Chapter 4

Reversible (Patho)Physiologically Relevant Test Interventions: Rationale and Examples

Kathleen Cantow, Mechthild Ladwig-Wiegard, Bert Flemming, Andrea Fekete, Adam Hosszu, and Erdmann Seeliger

Abstract

Renal tissue hypoperfusion and hypoxia are early key elements in the pathophysiology of acute kidney injury of various origins, and may also promote progression from acute injury to chronic kidney disease. Here we describe test interventions that are used to study the control of renal hemodynamics and oxygenation in experimental animals in the context of kidney-specific control of hemodynamics and oxygenation. The rationale behind the use of the individual tests, the physiological responses of renal hemodynamics and oxygenation, the use in preclinical studies, and the possible application in humans are discussed.

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Key words Renal hemodynamics and oxygenation, In vivo methods, Rats, Test interventions

1 Introduction

Kidney diseases are a global health burden with steadily increasing incidence and prevalence [1–5]. Animal studies indicate that acute kidney injuries (AKI) of various origins share one common link in the pathophysiological chain of events, ultimately leading to AKI, as well as to progression from AKI to chronic kidney diseases (CKD): imbalance between renal oxygen delivery and oxygen demand [6–14]. Renal tissue hypoperfusion and hypoxia have also been suggested to play a pivotal role in the pathophysiology of other kidney diseases including diabetic kidney disease [15–19]. These pathophysiological concepts have largely been generated by preclinical studies that used either invasive quantitative probes or noninvasive functional magnetic resonance imaging (MRI) techniques to gain insight into renal hemodynamics and oxygenation. Thus, making ultimate statements on the role of renal hypoperfusion and hypoxia for these renal disorders is elusive

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because in vivo assessment of renal hemodynamics and oxygenation constitutes a challenge.

All modalities available in today's experimental and translational research practice have inherent shortcomings and methodological constraints. Invasiveness is the major disadvantage of the gold standard physiological probes such as perivascular flow probes for measurement of total renal blood flow, laser-Doppler-optodes for assessment of local tissue perfusion, and Clark-type electrodes or fluorescence-quenching optodes for measurements of local tissue partial pressure of oxygen (pO_2), which precludes their use in humans. While functional MRI including blood oxygenation-sensitized T_2* (aka blood oxygenation level-dependent MRI; BOLD-MRI) offers noninvasive techniques to obtain insight into renal perfusion and oxygenation, its major weakness is its qualitative nature. Before it can be used for quantitative characterization of renal tissue perfusion and oxygenation, it needs to be calibrated with the gold standard invasive techniques in various (patho)-physiological scenarios [20–24].

The control of renal hemodynamics and oxygenation under physiological as well as under pathophysiological conditions is complex and differs considerably from nonrenal tissue [8, 21, 23, 25–27]. Due to the considerable capacity of the organism's homeostatic control systems to—at least partially—compensate for disturbances of, or injury to, certain control elements, these alterations are often not easily detectable when studied by measuring baseline data only. In order to disentangle these complexities, dedicated reversible test interventions are conceptually appealing. In fact, such interventions can serve three main purposes. First, they are used to gain more insight into the control of renal hemodynamics and oxygenation in healthy animals and in animal models of various kidney diseases [8, 12, 25, 28–37]. Second, the tests are used to assess whether a given drug or contrast agent has beneficial or unwarranted effects on the control of renal hemodynamics and oxygenation [38–40]. Finally, dedicated reversible tests are used to achieve calibration of functional MRI data [22, 23].

In this chapter, specifics of the control of renal hemodynamics and oxygenation are outlined first. Then, the individual test procedures are described, and the rationale behind their use, the physiological response of renal hemodynamics and oxygenation, the use in preclinical studies and the possible application in humans are discussed.

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2 Specifics of Renal Hemodynamics and Oxygenation

Renal hemodynamics and oxygenation offer a number of striking differences when compared to nonrenal tissue. First, total renal blood flow (RBF) is huge when compared to virtually all other organs on a per gram basis: the kidneys receive about 20% of the cardiac output under resting conditions. Yet the distribution of blood perfusion differs substantially between the layers: while 100% of blood flowing into the kidney reaches the cortex, only 15% of blood that previously passes through the cortex, will reach the medulla. Even intralayer (cortex, outer medulla, and inner medulla) perfusion is quite heterogeneous [8, 41, 42]. In accordance with the high total RBF, the kidneys' oxygen extraction (the difference between the O_2 content in the renal arterial and the renal venous blood) is low as compared to the majority of nonrenal tissues. Yet the partial pressure of oxygen (pO_2) is low in the medulla and also varies considerably within the respective layers, in accordance with the different blood flow distribution [21, 23, 26, 43–47].

Second, the kidney differs from all other organs with regard to the relationship between metabolism and perfusion. More than 26 thousand millimoles of sodium (Na^+) are filtered in the human glomeruli every day, equivalent to more than 1.5 kg of table salt. To achieve sodium balance, the amount of salt excreted by the kidneys must exactly match the amount of ingested salt minus the amount of extrarenal loss. Thus, more than 99% of the filtered sodium must usually be reabsorbed from the tubules. Tubular resorption relies on active transport processes, which account for about 85% of the kidney's energy expenditure and therefore its O_2 consumption. The more sodium is filtered in the glomeruli, the more must be reabsorbed. As glomerular filtration rate (GFR), under the majority of circumstances, increases with increasing RBF, renal O_2 consumption also usually increases with increasing renal perfusion. This is in contradistinction to all other organs, where metabolism determines perfusion [8, 48].

Third, hormones such as angiotensin II and epinephrine, sympathetic vasomotor nerves, and paracrine mediators such as nitric oxide or adenosine, that control resistance vessels in nonrenal tissues, impinge on intrarenal resistance vessels too, thereby altering renal O_2 delivery. However, in the kidney, they additionally affect tubular sodium resorption and thus O_2 consumption. Furthermore, their effect on postglomerular vessels can result in divergent responses of RBF and GFR. Finally, adenosine exerts vasodilation in virtually all nonrenal vascular beds, but vasoconstriction in the renal cortex [8, 25, 48, 49].

Fourth, the kidney is equipped with efficient mechanisms of autoregulation, that is, the ability to dampen or even to abolish the

effects that changes in renal arterial pressure would otherwise inevitably have on RBF and GFR. The almost perfect autoregulation of RBF and GFR probably relies on the fact that not just one, but three mechanisms are involved. The first one, the myogenic response (aka Bayliss effect) acts not only on renal resistance vessels but also on brain and gut vessels. The second mechanism, the tubuloglomerular feedback (TGF), and the third one, hitherto just named "third mechanism", are kidney-specific. Renal autoregulatory mechanisms, in particular the TGF and the third mechanism, have been suggested to serve the purpose of balancing O_2 delivery, that is, RBF with metabolic and O_2 demands arising from tubular reabsorption. The outer medulla is particularly prone to imbalance between O_2 delivery and demand since this layer exhibits a high O_2 demand but low pO_2 [21, 34, 35, 50–52].

Fifth, intrarenal perfusion is also affected by changes in tubular volume. The tubular volume fraction is quite large and can rapidly change due to alterations in GFR, in tubular outflow toward the pelvis, in tubular fluid resorption, and modulation of the transmural pressure gradient. Since the renal capsule is rather tough, changes in tubular volume will result in circular distension or compression of intrarenal vessels [23, 36].

Finally, in addition to the heterogeneous intrarenal blood perfusion, three other factors substantially contribute to the low tissue pO_2 and, in particular, to the "physiological hypoxia" in the medulla. First, there is a considerable shunt diffusion of O_2 from arteries to veins in the cortex and from descending to ascending vasa recta in the medulla [53–55]. Second, the Fåhræus–Lindqvist effect lowers the hematocrit in the vasa recta supplying the medulla, which lowers the O_2 content of blood perfusing parts of the medulla [41, 42]. Third, plasma skimming at intrarenal vessel branches results in different hematocrit and therefore O_2 content of blood perfusing the daughter vessels [41, 56].

3 Dedicated Reversible Test Interventions

3.1 Short Periods of Occlusion of the Renal Artery or Renal Vein

Occlusions of the renal artery (alternatively: the suprarenal aorta) or of the renal vein emulate clinical conditions in which deficient renal perfusion results in deterioration of intrarenal oxygenation. If maintained for longer periods of time these conditions can cause AKI [57–59]. The rationale for performing both of these tests is that renal arterial occlusion and renal venous occlusion have similar effects with regard to renal perfusion and oxygenation, yet opposing effects with regard to intrarenal blood volume. With the onset of aortic occlusion, the inflow of blood into the kidney is abruptly stopped while outflow via the renal vein continues until pressures in intrarenal vessels and in the vena cava are equalized. With the onset of renal venous occlusion, outflow of blood is abruptly stopped

while inflow via the artery does not cease until the arterial pressure-induced distension of intrarenal vessels is counterbalanced by the resistance of the renal tissue including the rather tough capsule [22, 39, 60].

In both cases, renal tissue perfusion rapidly decreases and eventually approaches zero flow. As renal O_2 consumption remains unaltered at the early stage of occlusions, a rapid and massive decline in renal tissue pO_2 results, which, in turn, also reduces blood pO_2 and the O_2 saturation of hemoglobin (StO_2) in the intrarenal (micro)vasculature. This intrarenal deoxygenation of hemoglobin (Hb) is aggravated by a progressive rightward shift of the oxyHb dissociation curve during the occlusion due to the intrarenal accumulation of carbon dioxide (CO_2) [22, 39, 60].

Yet the opposing changes of renal blood volume have an impact on renal tissue oxygenation. The decrease in tissue pO_2 at the onset of the venous occlusion is much slower than at the onset of the arterial occlusion. While renal O_2 consumption is similar during both kinds of occlusions, the transiently maintained inflow of oxygenated blood at the onset of venous occlusion increases the intrarenal reservoir of O_2 [22, 39, 60].

The opposing changes in renal blood volume have a massive impact on the changes in blood oxygenation-sensitized T_2^* (and its reciprocal value, R_2^*), because T_2^* reflects the amount of deoxygenated Hb (deoxyHb) per tissue volume (voxel) [23]. In case of the venous occlusion with its increase in the vascular volume fraction and thus the increasing amount of deoxyHb per volume, tissue T_2^* massively decreases [60]. With the arterial occlusion's decrease of deoxyHb per volume, the decrease in T_2^* is small. In fact, it was found significantly smaller than the decrease in T_2^* measured during hypoxemia (8% inspiratory oxygen fraction), which is diametrically opposed to the effects of arterial occlusion versus hypoxemia on tissue pO_2 (*see* Fig. 1) [22, 23, 36].

Short-time (1–3 min) occlusions of the renal artery (or of the suprarenal aorta) have been used in several studies for different scientific purposes. In order to gauge the effects on T_2^* and T_2 of bolus injections of an X-ray contrast medium into the thoracic aorta of healthy rats, the effect of arterial occlusion (and that of hypoxemia) was quantified in the same rats [36]. En route to calibration of T_2^* with quantitative physiological measurements by means of a dedicated hybrid MR-PHYSIOL setup (*see* the chapter by Cantow K et al. "Monitoring Renal Hemodynamics and Oxygenation by Invasive Probes: Experimental Protocol"), suprarenal aortic occlusion was used [22]. In order to ascertain that the superparamagnetic iron oxide nanoparticle (USPIO) preparation, ferumoxytol is suitable as a contrast medium for MR-based assessment of the renal blood volume fraction; its possible unwarranted effects on control of renal hemodynamics and oxygenation were tested by interventions including suprarenal aortic occlusion in rats (*see* Fig. 2)

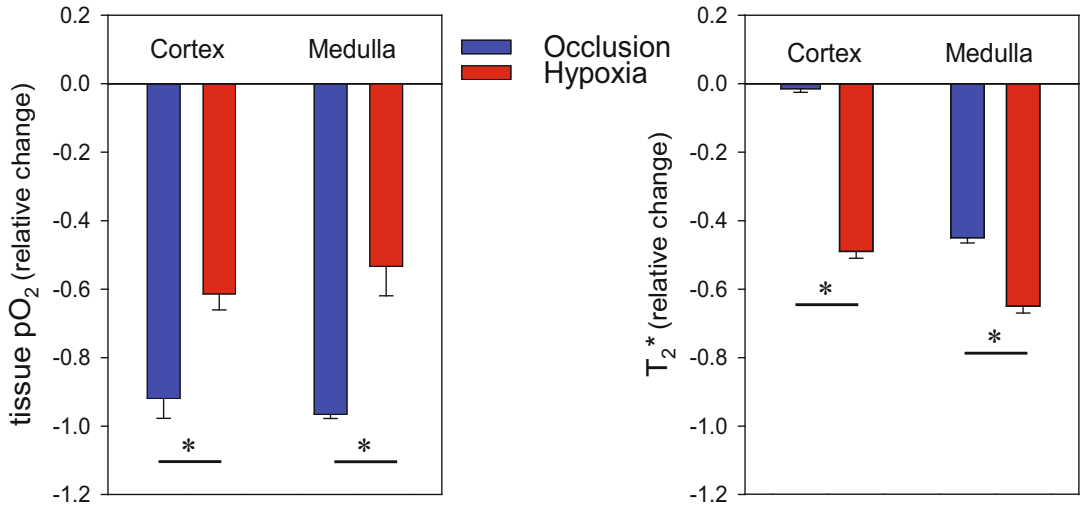

Fig. 1 Comparison of relative changes in renal cortical and medullary tissue pO_2 quantified by invasive gold standard fluorescence quenching optodes (left panel) versus relative changes in renal cortical and medullary T_2* (so-called BOLD-MRI, right panel), during short-term occlusion of the suprarenal aorta and short-term hypoxia (8% inspiratory O_2 fraction), respectively, in anesthetized rats. Data are mean ± SEM, redrawn from Refs. 22, 36

[38]. Implementing a setup that combines classical invasive probes for RBF, tissue perfusion, and pO_2 with newly developed near infrared spectroscopy (NIRS) techniques that enable monitoring of the amount of Hb per tissue volume and the O_2 saturation of Hb (StO_2) of intrarenal blood (termed PHYSIOL-NIRS), aortic occlusion was used as one of the test interventions [39]. By means of a dedicated deconvolution procedure developed by our group, the time course of RBF upon the release of the occlusion can be analyzed. This "step-response" analysis allows us to determine the strength of each of the three mechanisms of RBF autoregulation in the whole kidney in vivo, in both healthy rats and rat models of AKI [30, 34, 35].

Short-time (1–3 min) occlusions of the renal vein were also used for different purposes. In order to establish an optimum dose of the USPIO ferumoxytol in rats for the purpose of T_2*-based quantification of the renal blood volume fraction in a 9.4T small animal scanner, renal venous occlusion was chosen as the combined effects of the decrease in O_2 delivery and the increase in the blood volume fraction; thus, deoxyHb per volume was expected to result in a most prominent decrease in T_2* (*see* Fig. 3) [60].

Both renal arterial occlusion and renal venous occlusion was performed in the same healthy rats in the PHYSIOL-NIRS setup in order to directly compare their effects [39].

As the implementation of vascular occluders necessitates invasive techniques, these tests can be performed in preclinical studies only.

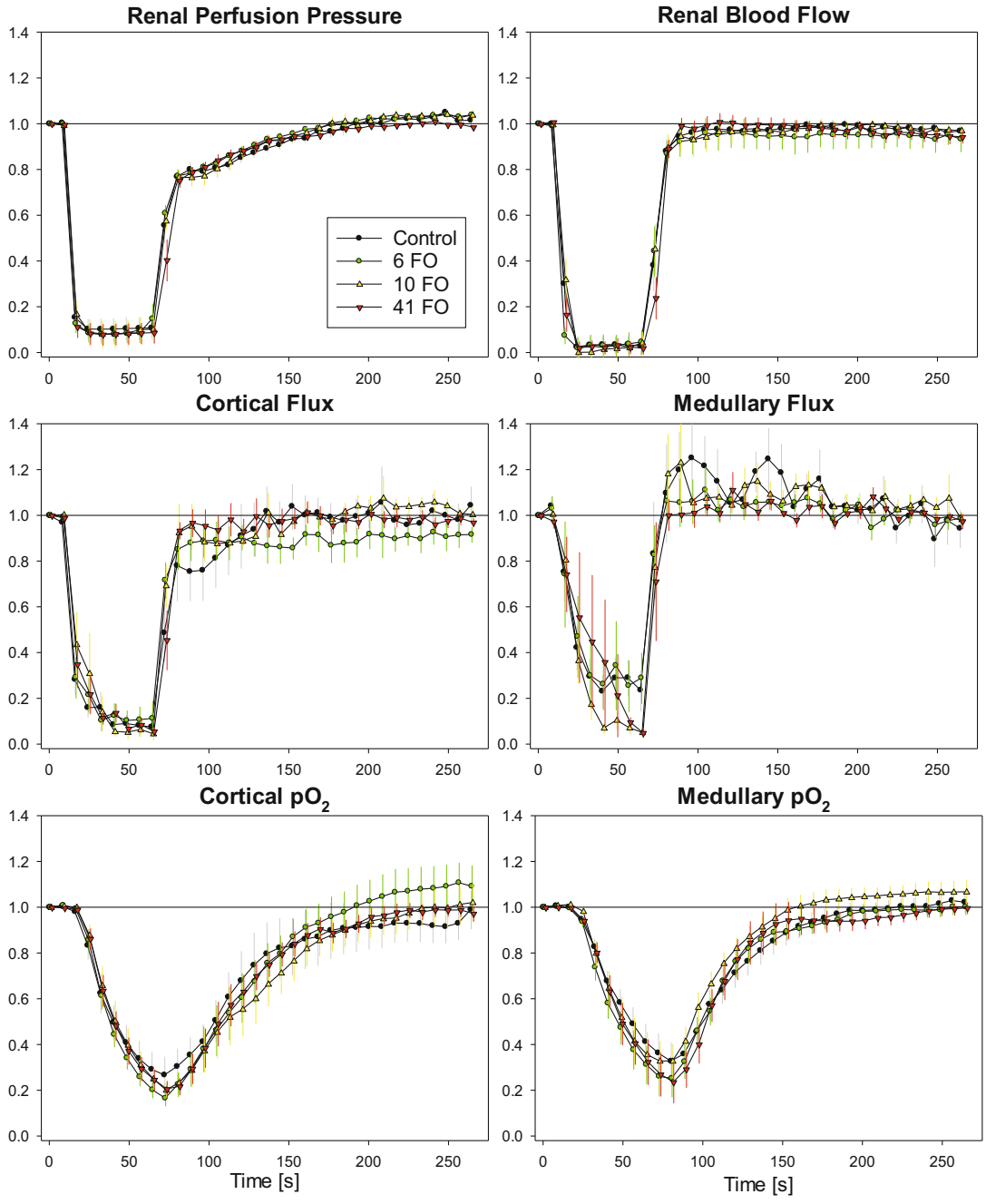

Fig. 2 In order to study whether the USPIO preparation, ferumoxytol (FO), exerts unwarranted effects on regulation of renal hemodynamics and oxygenation, a short-term suprarenal aortic occlusion was employed as test intervention in anesthetized rats [38]. Here, the relative changes (mean ± SEM) in hemodynamics and tissue oxygenation are depicted with FO dosages of 6, 10, and 41 mg Fe/kg body mass, or vehicle (Control)

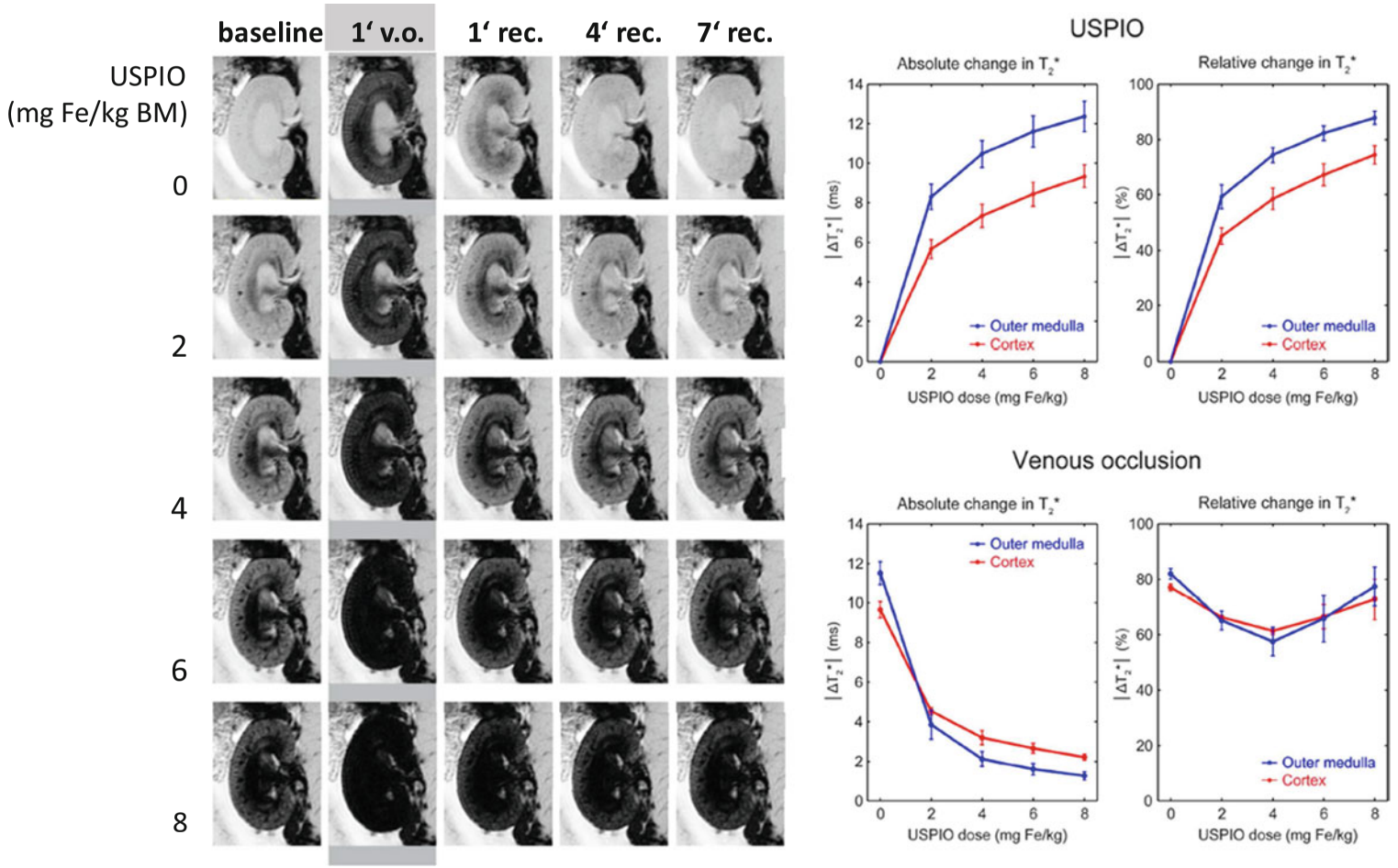

Fig. 3 Left panel: T_2*-weighted images (echo time = 3.6 ms, spatial resolution = 226 × 422 μm) obtained by a 9.4 T small animal MR scanner (Bruker Biospin, Biospec 94/20) of a rat kidney in vivo at baseline, during occlusion of the renal vein (v.o.), and at the beginning of the recovery phase (rec.) without the USPIO ferumoxytol (top row), and with four increasing doses of ferumoxytol [60]. Right panels: Comparison of the renal cortical and medullary T_2* sensitivity to USPIO injection and the T_2* sensitivity to the venous occlusion at different USPIO doses. Data are mean ± SEM (n = 4 rats) of cortical and medullary ROIs [60]

3.2 Servocontrolled Changes in Renal Arterial Pressure

Dynamic changes in renal arterial pressure according to different time courses of pressure reduction followed by pressure restoration—be it staircasewise or rampwise changes—enable insights into control of renal hemodynamics and oxygenation including the degree of autoregulation's efficiency and the contributions of the three autoregulatory mechanisms in vivo. This is achieved by a servocontrol system developed by our group, that was utilized to help disentangle the complexities of renal physiology and pathophysiology [30, 31, 33, 34]. Moreover, a study that employed such an intervention in a rat model that emulates an early stage of diabetic kidney disease (a type 1 diabetes mellitus–like model induced by administration of streptozotocin 4 weeks before obtaining the data on renal perfusion and oxygenation) unmasked alterations in the control of renal perfusion and oxygenation that would have gone undetected when only baseline data had been obtained [32]. Data on medullary tissue pO_2 obtained by invasive probes in this model have been inconsistent, which, among other reasons, may be caused by the spatial heterogeneity of pO_2 within the renal medulla [15–17]. A recent study did not find any significant differences in baseline data on RBF and cortical and medullary tissue pO_2 among healthy control rats, diabetic rats, and diabetic rats treated with the antidiabetic liraglutide (a glucagon-like peptide 1 agonist approved for patients suffering from type 2 diabetes). However, as depicted by Fig. 4, the response to ramp-wise reduction and restoration of renal arterial pressure differed considerably among these groups [32].

Again, as the implementation of a vascular occluder necessitates invasive techniques, such studies can be performed in preclinical studies only.

3.3 Short Periods of Changes in the Inspiratory Gas Mixture

Hyperoxia, hypoxia, and hypercapnia primarily alter blood oxygenation. Renal O_2 delivery is determined by renal perfusion and by the arterial O_2 content. The latter is determined, among other factors, by the inspiratory fraction of oxygen (FiO_2), and, due to the effect of CO_2 on the oxyHb dissociation curve, also by the inspiratory fraction of CO_2 ($FiCO_2$).

Increasing the FiO_2 from 21% (normoxia) to 100% (hyperoxia) results in a substantial increase in arterial pO_2 (usually four- to fivefold), whereas the increase in arterial O_2 content is very small, because most of the Hb in arterial blood is already O_2 saturated under normoxic conditions. Yet the increase in arterial pO_2 enhances the driving force for diffusion of O_2 from intrarenal vessels to tissue as well as from intrarenal arteries to veins. As a consequence, the increase in renal tissue pO_2 is substantial, whereby medullary pO_2 increases less than cortical pO_2, due to arteriovenous diffusive O_2 shunting, which reduces the O_2 content of arterial blood that perfuses the medulla [22, 26, 39, 53–55]. Renal T_2* changes exerted by hyperoxia are small

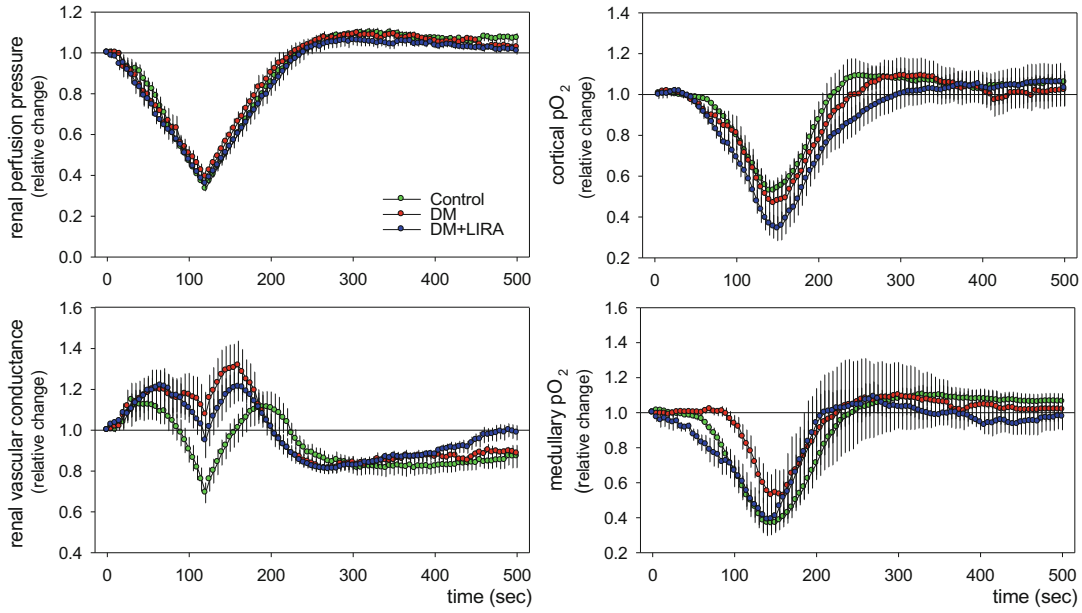

Fig. 4 Changes of invasively measured parameters of renal hemodynamics and oxygenation during ramp-wise reduction in renal perfusion pressure followed by ramp-wise pressure restoration in anesthetized rats. Conductance values (the reciprocal of vascular resistance) were calculated by dividing the respective perfusion values by renal perfusion pressure, in order to distinguish flow changes that result from passive circular distension/compression of vessels from those actively exerted by vascular smooth muscles. Three groups were studied: a healthy control group, a group in which a diabetes mellitus type 1-like disorder (DM) was induced by streptozotocin 4 weeks before obtaining the data, and a third group in which DM was induced and the antidiabetic liraglutide administered for 3 weeks (DM + LIRA). Values (mean ± SEM) are given as relative changes from baseline [32]

[22]. While the amount of deoxyHb in arterial blood is barely changed, the increase in blood pO_2 in intrarenal veins that results from the higher arteriovenous pO_2 difference decreases venous deoxyHb. While primarily altering blood oxygenation, the hyperoxic stimulus has also secondary effects: it results in vasoconstriction, preferentially in nonrenal vascular beds, which leads to an increase in arterial pressure [22, 31, 39].

The primary effect of reducing the FiO_2 (typically to either 8% or 10%, with durations of 3–12 min, in rat studies) is a decrease in oxygenation of arterial blood (hypoxemia) with the consequent reduction in renal O_2 supply. With ongoing O_2 consumption, this does per se result in a decrease in renal tissue pO_2. Yet renal O_2 supply is further diminished by hypoxia-induced extrarenal vasodilation that results in a drop in arterial pressure with ensuing decrease in RBF [22, 31, 39, 59]. Whether this is aggravated of alleviated by constriction or dilation, respectively, of the renal vasculature depends on the degree of hypoxia: in anesthetized rats, FiO_2 of 8% results in renal vasoconstriction while 10% results in vasodilation [22, 31, 39]. The combined effect of hypoxemia and

reduced RBF on renal O_2 supply leads to a major mismatch with O_2 consumption, that massively reduces tissue pO_2 as well as T_2* (*see* Fig. 1) [22, 39]. A further secondary effect of arterial hypoxemia is increased ventilation triggered by arterial chemoreceptors. The ensuing decrease in arterial pCO_2 shifts the oxyHb dissociation curve to the left, that is, O_2 is hindered from being released by Hb, which further aggravates the tissue hypoxia [61, 62].

With the hypercapnic stimulus (increasing $FiCO_2$ to 5%) the opposite effect is achieved, namely a rightward shift of the oxyHb dissociation curve. This would per se result in a decrease in StO_2 and an increase in blood and tissue pO_2. However, while the increase in tissue pO_2 is substantial, the StO_2 decrease is meagre [39]. The major reason that StO_2 does not decrease much is that increased pCO_2 of arterial blood is a very strong stimulus for ventilation, again mediated by arterial chemoreceptors [61, 62].

Hyperoxic, hypoxic, and hypercapnic tests have been used in a multitude of preclinical in vivo studies. With regard to the kidney this includes but is not limited to studies on the control of renal hemodynamics and oxygenation in healthy animals and models of kidney diseases [26, 27, 31, 53–55, 59], experiments that aimed at calibration of T_2* by means of the MR-PHYSIOL setup (*see* Fig. 5), [22] studies on the T_2* effect of an X-ray contrast medium, [36] assessment of possible unwarranted effects of the USPIO ferumoxytol, [38] and experiments en route to the PHYSIOL-NIRS setup [39].

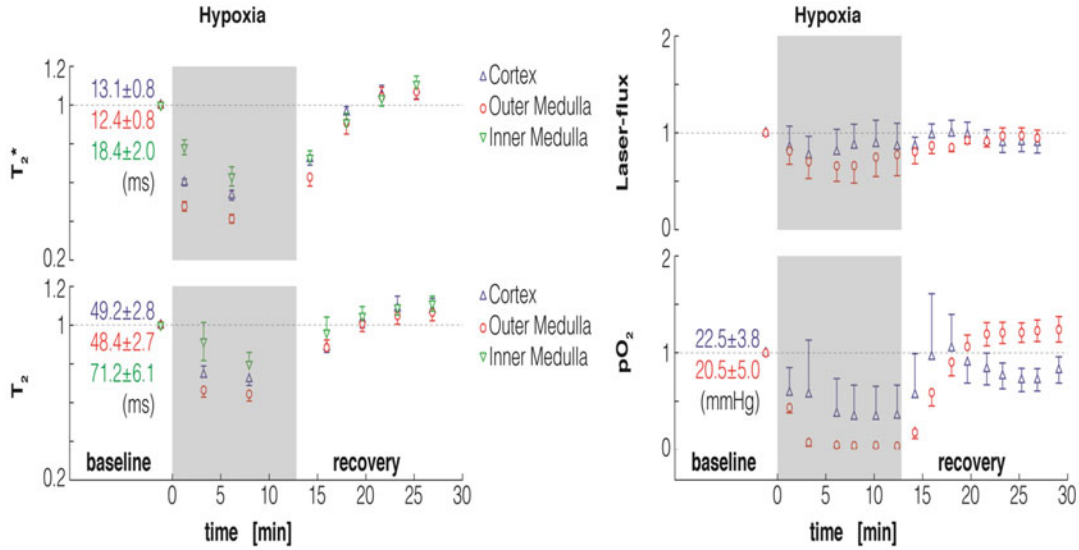

Fig. 5 Time courses of selected invasively measured data and MR parameters acquired simultaneously throughout baseline, a period of hypoxia (FiO_2 = 8%), and recovery in anesthetized rats by means of a dedicated MR-PHYSIOL hybrid setup (for details see text). Data (mean ± SEM) are relative changes from baseline, redrawn from Ref. 22

Given the broad therapeutic use of pure oxygen, short-term tests with 100% FiO_2 should pose no problem for studies in humans, whereas hypoxic challenges are precluded in humans, for obvious ethical reasons. Hypercapnia has been used for decades in humans, in particular, for the study of cerebrovascular reactivity, and should thus be employed in preclinical and clinical studies on renal hemodynamics and oxygenation as well [63, 64].

3.4 Administration of Drugs and Endogenous Vasoactive Substances

Furosemide is the "classic" loop diuretic: its major action is the inhibition of the sodium–potassium–two-chloride cotransporter in the apical membrane of tubular epithelial cells of the thick ascending limb of Henle's loop. The primary effect is an increase in urine flow rate and in urinary sodium and potassium excretion. As less tubular resorption necessitates less renal O_2 consumption, administration of furosemide leads to an increase in renal tissue pO_2 [65, 66]. In accordance, increases in renal T_2* (or decreases in its reciprocal value R_2*) have been observed in a multitude of preclinical as well as clinical studies (*see* Fig. 6) [37, 66–69]. It must be noted, however, that the increase in T_2* upon furosemide does not solely rely on improved oxygenation. First, the increase in tubular fluid downstream of the thick ascending limb will increase the transmural pressure gradient, thereby compressing intrarenal

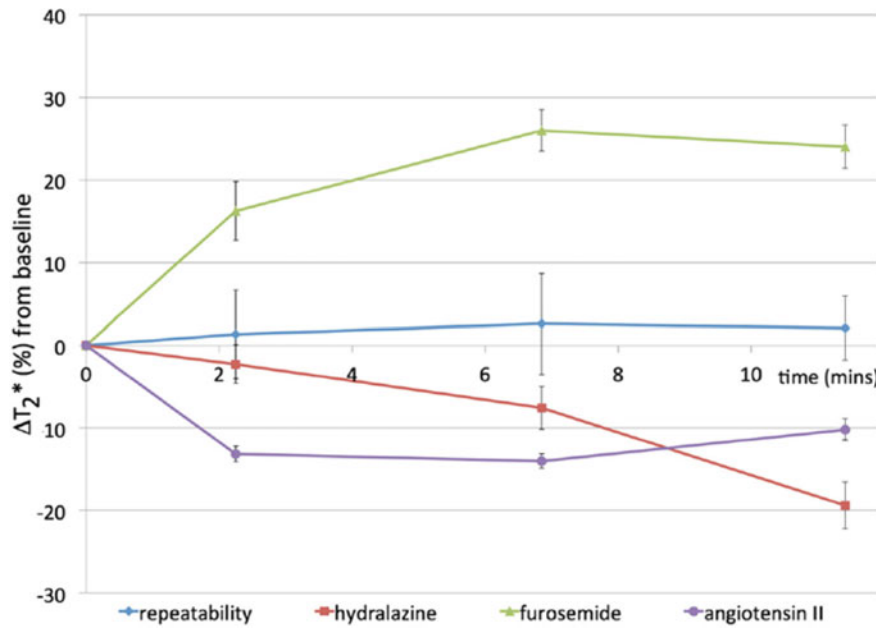

Fig. 6 Effect of injections of furosemide (5 mg/kg body mass), hydralazine (5 mg/kg), angiotensin II (0.5 μg/min/kg), and saline (repeatability) on renal BOLD as recorded by means of a 1.5 T clinical MR scanner (Magnetom Avanto, Siemens Healthcare), using a multiple gradient echo sequence (TR = 300 ms, TE = 5, 10, 20, 30, and 40 ms, voxel size 0.6 × 0.6 mm in-plane and 5 mm slice thickness) in rats in vivo. Data are mean ± SEM of median ROI values [37]

vessels with the ensuing decrease in the amount of deoxyHb per tissue volume. Second, furosemide inhibits the TGF, thereby compromising renal autoregulation with the possible consequence of an increase in RBF [34, 35, 50].

The use of the furosemide test in both preclinical and clinical MR studies is nowadays as widespread that it is almost regarded as a gold standard. However, whether it fulfils all expectations regarding its use as a diagnostic tool in patients suffering from various kidney diseases, remains to be seen [68].

Administration of furosemide is a reversible intervention insofar as its direct effects vanish with the excretion of the drug. However, it leaves the organism with deficits in water, sodium, and potassium. These should ideally be replenished—be it per os or by means of infusions of a balanced electrolyte solution.

Bolus injections of adenosine cause a rapid drop in arterial pressure due to its vasodilatory effect on nonrenal resistance vessels. In the renal cortical vascular bed, it exerts vasoconstriction [39, 49]. The consequence of these two effects is a substantial decrease in RBF followed by a smaller decrease in cortical tissue pO_2. All these effects vanish rapidly, lasting less than a minute for the hemodynamics and less than 2 min for the cortical pO_2 in rats [39].

Whereas the role of adenosine in various renal control mechanisms including the TGF as well as the potentially beneficial effect of adenosine receptor antagonists for prevention of X-ray contrast media-induced AKI have been intensively studied, [49, 70] the adenosine test has seldom been used to study renal hemodynamics and oxygenation. This may appear surprising, as injections of adenosine—be it intravenously or into coronary arteries—in patients suffering from coronary disease is quite established [71, 72]. While the risk for a decrease in renal cortical pO_2 in patients must not be ignored—even if it is lasting less than 2 min, the test should at least find wider use in preclinical studies.

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