Published in final edited form as:

Adv Exp Med Biol. 2013 ; 765: 329–334. doi:10.1007/978-1-4614-4989-8_46.

Renal Oxygenation and Function of the Rat Kidney: Effects of Inspired Oxygen and Preglomerular Oxygen Shunting

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Abstract

We investigated the hypothesis that a preglomerular diffusional shunt for O₂ stabilized renal PO₂ and that changes in intrarenal PO₂ determined nephron nitric oxide (NO) availability for blunting of the tubuloglomerular feedback (TGF) response. The inspired O₂ content of anesthetized rats was changed from normal (21%) to low (10%) or high (100%) for 30–45 min. Direct recordings of PO₂ in the lumens of proximal and distal tubules demonstrated significantly ($P < 0.05$) lower values at all sites in spontaneously hypertensive rats compared to normotensive Wistar Kyoto (WKY) rats. Low inspired O₂ did not change intratubular PO₂, but high inspired O₂ increased PO₂ modestly (25–50%; $P < 0.01$) in both strains and at both sites. Addition of 7-nitroindazole (7-NI; 10⁻⁴ M) to artificial tubular fluid perfusing the loop of Henle of WKY nephrons to block neuronal (type 1) nitric oxide synthase in the macula densa increased TGF but this increase was less ($P < 0.01$) in nephrons of rats breathing high vs. normal inspired O₂ (1.8 ± 0.4 vs. 3.4 ± 0.3 mmHg; $P < 0.01$). In conclusion, the PO₂ in the renal tubules was effectively buffered from even extreme changes in arterial PO₂, consistent with a functionally important preglomerular O₂ diffusional shunt. However, high inspired PO₂ increased intratubular PO₂ sufficiently to blunt the effects of NO derived from the macula densa, likely reflecting bioinactivation of NO by reactive oxygen species generated at increased PO₂ levels. Thus, the preglomerular diffusional shunt appeared to stabilize intrarenal PO₂ during changes in arterial oxygen and to protect NO signaling within the kidney.

Keywords

Kidney; Oxygenation; Shunting

1 Introduction

We used an ultramicro platinum-iridium microelectrode to measure the PO_2 in the outer cortex of the rat's kidney. The PO_2 values averaged 40–42 mmHg in the proximal and distal tubules, 30 mmHg in the outer medulla and 48 mmHg in the efferent arteriole. Since these values were clearly lower than the mean value of 55 mmHg recorded simultaneously in the renal vein [1], we concluded that a preglomerular diffusional O_2 shunt, likely between the arcuate and/or interlobular arteries and veins, limited the supply of O_2 to the renal tissues. However, its functional significance remained unclear. The prime function of a shunt is to stabilize down-stream gas tensions. Therefore, we measured the PO_2 within the rat's kidney during short-term (30–45 min) changes in inspired O_2 from normal (21%) to low (10%) or high (100%) values in normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) since the latter have reduced intrarenal PO_2 related to oxidative stress [1].

To further evaluate the functional significance of a shunt that restricted PO_2 in the renal tissues, we investigated intrarenal nitric oxide (NO) signaling at normal and high inspired O_2 . NO is bioinactivated by superoxide anion ($O_2^{\bullet-}$). $O_2^{\bullet-}$ production from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase increased with PO_2 [2]. NO produced by neuronal nitric oxide synthase (nNOS) in the macula densa blunted the tubuloglomerular feedback (TGF) response in normal rats, but this effect was lost in SHR because of inactivation of NO by $O_2^{\bullet-}$ [1, 3]. To test the role of inspired O_2 on intrarenal NO, the loop of Henle (LH) was perfused with artificial tubular fluid (ATF) while measuring the proximal stop flow pressure (PSF) upstream from a wax block in the proximal tubule during the addition of vehicle or 7-nitroindazole (7-NI; 10^{-4} M) to block nNOS in the macula densa. The degree to which the TGF was increased by blockade of nNOS was used as a marker of intra-renal NO signaling from the tubules (macula densa) to the afferent arterioles. We compared the effects of nephron perfusion of 7-NI while rats inspired normal O_2 or high O_2 to test the hypotheses that a function of the O_2 shunt was to reduce the normal levels of intrarenal O_2 sufficiently to preserve NO signaling within the kidney.

2 Methods

The methods to measure PO_2 in proximal and distal tubules with an ultramicro, coaxial, platinum/iridium micropipette [1] and to study the role of NO from macula densa nNOS in the regulation of TGF [1, 3] have been published. In brief, adult male Sprague Dawley rats (Harlan, Madison, WI, USA) were anesthetized with thiobarbital (Inactin, 100 mg/kg intraperitoneally; Research Biochemicals Inc., Natick, MA, USA). A catheter was placed in a jugular vein for fluid infusion and in a femoral artery for recordings of mean blood pressure (MAP). The bladder was catheterized and a tracheotomy tube inserted to facilitate breathing. The left kidney was exposed by a flank incision and immobilized in a Lucite cup. After surgery, the rats were infused with saline solution containing 1% bovine serum albumin at a rate of 1.5 mL/h and the studies begun after 60 min of stabilization. Micropuncture measurements to determine intrarenal PO_2 and proximal tubular stop flow pressure (PSF) were conducted while the rats inhaled three different gas mixtures containing 10, 21, and 100% oxygen, respectively. We perfused the loop of Henle (LH) with ATF while measuring the PSF during the addition of vehicle or 7-NI (10^{-4} M) to block nNOS.

3 Results

Values for normal PO_2 in proximal and distal tubules [1] and TGF responses to 7-NI in SHR and WKY rats [1, 3] were similar to our previous publications. PO_2 values were lower in SHR than WKY, confirming a previous report [1] (Fig. 46.1). At 30–45 min after reducing the inspired O_2 from 21% (air) to 10%, there were no significant changes in the PO_2 recorded in the proximal or distal tubules of either WKY or SHR rats. After switching from

normal to 100% inspired O₂, there were significant increases at both sites and in both strains. However, despite a fivefold increase in inspired O₂, the intratubular PO₂ values increased on average by only 25–50%.

The TGF was quantitated from the reduction in PSF (an index of glomerular capillary pressure) recorded by a pressure pipette upstream from a wax block in the proximal tubule of the test nephron during perfusion of its macula densa segment from the late proximal tubule with ATF. The addition of 7-NI to ATF increased the TGF by 3.4 ± 0.3 mmHg in rats breathing room air similar to prior reports [1, 3]. After changing from normal to low inspired O₂, there were no significant changes in the response to 7-NI. However, 30–45 min after changing from normal to 100% inspired O₂, the increase of TGF with nephron perfusion of 7-NI was reduced significantly by 50% to 1.8 ± 0.4 mmHg ($P < 0.01$). This indicated that an increase in intratubular PO₂ above normal values during breathing of 100% O₂ reduced NO signaling within the renal cortex.

4 Discussion

The main new findings of this study were that the directly measured intranephron PO₂ values in the outer cortex of the rat's kidney were sufficiently buffered from contemporary changes in inspired O₂ that there were no significant reductions in PO₂ in the proximal or distal tubules of normal or hypertensive rats when the inspired O₂ was reduced from 21 to 10%. Moreover, during a fivefold increase in inspired O₂ from 20 to 100%, the increases in intranephron PO₂ were limited to 25–50% at both sites. Reducing the inspired O₂ from 21 to 10% did not affect nephron/vascular signaling by NO, as indexed from the increase in TGF responses during blockade of macula densa nNOS. However, increasing the inspired O₂ from 21 to 100%, which increased the distal nephron PO₂ from 39 to 65 mmHg, reduced this index of NO signaling by 50%. We concluded that there was reduced NO bio-availability in the cortical nephrons at high inspired O₂, likely due to increased intrarenal superoxide anion (O₂^{•-}) generation which bioinactivated NO at high tissue PO₂ levels.

Our results confirmed that the levels of PO₂ within the tubules, even of the most well-oxygenated part of the outer cortex of the kidney, were well below those in the renal vein. What could be the evolutionary selective advantage of an O₂ shunt that clearly reduced the PO₂ levels in the kidney substantially (Fig. 46.1a)? They point to some possible explanations. First, the shunt stabilized the intrarenal PO₂ and limited the fall in renal PO₂ during arterial hypoxemia which might otherwise put the kidney at risk for hypoxic injury (Fig. 46.2b). Indeed, the human kidney functions remarkably well during even prolonged hypoxemia [4]. Second, the finding that an increase in intrarenal PO₂ from 40 to about 65 mmHg curtailed the function of NO within the renal cortex suggested that an O₂ shunt that normally restricted the PO₂ levels in the kidney may be necessary for fully effective NO signaling. We found that O₂ availability limited NADPH oxidase activity in renal tissues [2]. Thus, a relatively low level of tissue PO₂ may be needed to restrict renal O₂^{•-} generation and to preserve NO signaling. Nevertheless, the kidney is at substantial risk of hypoxic/ischemic damage during hypotension and shock. Redfors et al. demonstrated a sharp reduction in oxygenation of the kidney of patients with acute kidney injury after cardiac surgery [5]. Indeed, these findings may explain the extreme vulnerability of the kidney to shock in contrast to its relative immunity from the effects of hypoxemia. A reduction in renal tissue PO₂ due to ROS accumulation during shock should reduce the PO₂ in the venous shunt pathway and enhance the gradient for O₂ diffusion from the artery to the vein. Moreover, a reduction in renal blood flow (RBF) during shock should prolong the time that blood spends in the shunt pathway. These two together would enhance O₂ shunting and further curtail renal tissue PO₂ values (Fig. 46.2c).

A potential advantage of the shunt relates to reversed diffusion of gases that are produced within the kidney and thereby have higher concentrations in venous than arterial blood (Fig. 46.2d). Notably, the PCO_2 levels in the proximal or distal tubule, interstitium, efferent arteriole, or Bowman space are 15–25 mmHg above those in arterial or renal venous blood [6]. This may, in part, represent reverse shunting of CO_2 produced in the kidney from the renal venous to the arterial systems. Renal CO_2 is produced by the reaction of secreted H^+ with filtered HCO_3^- or as a byproduct of cellular respiration. The kidney requires a huge supply of CO_2 to generate sufficient H^+ and HCO_3^- from the reaction of CO_2 and H_2O to reabsorb two-thirds of the filtered sodium. Indeed, the normal arterial level of PCO_2 of 35 mmHg was suboptimal for tubular sodium transport. Thus, an increase in the PCO_2 of the bath of a rabbit isolated perfused proximal tubule from 35 to 70 mmHg increased the absolute reabsorption of fluid and sodium by 30% [7]. The kidney also has a unique requirement for NH_3 to generate approximately 50 mmol of NH_4^+ daily for the excretion of H^+ in the urine. The partial pressure of NH_3 in the kidney exceeds that in the arterial blood [8]. A reverse shunt for NH_3 from the renal venous to the arterial systems could not only enhance renal NH_3 levels sufficiently to buffer urinary H^+ as NH_4^+ but also would limit NH_3 produced in the proximal tubules from escaping into the systemic circulation where it can lead to toxic effects on the brain.

In conclusion, these experiments highlight the quantitative importance of the preglomerular diffusional shunt for O_2 , indicate some of its functions, and suggest additional functions for trapping of CO_2 and NH_3 in the kidney to provide the very high fluxes required for active Na^+ reabsorption and H^+ excretion.

Acknowledgments

CSW and WJW were supported by grants from the NIH (DK-36079; DK-49870; HL-68686) and from funds from the George E. Schreiner Chair of Nephrology.

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Fig. 46.1.

Mean $\pm$ SEM values ($n = 4-6$) for partial pressure of oxygen (PO₂) in proximal (a) and distal tubules (b) and absolute change in tubuloglomerular feedback (TGF) response (c) before and after intratubular administration of 7-nitroindazole (10^{-4} M) at different inspired oxygen contents. Data are shown for control Wistar Kyoto (WKY) rats (open circles and broken lines) and hypertensive SHR (closed circles and continuous lines). Significance of difference between SHR and WKY: *, $P < 0.05$; **, $P < 0.01$. Significance of difference between values at normal or high inspired O₂: ‡, $P < 0.05$.

Fig. 46.2.

Diagrammatic representation of a preglomerular diffusional gas shunt for O_2 in (a–c) or for CO_2 in (d). (a) Depicts effects in the normal kidney. (b) Depicts decreased O_2 shunting at low arterial PO_2 values. (c) Depicts increased O_2 shunting during shock. (d) Depicts shunting of CO_2 from the preglomerular venous to arterial systems in the normal kidney. For explanation, see Sect. 4