Pronounced kidney hypoxia precedes albuminuria in type 1 diabetic mice

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Franzén S, Pihl L, Khan N, Gustafsson H, Palm F. Pronounced kidney hypoxia precedes albuminuria in type 1 diabetic mice. Am J Physiol Renal Physiol 310: F807–F809, 2016. First published March 2, 2016; doi:10.1152/ajprenal.00049.2016.—Intrarenal tissue hypoxia has been proposed as a unifying mechanism for the development of chronic kidney disease, including diabetic nephropathy. However, hypoxia has to be present before the onset of kidney disease to be the causal mechanism. To establish whether hypoxia precedes the onset of diabetic nephropathy, we implemented a minimally invasive electron paramagnetic resonance oximetry technique using implanted oxygen sensing probes for repetitive measurements of in vivo kidney tissue oxygen tensions in mice. Kidney cortex oxygen tensions were measured before and up to 15 days after the induction of insulinopenic diabetes in male mice and compared with normoglycemic controls. On day 2, kidney hypoxia developed pronounced intrarenal hypoxia 3 days after the induction of diabetes, which persisted throughout the study period. On day 16, diabetic mice had glomerular hyperfiltration, but normal urinary albumin excretion and normal blood pressure. Kidney oxygen tensions were determined to define the temporal relationship between intrarenal hypoxia and disease development. Diabetic mice developed pronounced intrarenal hypoxia 3 days after the induction of diabetes, which persisted throughout the study period. On day 16, diabetic mice had glomerular hyperfiltration, but normal urinary albumin excretion. In conclusion, intrarenal tissue hypoxia in diabetes precedes albuminuria thereby being a plausible cause for the onset and progression of diabetic nephropathy.

Diabetic nephropathy; kidney tissue oxygen tensions; intrarenal hypoxia

Diabetic nephropathy is a common cause for end-stage renal disease (9), affecting approximately one-third of all diabetes patients. Although substantial efforts have been directed toward identifying the mechanisms, surprisingly little is known about the pathways preceding diabetic nephropathy. However, intrarenal tissue hypoxia has been proposed as a unifying mechanism for chronic kidney disease (10), including diabetic nephropathy (5). Indeed, intrarenal hypoxia per se, without confounding factors such as hyperglycemia, oxidative stress, or hypertension, is sufficient to initiate the development of chronic kidney disease (4). To solidify intrarenal hypoxia as a mechanism for chronic kidney disease, it is pivotal that hypoxia develops before even the earliest sign of nephropathy. Although previous efforts have addressed this issue, it has proven difficult to repetitively determine absolute kidney oxygen tensions. In this study, we utilized a novel technique allowing for repetitive measurements of absolute intrarenal oxygen levels in vivo to investigate the development of diabetes-induced intrarenal hypoxia compared with development of kidney disease.

Materials and Methods

Animals and materials. Diabetes was induced by a single intravenous injection of alloxan (75 mg/kg) in male NMRI mice (n = 13) and the results were compared with the corresponding normoglycemic controls (n = 12). All animals were given food and water ad libitum and all procedures were approved by the local ethical committee and performed according to national guidelines for the care and use of experimental animals.

Repetitive measurements of kidney oxygen tension using EPR oximetry. Oxygen-sensing lithium phthalocyanine (LiPc) probes were synthesized at the EPR Center, Geisel School of Medicine, and aggregates of LiPc crystals were loaded into 23-gauge needles to produce a solid pellet, as previously described (6). A single LiPc pellet was implanted into the left kidney cortex of each mouse as described previously (3).

Nine days thereafter, mice were anesthetized by Isoflurane (2% in air) and placed in the electron paramagnetic resonance (EPR) spectrometer for measurements of baseline oxygen tension using EPR oximetry (7). Diabetes was induced 2 days later with the use of alloxan. Additional measurements of oxygen tensions were performed 3, 11, and 15 days after the induction of diabetes.

Measurements were performed using a Bruker Elexsys ES450 L band EPR spectrometer equipped with an ES450 GCL Triple axis coil set (gradient field strength up to 40 G/cm) and an ES450 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. No EPR signal could be detected for the empty resonator. The recorded EPR spectra were imported into MATLAB and peak-to-peak line widths were analyzed using an in-house developed MATLAB script. Oxygen tensions were calculated by comparison of the EPR line width for the LiPc probe with spectra obtained from a calibration probe made from the same batch and measured at different oxygen tensions.

Urinary albumin excretion and glomerular filtration rate. On day 16 after the induction of diabetes, all mice were placed in metabolic cages to determine 24-h urinary albumin excretion to determine 24-h urinary albumin excretion (Mouse Albumin ELISA Kit; Bethyl Laboratories, Montgomery, TX) corrected for creatinine (Abbott Laboratories, Abbott Park, IL). Conscious glomerular filtration rates were determined as previously described (2).

Statistical analysis. Repeated-measurements 2-by-2 ANOVA followed by Bonferroni post hoc test was used to analyze differences in kidney oxygen tensions between control and diabetic mice over time. Unpaired Student’s t-test was used to analyze differences in blood glucose, urinary albumin excretion, and glomerular filtration rates between the two groups. All data are displayed as means ± SE and P < 0.05 was considered significant.
RESULTS AND DISCUSSION

We have taken advantage of a novel in vivo EPR technique to repeatedly monitor tissue oxygenation using implantable oxygen-sensing LiPc particulates. By monitoring oxygen levels in the kidney cortex of normoglycemic control mice before and immediately after the onset of chemically induced insulinopenic diabetes, we can demonstrate that a pronounced intrarenal hypoxia develops within the first 3 days after diabetes in this animal model (Fig. 1). Intrarenal hypoxia persisted throughout the 15-day study period. Furthermore, the urinary albumin excretion on day 16 after the onset of diabetes was not significantly different compared with normoglycemic control mice (Fig. 2), even though the diabetic mice had a significant glomerular hyperfiltration (Fig. 3). We previously reported a progressive increase in diabetes-induced glomerular leakage in several different mouse strains (2). An increase in urinary protein excretion was evident during week 5 to week 10 after the onset of diabetes.

Previous studies also reported reduced kidney oxygenation within the first few days after diabetes onset in rats using both blood oxygen level-dependent (BOLD) magnetic resonance imaging and fluorescence optodes (1, 13). Furthermore, Manciotham and co-workers (8) also reported pronounced intrarenal hypoxia in the early phase in the remnant kidney model using pimonidazole staining and hypoxia-inducible factor (HIF) expression analysis. However, to the best of our knowledge, the present study is the first to report pronounced intrarenal hypoxia in early diabetes using a technique allowing for repetitive quantification of absolute oxygen tensions during the disease progression.

The nature of the EPR oximetry technique using implanted oxygen-sensing probes allows for precise determination of absolute tissue oxygen tensions in a defined space. The line width of EPR signal from the LiPc probe is proportional to the oxygen availability and the technique does not consume oxygen per se. The implanted LiPc probe is likely to eventually induce fibrosis development which potentially could influence oxygen diffusion and thus also the accuracy of the measurement. However, in a previous validation effort we demonstrated that both cortical and medullary measurements are stable for at least 25 days (3), which is well within the time frame of the current study.

It has previously been reported that increased kidney oxygen consumption, secondary to mitochondrial leak respiration and inefficient tubular sodium transport, results in intrarenal hypoxia in diabetes due to lack of metabolic control of renal blood flow (5, 12). Interestingly, the development of intrarenal hypoxia is not paralleled by increased expression of HIFs. It has been reported that defective HIF signaling in hyperglycemia may be due to covalent modification of the coactivator p300 (14) and we demonstrated that pharmacological activation of the HIF system during the induction of diabetes prevents diabetic nephropathy via normalization of kidney oxygen metabolism (11).

In conclusion, in this study we provide further evidence for a potential role of intrarenal tissue hypoxia in diabetic nephropathy since pronounced intrarenal hypoxia develops already within the first 3 days after the onset of hyperglycemia. This finding also implies that it may be useful to monitor intrarenal
oxygen levels in diabetic patients to select those who should receive closer monitoring since intrarenal hypoxia can be utilized as an early biomarker of developing kidney disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.F., N.K., H.G., and F.P. conception and design of research; S.F. and L.P. performed experiments; S.F., L.P., and H.G. analyzed data; S.F., L.P., N.K., H.G., and F.P. interpreted results of experiments; S.F. drafted manuscript; S.F., L.P., N.K., H.G., and F.P. approved final version of manuscript; F.P. prepared figures.

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