under an Olympus BX41 microscope (Olympus). For quantitative analysis, three random images from each kidney were assessed for the thickness of the fibrotic tissues surrounding the electrodes. Measurements were taken at four points (0, 90, 180, and 270° relative to the centre of the electrode position) and the fibrotic layer was considered to terminate once at its point of contact with a neighboring cell.

Calculation of oxygen tension. The CPE measures the molar concentration of oxygen (the amount of oxygen) by electrochemical reduction of the oxygen present in the fluid surrounding the tip of the electrode (2). During the calibrations, the exact molar concentration at each calibration step is dependent on the solubility of oxygen in the solution. Solubility depends on osmolality, temperature, and pH. Changes in the solubility will change the amount of oxygen that can be dissolved in the solution, which will change the electrical current measured (see Henry’s law in Ref. 3). However, with a constant gas partial pressure the partial oxygen pressure (PO2) will not depend on solubility. Therefore, and to enable the comparison with existing literature, oxygen recordings are reported as PO2 using: 1 μM = 0.631 mmHg.

Statistics. Ordinary least-squares regression analysis and analysis of covariance were used to determine whether the linear relationships between PO2 and current changed during the period of implantation (28). Between animal means ± SE of PO2 measured over each 24-h period were calculated. For studies of the effects of altered inspired oxygen, means ± SE of PO2, over the final 5 min of each 30-min period of gas exposure, were calculated. Measurements made serially over time were subjected to repeated-measures ANOVA (30). We tested the hypothesis that tissue PO2 varied over the period of telemeter implantation. The error mean square for within-subject variability in tissue PO2 was used for statistical power calculations to determine the ability of the CPE-telemeter technology to detect changes in kidney tissue PO2 under chronic conditions. We also used repeated-measures ANOVA to test the hypothesis that the magnitude of responses to altered inspired oxygen concentration changed over the period of implantation. Statistical analyses were performed using either SPSS Statistics 19 (IBM), SYSTAT (Version 13; Systat Software, Chicago, IL), or nQuery Advisor (Version 6.02; Statistical Solutions, Cork, Ireland).

RESULTS

Acute studies in anesthetized rats. In anesthetized rats the measurements of renal tissue PO2, obtained by both the Clark electrode and the CPE-telemeter system, responded rapidly to changes in inspired oxygen content (Fig. 2). There was relatively good agreement between the two methods, in absolute levels of PO2 at the three depths of electrode insertion, although at a depth of 2 mm this agreement was less convincing (Table 1).

Changes in tissue PO2, induced by ventilating the rats with hypoxic (18 and 10% oxygen) and hyperoxic (40 and 100% oxygen)
was good agreement between the calibration slope reported by

5.42 (12.6 to 23.4)/H11002

The corresponding changes for the CPE-telemetry system were

(62.4%) or 6 mm (79.2%).

measurements at 2 mm (43.6%) than for those derived at 4 mm

for less of the total variance in the data set derived from

three electrode depths. However, the lines of best fit accounted

provided no evidence of proportional or fixed bias at any of the

at each depth of electrode insertion, absolute values of tissue PO2 differed systematically between the Clark electrode and carbon paste electrode (CPE)-telemetry system. Because 3 comparisons were made (1 at each depth), the Bonferroni correction was applied with k = 3 (29). Across all 3 depths, PSmethod = 0.22 (df = 1,172).

oxygen) gas mixtures, were remarkably similar for the two

methods of measurement (Fig. 3). The linear regression relation-

ship had a slope of 0.90 with 95% confidence limits including unity (0.5–0.7) and an x-intercept of 1.6 ΔmmHg with 95% confidence limits including zero (−0.9 to 4.1) ΔmmHg. This significant relationship accounted for 55% of the variance in the total data set (n = 73).

Individual regression analyses were performed for the data

at each depth of electrode insertion (Table 2). These analyses

provided no evidence of proportional or fixed bias at any of the

three electrode depths. However, the lines of best fit accounted

for less of the total variance in the data set derived from

measurements at 2 mm (43.6%) than for those derived at 4 mm

(62.4%) or 6 mm (79.2%).

The CPE-telemetry system and the Clark electrode were able
to detect small changes in tissue PO2 when the ventilation gas
was changed from 21 to 18% oxygen. The mean (95% confidence limits) changes measured by the Clark electrode were 7.5 (−17.6 to 32.7) ΔmmHg at 2 mm, −4.7 (−8.6 to −0.63) ΔmmHg at 4 mm, and −6.7 (−8.1 to −0.6) ΔmmHg at 6 mm. The corresponding changes for the CPE-telemetry system were 5.42 (−12.6 to 23.4) ΔmmHg at 2 mm, −3.34 (−6.2 to −0.4) ΔmmHg at 4 mm, and −4.8 (−5.8 to −3.9) ΔmmHg at 6 mm.

Chronic studies: CPE-telemetry system calibration. There was good agreement between the calibration slope reported by

the manufacturer of the electrode (BlueBox Sensors) and that determined in our laboratory before implantation (preimplantation, not shown). However, the slope of the calibration curve after electrode explantation (postexplantation) was considerably greater than that determined before telemeter implantation (P = 0.01; Fig. 4) and that provided by the manufacturer (P = 0.02). Furthermore, the current measured under anoxic conditions was negligible before telemeter implantation (−5.3 ± 2.6 nA) but not after explantation (−67.6 ± 14.6 nA). Postimplantation calibration relationships were only determined for a subset of the telemeters used in this study. Consequently, all values of tissue PO2 reported herein are based on the preim-

plantation calibration relationship, unless otherwise stated (i.e., see Fig. 7A).

Neither bending the CPE tip nor exposing the CPEs to

hyperosmolarity significantly altered the calibration of the

CPE. Before bending the electrode, the linear regression relation-

ship had a slope of −1.68 nA/mmHg and an x-intercept of −7.03 nA. After bending, the corresponding linear regression coefficients were −1.62 nA/mmHg and −7.49 nA, respectively (Fig. 5A). Under isosmotic conditions (300 mosmol/l), the calibration relationship had a slope of −1.37 nA/mmHg and an x-intercept of −2.53 nA. Under hyperosmotic conditions (1,000 mosmol/l), the corresponding regression coefficients were −1.34 nA/mmHg and −5.41 nA, respectively (Fig. 5B). Neither epinephrine (1 μM) nor norepinephrine (1 μM) altered the calibration relationships between oxygen content and current. Under baseline conditions, the calibration relationship had a slope of −1.53 nA/mmHg and an x-intercept of −5.64 nA. During exposure to epinephrine, the corresponding regression coefficients were −1.38 nA/mmHg and −7.39 nA, respectively; and during exposure to norepinephrine, the corresponding regression coefficients were −1.69 nA/mmHg and −3.41 nA, respectively (Fig. 5C).

With the use of the preimplantation calibration relationships, the kidney PO2 measured under anesthesia by the CPE-telemeter system varied widely between rats, the maximum value being 271 mmHg and the lowest being 11 mmHg, with a mean of 88 ± 16 mmHg. After cardiac arrest, the measured value of kidney tissue PO2 reduced sharply in all of the rats until it reached a plateau value which averaged 18 ± 5 mmHg. This represents the zero offset of the telemeter system, since renal tissue PO2 rapidly approaches zero after cardiac arrest (32). Consequently, all values of tissue PO2 reported below have had this zero offset value subtracted from them.

Table 1. Absolute values of kidney tissue PO2 in anesthetized rats

<table>
<thead>
<tr>
<th>Inspired Oxygen, %</th>
<th>Clark Depth, 2 mm</th>
<th>CPE</th>
<th></th>
<th>Clark Depth, 4 mm</th>
<th>CPE</th>
<th></th>
<th>Clark Depth, 6 mm</th>
<th>CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.8 ± 2.8</td>
<td>8.6 ± 3.9</td>
<td></td>
<td>3.6 ± 2.0</td>
<td>3.4 ± 1.3</td>
<td></td>
<td>7.3 ± 3.0</td>
<td>6.4 ± 2.3</td>
</tr>
<tr>
<td>18</td>
<td>19.2 ± 4.4</td>
<td>10.0 ± 3.3</td>
<td></td>
<td>21.9 ± 5.3</td>
<td>15.3 ± 3.3</td>
<td></td>
<td>21.1 ± 4.7</td>
<td>26.6 ± 7.6</td>
</tr>
<tr>
<td>21</td>
<td>24.2 ± 3.3</td>
<td>16.0 ± 4.2</td>
<td></td>
<td>24.0 ± 4.9</td>
<td>19.4 ± 3.1</td>
<td></td>
<td>25.4 ± 3.2</td>
<td>28.2 ± 5.8</td>
</tr>
<tr>
<td>40</td>
<td>46.6 ± 5.9</td>
<td>26.1 ± 5.1</td>
<td></td>
<td>32.6 ± 4.2</td>
<td>26.9 ± 2.7</td>
<td></td>
<td>31.2 ± 3.3</td>
<td>34.6 ± 5.4</td>
</tr>
<tr>
<td>100</td>
<td>46.6 ± 9.6</td>
<td>54.0 ± 2.8</td>
<td></td>
<td>22.3 ± 5.1</td>
<td>31.0 ± 3.2</td>
<td></td>
<td>34.5 ± 4.7</td>
<td>36.4 ± 5.6</td>
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</table>

PMethod = 0.21 (df = 1,46). Values are the means ± SE of tissue PO2 (mmHg, n = 7). PMethod is the outcome of analysis of variance, testing the specific hypothesis that, at each depth of electrode insertion, absolute values of tissue PO2 differed systematically between the Clark electrode and carbon paste electrode (CPE)-telemetry system. Because 3 comparisons were made (1 at each depth), the Bonferroni correction was applied with k = 3 (29). Across all 3 depths, PMethod = 0.22 (df = 1,172).

Fig. 3. Changes in kidney tissue PO2 in anesthetized rats, measured by Clark electrode and the CPE-telemetry system. Kidney PO2 was changed by altering inspired oxygen content. Measurements were made sequentially at different depths below the cortical surface. Symbols represent individual data points. Solid line represents the line of best fit, calculated by ordinary least products regression analysis (r = 0.74, P < 0.001, PO2,telemetry = 1.6 + 0.90 * PO2,clark).
Table 2. Regression coefficients and Pearson product moment correlation coefficients for relationships between the 2 methods of measurement at the 3 depths of electrode insertion

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>a</th>
<th>a (Low)</th>
<th>a (High)</th>
<th>b</th>
<th>b (Low)</th>
<th>b (High)</th>
<th>Pearson’s r</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>22</td>
<td>2.7</td>
<td>(−2.8)</td>
<td>(8.52)</td>
<td>0.79</td>
<td>(0.53)</td>
<td>(1.11)</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>2.8</td>
<td>(−0.3)</td>
<td>(5.7)</td>
<td>0.83</td>
<td>(0.64)</td>
<td>(1.23)</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>0.5</td>
<td>(−2.0)</td>
<td>(3.2)</td>
<td>1.27</td>
<td>(0.96)</td>
<td>(1.50)</td>
</tr>
<tr>
<td>Overall</td>
<td>73</td>
<td>1.6</td>
<td>(−0.9)</td>
<td>(4.1)</td>
<td>0.90</td>
<td>(0.72)</td>
<td>(1.14)</td>
</tr>
</tbody>
</table>

Tissue PO₂ was measured simultaneously by CPE and Clark electrode. Values of a and b refer to the relationship PO₂,CPE = a + b * PO₂,Clark determined by ordinary least squares regression analysis. (Low) and (High) refer to the calculated 95% confidence limits for a and b.

Chronic studies: stability of tissue PO₂ in conscious rats. Repeated-measures ANOVA revealed no significant difference between the mean medullary tissue PO₂ measured in the Wistar rats compared with the Sprague-Dawley rats (P = 0.99). Hence, the observations in the two strains were analyzed as a single data set.

Most of the within-rat variability occurred within the first 6 h after surgery, during which renal medullary tissue PO₂ gradually increased. With the use of the preimplantation calibration relationship and subtracting the zero offsets found during cardiac arrest, medullary tissue PO₂ increased on average from 72 ± 16 mmHg during the first hour postimplantation to 97 ± 14 mmHg after 24 h (Fig. 6). Within rat averages were clearly outside the range of previous estimates of medullary kidney tissue PO₂, 10–40 mmHg in most cases (7, 9, 15, 21, 23). Using the postimplantation calibration relationships determined in a subset of animals did not bring the values in the realm of previously recorded physiological values, with PO₂ averaging 54 ± 5 mmHg after 24 h (Fig. 7A). Therefore, for subsequent analysis of the complete data set the recorded values of each individual rat were normalized to the mean levels obtained between days 5 and 19 after implantation. All values were derived using the preimplantation calibration relationship and subtraction of the zero offsets (Fig. 7B). This approach demonstrated that the measured PO₂ was relatively labile during the first 5 days after implantation but thereafter remained relatively stable for the subsequent 14 days, although there was a tendency for the measured PO₂ to increase from 95 ± 6% of baseline on day 14 to 122 ± 12% when the experiment was terminated on day 19.

To translate how the stability and reproducibility of the CPE-telemetry system in untreated rats can facilitate researchers to study long-term trends in kidney tissue oxygenation, we performed power calculation. Power calculations were performed using values of medullary tissue PO₂ obtained over days 13–19 after implantation, derived using preimplantation calibration relationships and subtracting the zero offsets. We examined two scenarios. The first was a transient divergence of medullary PO₂ in an intervention group compared with a control group, of 25 or 40% on day 14 and 12% on day 15, with values returning to those of the control group from days 16 to 19 (transient effect scenario). The second scenario was a slowly developing effect, commencing from day 14, reaching a peak effect of 24 or 40% on day 19 (gradual effect scenario). nQuery Advisor 6.02 was used to estimate minimal group size for 80% power to attain P = 0.05 for the group × time interaction and applying the Greenhouse-Geisser adjustment (30). Minimum sample size was calculated at 15 per group or 8 per group for transient effects of 25 or 40%, respectively. For the gradual effect scenario, groups sizes of 17 or 9 would be required for changes of 25 or 40%, respectively.

Chronic studies: reproducibility of responses to altered inspired oxygen content in conscious rats. This analysis was performed on data using the preimplantation calibration relationship and subtraction of the zero offsets before normalization of medullary PO₂ to the average value during inspiration of room air. When rats were exposed to hyperoxia, kidney tissue PO₂ increased by 60 ± 20%. When rats were exposed to hypoxia kidney tissue PO₂ decreased by 58 ± 5%. The magnitude of responses to altered inspired oxygen concentration did not significantly change over the period of implantation (i.e., across the 4 trials carried out at 3- to 4-day intervals) (P = 0.24; Fig. 8). The video recordings of the rats during these experiments did not reveal any overt correlation between locomotor activity and the signal from the telemeter.

Histology. Renal morphology in rats after 3 wk of electrode implantation appeared to be normal except for a relatively thin layer of fibrotic tissues (50.7 ± 3.6 μm) in the immediate proximity of the implanted electrodes (Fig. 9). As the kidneys were perfusion fixed, the tubules had a dilated appearance.

DISCUSSION

We report the development and validation of a novel technology combining a rechargeable telemeter (using wireless power transfer; Ref. 8) coupled with a CPE to allow for the continuous and long-term assessment of kidney tissue PO₂ in the freely moving rat. We were able to record a relatively...
stable level of medullary tissue PO2 over a 19-day period. We were also able to show reproducible responses of medullary tissue PO2 to systemic hypoxia and hyperoxia across the 3-wk period of implantation. Currently, the technology does have the limitation that it only provides relative changes in, rather than absolute levels of, kidney tissue PO2. Nevertheless, we propose that this technology provides a means to study the temporal relationships between tissue hypoxia and renal dysfunction and cellular damage to better understand the contribution of hypoxia to the initiation and progression of renal disease.

Fig. 5. Relationships between current measured by the telemeter and PO2, and the effects of bending the electrode, altering the osmolarity of the calibration solution or adding epinephrine or norepinephrine. A: average calibration relationship ($n = 8$) before (○) and after bending (●). B: average relationship when the calibration was performed in solutions with osmolarities of 300 mosmol/l (●) and 1,000 mosmol/l (○). C: average relationship when the calibration was performed in the absence (○) or presence of 1 μM epinephrine (●) or 1 μM norepinephrine (●). Dashed lines show lines of best fit determined by ordinary least squares regression analysis. Before bending: current = $-7.03 - 1.68 \times PO2$; and after bending: current = $-7.49 - 1.62 \times PO2$. At 300 mosmol/l: current = $-2.53 - 1.37 \times PO2$; and at 100 mosmol/l: current = $-5.41 - 1.34 \times PO2$. In the absence of catecholamines: current = $-5.64 - 1.53 \times PO2$; in the presence of 1 μM epinephrine: current = $-7.39 - 1.38 \times PO2$; and in the presence of 1 μM norepinephrine: current = $-3.41 - 1.69 \times PO2$.

Fig. 6. Medullary tissue PO2 over the 24 h following telemeter implantation. Values were derived using the preimplantation calibration relationship and subtraction of zero offset values and are shown as means ± SE of all 14 rats.

Fig. 7. Mean daily medullary tissue PO2 across a 19-day period. A: subgroups of animals ($n = 6$) in which telemeter calibration relationships were determined before implantation and after explantation. ○: Data derived using the preimplantation calibration relationship. ●: Data derived using the postexplantation calibration relationship. All values presented are after subtraction of the zero offset values. B: medullary tissue PO2 across a 19-day period, expressed as a percentage of the average values on days 5–19 of all 14 rats. All data are shown as means ± SE.
Our initial studies focused on the validity of the CPE-telemetry system. We found, in anesthetized rats, that the CPE-telemetry system exhibited no fixed or proportional bias when compared with the gold standard Clark electrode. We also found that neither electrode attachment nor the bending of the electrode before implantation altered the calibration of the CPE. Moreover, the stability of the CPE-telemeter calibration relationship in the face of changes in the osmolarity of the calibration fluid provided evidence that the hyperosmolarity of the medullary interstitium, and the potential for medullary osmolarity to change in response to physiological and pathophysiological challenges, would not confound our measurements.

Subsequent studies focused on the utility of the CPE-telemetry system for chronic measurement of renal medullary tissue PO2 in unrestrained rats. CPEs have previously been used extensively in neuroscience for the monitoring of PO2 in a variety of brain regions (4–6, 24–26, 38). Most recently, we have used the telemetry system to chronically measure PO2 in the rat striatum and observed values in the normal physiological range (38). In contrast, values of medullary tissue PO2 estimated in the current study were considerably greater than those reported previously in anesthetized rats (7, 9, 15, 21, 23). The overestimation of medullary PO2 using the CPE-telemetry approach appears to be partly due to changes in the calibration parameters for the CPE, since the current measured at any particular PO2 in vitro was greater after telemeter explantation than before implantation. There also appears to be a zero offset applicable to the in vivo setting, since a positive value of medullary tissue PO2, averaging 18 ± 5 mmHg, was measured at death, when we know kidney tissue PO2 is negligible (32).

Two observations indicate that these changes in the performance of the CPE-telemetry system occur relatively soon after implantation. Firstly, there was good agreement between the CPE-telemetry system and the gold standard Clark electrode in our acute studies. Secondly, variability in the measured medullary tissue PO2 was most marked in the first 6 h after implantation and on average increased over the first 24 h of implantation. Indeed, in rats subjected to no interventions other than implantation of a CPE-telemeter unit, measured medullary

![Fig. 8. Responses of medullary tissue PO2 to altered inspired oxygen content.](image)

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![Fig. 9. Representative kidney section stained with Masson’s trichrome of rats after 3 wk of electrode implantation.](image)
tissue PO₂ remained relatively stable over the period 5–19 days after implantation.

What factors could account for the overestimation of medullary tissue PO₂ by the CPE-telemetry system? Bolger et al. (5) have previously demonstrated the lack of impact on CPE current of a range of substances that might be expected in brain or kidney tissue, including ascorbic acid (500 μM), homovanillic acid (10 μM), L-glutathione (50 μM), L-cysteine (50 μM), uric acid (50 μM), serotonin (10 nM), L-tryptophan (100 μM), dehydroascorbic acid (100 μM), L-tyrosine (100 μM), dopamine (50 nM), 3,4-dihydroxyphenylacetic acid (20 μM), and 5-hydroxyindoleacetic acid (50 μM). Furthermore, in the current study we established that neither norepinephrine (1 μM) nor epinephrine (1 μM) interfere with the telemeter unit calibration. Nevertheless, the environment of the renal medulla differs from that of the brain in a number of ways. Firstly, the interstitium of the medulla, but not the brain, is hyperosmotic (39, 42). We were able to exclude this as a potential confounding factor, because calibration relationships for the CPE-telemetry units were little affected by osmolarity in vitro. A second important difference between the environments of the brain and renal medulla is that lipids are far more abundant in the brain than in the renal medulla (17, 40). Differences in cellular and interstitial content between the kidney and brain may therefore have affected the interactions between the silicon oil and the tissue surrounding the electrodes. It has been documented that the presence of electroactive species, lipids, and proteins can interfere with the function of CPEs (5, 19, 34). The difference in lipid, protein, and electroactive species profiles between the brain and kidneys may result in a difference in electrode-tissue reaction and hence the apparent overestimation of medullary tissue PO₂ by the CPE-telemetry system.

Since we were unable to identify the causes of the altered calibration of the CPE-telemetry system after implantation, and the reasons for the presence of an additional zero offset under in vivo conditions, we subsequently used relative values in our analysis instead of absolute concentration. This offset places restraints on study design, because it makes direct between-animal comparisons problematic. However, it does not limit the use of within-animal experimental designs in which changes in PO₂ are determined from a baseline established before the intervention.

The CPE-telemetry system can detect relatively small changes in kidney tissue PO₂. We have previously found that the threshold for detection of reduced cortical and medullary tissue oxygen tension induced by hypoxemia in anesthetized rabbits, by both Clark electrodes and fluorescence optodes, is crossed at an inspired oxygen concentration of 17–18% (12). This equates to an oxygen partial pressure at altitudes of only ~1,500–2,000 m. Our current findings in anesthetized rats indicate that the CPE-telemetry system is at least as sensitive as the Clarke electrode to changes in renal tissue PO₂, as assessed by the response to ventilation with 18% oxygen. In conscious rats, after chronic implantation of a CPE-telemetry unit, reductions in medullary tissue PO₂ induced by exposure to 10% oxygen, and increases in medullary tissue PO₂ induced by exposure to 100% oxygen were reproducible over a period of 2 wk. Thus the CPE-telemetry system appears to be well suited for application to experimental paradigms in which acute responses to test stimuli are repeatedly assessed in conscious rats in a within-animal design.

The CPE-telemetry system also has the potential to detect changes in medullary tissue PO₂ over longer periods of days to weeks. Power calculations based on data collected over days 13–19 after telemeter implantation indicated that group sizes of 15 would be required to detect a transient 25% change in medullary tissue PO₂ occurring over 2 days, while group sizes of 17 would be required to detect a slowly developing change of 25%. Fewer animals would be required for detection of larger (40%) transient (n = 8) or slowly developing (n = 9) changes.

There is clearly room for improvement of the system. One potential refinement would be the implantation of an inflatable cuff around the renal artery at the time of telemeter implantation. This would allow the zero offset of the telemeter to be assessed periodically across the course of an experiment to assess the potential for baseline drift. Unfortunately, it is not possible to calibrate the CPE-telemetry system, or any other system for measurement of kidney tissue PO₂, under in vivo conditions.

To assess whether the implantation of electrodes in the kidney induces inflammatory and/or fibrotic responses, electrodes without the telemeter unit were implanted in a subgroup of rats into the left and right kidneys. Kidney histopathology was examined 3 wk after electrode implantation. The presence of only a thin (~50 μm) layer of fibrotic tissue surrounding the electrodes after 3 wk suggests that any tissue reaction that arises from electrode implantation does not confound kidney tissue PO₂. The stability of tissue kidney tissue PO₂ measurements also supports the proposition that the implantation of electrodes did not result in significant tissue injury, which would otherwise have resulted in a transient change in kidney tissue PO₂.

Conclusions and future directions. A new telemetric method for long-term monitoring of renal medullary oxygenation has been developed. Currently, the method can only detect relative changes in medullary oxygen tension, but further development should allow measurement of absolute values. This exciting technology opens up a unique opportunity to examine the overall temporal relationships between tissue hypoxia and the progression of both acute and chronic renal disease. The system provides uninterrupted data monitoring in freely moving rats and constitutes a new tool that enables researchers to study long-term trends in kidney tissue oxygenation. Future applications of this technology should include investigations of the role of renal tissue hypoxia in the pathogenesis of acute kidney injury and chronic kidney disease. For example, Fine et al. (14) identified two critical unproven elements of their “chronic hypoxia” hypothesis for the pathogenesis of chronic kidney disease. Their hypothesis predicts that hypoxia should be 1) present during the early stages of kidney disease, before histological evidence of scarring; and 2) persist throughout the course of the disease. Thus our new method provides a means to further test their hypothesis. On the other hand, given the presence of some variation in PO₂ measured over periods of weeks in the current studies, it may be more efficient to first focus on acute kidney injury induced by insults such as ischemia-reperfusion injury, sepsis, and radiocontrast administration (13).
REFERENCES


