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DISSERTATION

Functional TRPV1 and TRPV4 channels in the murine renal vasculature

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Zusammenfassung

Transiente Rezeptorpotential Vanilloid 1- (TRPV1) und 4- (TRPV4) Kationenkanäle fördern die endothelabhängige Relaxation mesenterialer Arterien der Maus. TRPV1-positive sensorische Nerven sind in der Niere weit verbreitet. Unbekannt ist jedoch, welche Rolle TRPV1 und TRPV4 im renalen Gefäßsystem spielen und ob sich eine Hemmung dieser Kanäle nachteilig auf akute Nierenschädigung (AKI) auswirkt. Die untersuchte Hypothese war, dass TRPV1/4 eine Funktion bei der endothelabhängigen Vasodilatation von Nierenarterien haben und es wurde die Rolle von TRPV1-Kanälen in I/R-induziertem AKI untersucht. Die Verteilung funktioneller TRPV1/4-Kanäle in verschiedenen Segmenten des Nierengefäßsystems wurde untersucht. Mesenteriale Arterien dienten als Kontrollgefäße. Auch K_V7.1-Kanäle (spannungsgesteuerte K^+-Kanäle) werden in renalen Arterien exprimiert und es wurde vermutet, dass ihre Stimulation eine starke Relaxation erzeugt. Daher wurde weiterhin die Hypothese getestet, dass Kv1.7-Kanäle den arteriellen Tonus kontrollieren. Die Ergebnisse zeigen, dass der TRPV1-Agonist Capsaicin Maus-Mesenterialarterien mit einer EC50 (Konzentration der Substanz, die eine halbmaximale Reaktion hervorruft) von 25 nM, große Nierenarterien der Maus oder die absteigenden Vasa recta der Ratte jedoch erst bei >100-fach höherer Konzentration relaxierte. Der TRPV4-Agonist GSK1016790A relaxierte große Nierenarterien, Mesenterialarterien und Vasa recta mit einer EC50 von 18 nM, 63 nM bzw. ~10 nM. Diese Effekte waren endothelabhängig und wurden durch den TRPV4-Antagonisten AB159908 (10 µM) inhibiert. Capsaicin und GSK1016790A führten zu vaskulärer Dilatation bei isolierten perfundierten Nieren (Maus) mit einer EC50 von 23 nM bzw. 3 nM. Der K_V7.1-Öffner R-L3 relaxierte konzentrationsabhängig Mesenterial- und Nierenarterien (EC50 ~1,4 µM) von Wildtyp-Mäusen (*Kcnq1*+/+). Diese Relaxation wurde durch die K_V7.1-Inhibitoren Chromanol-293B und HMR1556 oder XE991 (pan-K_V7-Blocker) nicht beeinflusst. Capsaicin milderte die Folgen von AKI bei Mäusen, analysiert durch Serum-Kreatinin-Spiegel, tubuläre Schäden, das Auftreten von Neutrophilen-Gelatinase-assoziierten Lipocalinen (NGAL) und Ly-6B.2-positiven polymorphonukleären inflammatorischen Zellen in geschädigten Nieren. Die Ergebnisse zeigen, dass TRPV1 eine enge, diskrete Verteilung in Widerstandsgefäßen und TRPV4 eine allgemeinere Verteilung entlang verschiedener Gefäßabschnitte bei Mäusen aufweist. Die Aktivierung von TRPV1-Kanälen verringert die Schäden bei I/R-induzierter AKI. K_V7.1-Kanäle sind nicht erforderlich für die Kontrolle des arteriellen Tonus durch alpha1-adrenerge Vasokonstriktoren bei Nierenarterien in Mäusen.

Abstract

Transient receptor potential vanilloid 1 (TRPV1) and 4 (TRPV4) cation channels promote endothelium-dependent relaxation of mouse mesenteric arteries. TRPV1-positive sensory nerves are widely distributed in the kidney. However, it is unknown what role TRPV1 and TRPV4 play in the renal vasculature, and it is unclear whether inhibition of these channels is detrimental in AKI or not. Here, the hypothesis was tested that TRPV1 and/or TRPV4 play a role in endothelium-dependent vasodilation of renal blood vessels, and the role of TRPV1 channels in I/R-induced AKI was examined. The distribution of functional TRPV1/4 along different segments of the renal vasculature was studied. Mesenteric arteries were examined as control vessels. In addition, $K_V7.1$ voltage-gated potassium channels are expressed in renal arteries and have been suggested to evoke profound relaxation upon stimulation. Here, the hypothesis that $K_V7.1$ channels control arterial tone was tested. The results showed that the TRPV1 agonist capsaicin relaxed mouse mesenteric arteries with an EC_{50} (the concentration of the drug that elicits a half-maximal response) of 25 nM, but relaxation of large mouse renal arteries or rat descending vasa recta was only observed at >100-fold higher concentrations. The TRPV4 agonist GSK1016790A relaxed large conducting renal arteries, mesenteric arteries, and vasa recta with EC_{50} of 18 nM, 63 nM, and approximately 10 nM, respectively. These effects were endothelium-dependent and inhibited by a TRPV4 antagonist, AB159908 (10 μM). Capsaicin and GSK1016790A produced vascular dilation in isolated mouse perfused kidneys at EC_{50} of 23 nM and 3 nM, respectively. R-L3 (a $K_V7.1$ opener) produced concentration-dependent relaxation (EC_{50} of approximately 1.4 μM) of wild-type (*Kcnq1*+/+). This relaxation was not affected by chromanol-293B ($K_V7.1$ inhibitor), HMR1556 ($K_V7.1$ inhibitor), or XE991 (pan-K_V7 blocker). Capsaicin ameliorated the outcome of AKI mice, as measured by serum creatinine levels, tubular damage, neutrophil gelatinase-associated lipocalin abundance, and Ly-6B.2 positive polymorphonuclear inflammatory cells in injured kidneys. These results demonstrate that TRPV1 has a narrow, discrete distribution in the resistance vasculature and that TRPV4 has a universal, widespread distribution along different vascular segments in mice. Activation of TRPV1 channels ameliorates I/R-induced AKI, but inhibition of these channels does not affect the outcome of AKI. $K_V7.1$ channels are not required for the control of arterial tone by alpha1 adrenergic vasoconstrictors in the renal arteries of mice.

1. Introduction

1.1 Functional TRPV1/4 in kidney vasculature

Transient receptor potential vanilloid 1 (TRPV1), a polymodal nonselective cation channel, is expressed in sensory neurons and in non-neuronal tissues[1]. Apart from its role as a potent mediator of analgesic effects, TRPV1 also exerts effects in the cardiovascular system[2]. Drugs that target TRPV1 and/or the downstream pathways activated by TRPV1 have been reported to prevent ischemia-induced injury in the kidney[3] and in the lung[4], and to reduce renal injury in mice with deoxycorticosterone acetate (DOCA)-salt hypertension[5]. The underlying processes are largely unknown, but may involve vascular mechanisms.

Yang et al. identified TRPV1 channels in the endothelium of mouse mesenteric arteries[6]. Notably, TRPV1 activation was recently reported to decrease phosphorylation of endothelial nitric oxide synthase (eNOS) at threonine 497 (Thr497) in cultured bovine aortic endothelial cells, which led to increased nitric oxide (NO) production and vasodilation[7]. This finding is of particular interest because transient receptor potential vanilloid 4 (TRPV4) channels are regarded as key TRPV channels that promote endothelium-dependent relaxation in these and other arteries[8,9,10]. For instance, calcium sparklets were shown to provide elementary calcium influx through individual TRPV4 channels in mesenteric arteries[11].

Although TRPV1 and TRPV4 are expressed in several systemic vascular beds[5,12], the relative amount and regional distribution of functional TRPV1 and TRPV4 in the renal circulation are unknown. It was hypothesized that TRPV1/4 are involved in endothelium-dependent vasodilation of renal blood vessels.

1.2 K_V7.1 channels and the regulation of renal arterial vascular tone

K_V7.1 voltage-gated potassium channels are expressed in vascular smooth muscle cells (VSMCs) of diverse arteries, including renal arteries[13,14,15]. Based on pharmacological evidence using R-L3 (a K_V7.1 opener) and HMR1556 and chromanol 293B (K_V7.1 inhibitors), these channels have been suggested to evoke profound relaxation upon stimulation in various vascular beds in rats[15]. However, the specificity of these drugs *in vivo* is uncertain. *Kcnq1-/-* mice and pharmacological tools were applied to determine whether K_V7.1 plays a role in the regulation of arterial tone.

1.3 TRPV1 channels in acute kidney injury (AKI)

TRPV1-positive sensory nerves are widely distributed in the kidney, suggesting that TRPV1-mediated action may participate in the regulation of renal function under pathophysiological conditions[16]. Indeed, sodium excretion in response to sodium loading is impaired in models of salt-sensitive hypertension induced by surgical sensory denervation or by sensory nerve degeneration following capsaicin treatment[17]. Blockade of TRPV1 increases blood pressure in Wistar or Dahl salt-resistant rats fed a high, but not normal, salt diet suggesting that high salt intake may activate TRPV1 and confer a protective effect[18]. Chronic TRPV1 stimulation by systemic application of capsaicin lowers systemic blood pressure and may involve TRPV1 channels in sensory nerves and blood vessels[6].

Catheter-based renal denervation (RDN) is a novel technique to lower resistant hypertension by specifically targeting renal sympathetic nerves[19]. This procedure is expected to affect the function of TRPV1-rich primary sensory nerves of the renal sympathetic nervous system. Renal sympathetic nerves and circulating catecholamines are believed to be involved in the progressive renal tissue injury that accompanies ischemic acute renal failure[20,21]. However, further investigation is needed regarding the primary sensory nerves of the renal sympathetic nervous system to understand how they contribute to the pathogenesis of ischemia/reperfusion (I/R)-induced AKI.

Three studies have reported that systemic activation of TRPV1 by capsaicin ameliorates I/R-induced renal dysfunction[22,23,24]. Capsazepine is a potent blocker of TRPV1 channels[25,26], but this drug has not been studied in AKI. Deficiency of TRPV1 genes in mice has been shown to impair cardiac recovery following I/R injury[27]. It is unknown whether inhibition of TRPV1 channels is detrimental in AKI or not. Knowledge of these effects may have clinical implications by providing a greater understanding of the kidneys' ability to respond to ischemic stimuli after renal denervation therapy.

Objectives

Specifically, the following hypotheses were tested:

1) TRPV1 and TRPV4 cation channels promote endothelium-dependent relaxation of mouse mesenteric arteries. However, the role of TRPV1 and TRPV4 in the renal vasculature is largely unknown. It was hypothesized that TRPV1/4 are involved in endothelium-dependent vasodilation of renal blood vessels.

2) $K_V7.1$ voltage-gated potassium channels are expressed in renal arteries. Therefore, $K_V7.1$ could be involved in regulating arterial tone. To test this hypothesis, *Kcnq1-/-* mice and pharmacological tools were used to determine whether $K_V7.1$ plays a role in the regulation of arterial tone.

3) Stimulation of TRPV1 channels protects against I/R-induced AKI. However, it is unknown whether inhibition of these channels is detrimental in AKI or not. Therefore, the role of TRPV1 channels in I/R-induced AKI was investigated by modulating these channels with capsaicin (a TRPV1 agonist) and capsazepine (a TRPV1 antagonist) and by studying *Trpv1-/-* mice.

This study will demonstrate new findings regarding the function and expression of TRPV1 and TRPV4 channels in the renal vasculature. In addition, it will provide insight into the role of TRPV1 in renal vascular resistance, a response that is critical for the regulation of blood pressure. Moreover, this research aims to determine whether $K_V7.1$ plays a role in regulating renal arterial tone and whether inhibition of TRPV1 channels is detrimental in AKI or not.

2. Method

2.1 RNA isolation and quantitative real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from kidney, isolated fat-free renal tissue, and mesenteric artery tissue using the RNeasy RNA isolation kit (Qiagen GmbH, Hilden, Germany). RNA concentration and quality were measured with a NanoDrop-1000 spectrophotometer (PeqLab, Erlangen, Germany). Two micrograms of RNA were used for complementary DNA (cDNA) transcription (Life Technologies GmbH, Darmstadt, Germany). Quantitative analysis of target messenger RNA (mRNA) expression was performed with RT-PCR using the relative standard curve method. SYBR green analysis was conducted using an Applied Biosystems 7500 Sequence Detector (Technologies GmbH, Darmstadt, Germany). The mRNA levels of target genes were normalized against 18s ribosomal RNA levels[28].

2.2 Measurement of vascular reactivity. Vascular reactivity was measured in freshly isolated mesenteric arteries and main renal arteries as previously described[29]. In some experiments, the endothelium was mechanically removed using a hair by rubbing it along the endothelium. Before and after removing the endothelium, endothelial integrity and functionality were confirmed by the relaxant response to acetylcholine (Ach; 1 μM)[28].

2.3 Perfusion of kidney vasculature. Isolated kidney perfusion was performed as described previously[28,30].

2.4 Perfusion of outer medullary descending vasa recta (DVR). Animals were sacrificed under isoflurane anesthesia. The kidneys were removed, and transversal slices were made along the main axis of the renal medulla. DVR were manually isolated using sharpened forceps, and perfused using concentric micropipettes as described previously[28,31].

2.5 Cell culture and transfection. Human embryonic kidney 293 (HEK293) cells were grown in minimal essential medium (MEM) supplemented with Earl salts (PAA, Pasching, Austria) and with 10% fetal calf serum (PAA), 4 mM L-glutamine (PAA), 100 units/ml penicillin (PAA), and 100 μg/ml streptomycin (PAA) in the presence of 5% CO_2 at 37 °C. For the experiments, the cells were plated in 35 mm dishes and placed onto glass coverslips. One to two days after seeding, the cells were transfected with 1 μg of plasmid DNA coding for TRPV1 C-terminally fused to yellow

fluorescent protein (YFP) using X-tremeGENE 9 (Roche Diagnostic, Mannheim, Germany). The cells were used in experiments 15 h post-transfection[2,28,32].

2.6 Single cell fluorescence measurements of transfected HEK293. $[Ca^{2+}]_i$ measurements in single cells were carried out using the fluorescence indicator fura-2-AM in combination with a monochromator-based imaging system (FEI-T.I.L.L. Photonics, Gräfeling, Germany) attached to an inverted microscope (Axiovert 100; Carl Zeiss, Oberkochen, Germany)[33]. Cells were loaded with 2 μM fura-2-AM (Biomol, Hamburg, Germany) for 30 minutes at 37 °C in a standard solution composed of (in mM) 138 NaCl, 6 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 5.5 glucose, and 10 HEPES (adjusted to pH 7.4 with NaOH). For $[Ca^{2+}]_i$ measurements, fluorescence was excited at 340 and 380 nm[28].

2.7 Animal model of renal I/R. The model of I/R-induced AKI was established as previously described for rats[34,35].

2.8 Serum creatinine measurements. Serum creatinine levels were measured by an autoanalyzer (Beckman Analyzer; Beckman Instruments, Munich, Germany).

2.9 Histological evaluation of the kidneys. The kidneys were immersed in 4% buffered formalin for histopathological and immunohistochemical examinations. In total 51 mice underwent surgery; two of them died during the perioperative period and were excluded from the study[28]. Histomorphometric analysis was performed to evaluate the acute tubular necrosis (ATN) score semiquantitatively in mouse renal parenchyma using hematoxylin and eosin (H&E) staining. Slides were examined under a light microscope (200X magnification). Acute tubular injury (ATI) was observed in this study to assess the reversible tubular damage due to ischemia[35].

2.10 Neutrophil gelatinase-associated lipocalin (NGAL) immunohistochemistry. NGAL immunohistochemistry was performed on paraffin-fixed kidney sections. Nonspecific binding sites were blocked with 10% normal donkey serum for 30 minutes. Then sections were incubated with NGAL antibody (dilution: 1:300) overnight at 4 °C in a humid chamber. For fluorescence visualization of bound primary antibody, sections were further incubated with Cy3-conjugated secondary antibody for 1 h in a humid chamber at room temperature[33,35].

2.11 Immunohistochemical detection of Ly-6B.2 positive cells. Immunohistochemical staining of Ly-6B.2 positive cells was performed on paraffin-fixed kidney sections. Antigen retrieval was performed by incubating sections for 10 minutes at 37 °C in a trypsin solution (Sigma). After cooling down, nonspecific

binding sites were blocked with 10% normal donkey serum for 30 minutes. Then the sections were incubated with the rat anti-mouse Ly-6B.2 monoclonal antibody, clone 7/4 (dilution: 1:300) overnight at 4 °C in a humid chamber. For fluorescence visualization of bound primary antibody, sections were further incubated with Cy3-conjugated secondary antibody for 1 h in a humid chamber at room temperature[35,36].

2.12 Determination of endovanilloid profiles. Endovanilloid profiles were measured by Lipidomics GmbH (Berlin, Germany).

For a complete description of the methods see:

Chen L, Kaßmann M, Sendeski M, Tsvetkov D, Marko L, Michalick L, Riehle M, Liedtke WB, Kuebler WM, Harteneck C, Tepel M, Patzak A, and Gollasch M. Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. Acta Physiol (Oxf). 2015;213(2):481–91.

Chen L, Marko L, Kaßmann M, Zhu Y, Wu KY, and Gollasch M. Role of TRPV1 channels in ischemia/reperfusion-induced acute kidney injury. PlosOne. 2014;9(10):e109842.

Tsvetkov D, Kaßmann M, Tano JY, **Chen L**, Schleifenbaum J, Voelkl J, Lang F, Huang Y, and Gollasch M. Do KCNQ1 channels contribute to control of arterial vascular tone? British Journal of Pharmacology. 2017;174(2):150–162.

Copies of these publications are located in the **Appendix**.

3. Results

Project 1: Functional TRPV1 channels in the murine renal vasculature

The TRPV1agonist capsaicin relaxed mouse mesenteric arteries and isolated mouse perfused kidneys with anEC_{50} of 25nM and 23nM, but large mouse renal arteries or rat descending vasa recta only at >100-fold higher concentrations. The vasodilatory effect of capsaicin in the low-nanomolar concentration range was endothelium-dependent and absent in vessels of *Trpv1 -/-* mice.

Anesthetized C57BL/6 mice were subjected to 25 min of renal ischemia and 24 hrs of reperfusion. Mice were pretreated with capsaicin (0.3 mg/kg body weight) or capsazepine (50 mg/kg body weight). Capsaicin ameliorated the outcome of AKI, as measured by serum creatinine levels, tubular damage,neutrophil gelatinase-associated lipocalin (NGAL) abundance and Ly-6B.2 positive polymorphonuclear inflammatory cells in injured kidneys. Neither capsazepine nor deficiency of TRPV1 did deteriorate renal function or histology after AKI.

Project 2: Functional TRPV4 channels in the murine renal vasculature

The TRPV4 agonist GSK1016790A relaxed large conducting renal arteries, mesenteric arteries and vasa recta withEC50 of 18, 63nM and ~10 nM respectively. These effects were endothelium-dependent and inhibited by a TRPV4 antagonist, AB159908 (10μM). GSK1016790A produced vascular dilation in isolated mouse perfused kidneys with EC50 of 3 nM and the effects were inhibited in *Trpv4 -/-* kidneys.

Project 3: K_V7.1 channels in renal arteries

R-L3 produces similar concentration-dependent relaxations (EC50 ~1.4 μM) of wild-type (*Kcnq1+/+*) and *Kcnq1-/-* arteries pre-contracted with either phenylephrine or 60 mM KCl. This relaxation was not affected by 10 μM chromanol 293B, 10 μM HMR1556 or 30 μM XE991 (pan-K_V7 blocker).

4. Discussion

This study provides several new findings regarding the function and expression of TRPV1 and TRPV4 channels in the renal vasculature. First, functional TRPV1 channels are present in renal resistance arteries of mice as indicated by measurements in isolated kidneys, but they are nonfunctional in renal conduit arteries. Second, functional TRPV4 channels are expressed in both renal resistance arteries and renal conduit arteries. Third, the vasodilatory effect of capsaicin at low nanomolar concentrations is absent in arteries of *Trpv1-/-* mice. In contrast, the vasorelaxant effect of GSK1016790A is observed in mesenteric and renal resistance arteries of *Trpv1-/-* mice, but not in *Trpv4-/-* mice, indicating that the TRPV1- and TRPV4-mediated vasodilations are not functionally coupled. Nevertheless, endothelial NOS seems to be a common downstream mediator of both TRPV4- and TRPV1-dependent vasodilations, as indicated by our experiments using NG-Nitro-L-arginine methyl ester hydrochloride(L-NAME). Fourth, these findings provide compelling evidence that $K_V7.1$ channels do not contribute to vascular contraction in mouse mesenteric and renal arteries. A striking loss of functional $K_V7.1$ channels in R-L3 induced relaxations was observed. Fifth, this study showed for the first time that I/R–induced AKI is not affected by inhibition of TRPV1 channels.

4.1 TRPV1 in the kidney

Recent preclinical data indicate that activators of TRPV1 may improve the outcome of ischemic AKI[37]. Although a number of derivatives of arachidonic acid (AA), such as 5-,12-, and 20-lipoxygenase products, cytochrome P450 epoxygenase products (epoxyeicosatrienoic acids), and N-acyl dopamine conjugates of AA, have been identified as endogenous activators (“endovanilloids”) of TRPV1[2], their roles as TRPV activators in AKI are unclear. Activation of TRPV1 in the kidney *in vivo* or in isolated perfused kidneys increases the glomerular filtration rate (GFR) and enhances renal sodium and water excretion[38].

The present studies failed to detect functional TRPV1 channels in large renal arteries and DVR of kidneys. Instead, the findings revealed an additional role of TRPV1 in the renal circulation, namely in regulating preglomerular vascular resistance. This function is critical for the regulation of systemic blood pressure and may provide a mechanistic explanation for the stimulating effect of capsaicin on the GFR. Future

studies are needed to clarify the role of TRPV1 channels and endogenous “vanilloids,” such as the TRPV1 agonist 20-hydroxyeicosatetraenoic acid (20-HETE)[2], in hypertension and renal I/R injury. These results indicate that the role of TRPV1 channels in renal resistance vasculature should be considered in future studies. The renal resistance vasculature includes interlobular, afferent, and efferent resistances, so each of these vessels could serve as a putative target for TRPV1 action. Future studies should determine whether the distribution of functional TRPV1 channels is restricted to certain resistance vessels.

Ischemic acute renal failure is a frequent clinical syndrome with high morbidity and mortality[39]. Recent preclinical data indicate that activators of TRPV1 can improve the outcome of ischemic AKI[22,23,24]. In the present study, an isolated kidney I/R mouse model of AKI was used to study the effects of pharmacological inhibition of TRPV1 channels (using capsazepine) and genetic ablation of TRPV1 (using *Trpv1*-/- mice). For comparison, the effects of TRPV1 stimulation (using the TRPV1 agonist capsaicin) were studied. The results demonstrated that inhibition of TRPV1 channels does not affect the outcome of I/R-induced renal injury. Evaluation of three injury parameters demonstrated that TRPV1 inhibition does not cause harm in I/R-induced AKI. Although the relatively high concentration of capsaicin used in this study to activate TRPV1 *in vivo* may have additional effects not related to TRPV1, measurements of the three injury parameters demonstrated a beneficial role of capsaicin in I/R-induced AKI as previously reported by other groups for capsaicin and other TRPV1 agonists[22,23,24]. There were consistently fewer Ly-6B.2-positive cells in kidneys after I/R in capsaicin-treated mice. The beneficial effects of capsaicin may involve release of a substance from TRPV1-expressing nerve endings that exerts a protective action against I/R-induced kidney injury. One interesting candidate is somatostatin[40,41], which can act as an autocrine “endovannilloid” to modulate peripheral TRPV1 receptors[42,43]. To date, somatostatin has not been implicated in AKI. In addition, the measurements of other endovanilloids[44] in kidney tissue demonstrate that anandamide and/or 9,10- and 11,12-epoxides of linoleic acid (EpOMEs/DiHOMES), but not 20-HETE or epoxyeicosatrienoic acids (EETs), are possible candidates involved in the beneficial effects of capsaicin on the outcome of I/R-induced AKI. Although EpOMEs/DiHOMES[45] and anandamide analogs/receptor agonists[46] have been recently suggested as novel therapeutic targets in AKI, these data suggest that future studies on their roles in AKI should consider TRPV1 signaling pathways to understand their molecular actions.

These data are consistent with previous results demonstrating that systemic activation of TRPV1 channels by capsaicin or other agonists mitigates I/R-induced AKI[22,23,24]. There are concerns that this procedure may harm the kidney's ability to respond to ischemic stimuli later in life[2] because long-term clinical results on patients who have undergone this procedure are not yet available[47].

4.2 TRPV4 in the kidney

Northern blot analysis revealed strong expression of TRPV4 in the kidney; however, the regional distribution of functional TRPV4 channels in the renal vasculature is unknown[48]. The present results demonstrate that the TRPV4 agonist GSK1016790A produces endothelium-dependent relaxation of renal and mesenteric arteries. These effects were inhibited by the TRPV4-selective antagonist AB159908, suggesting that the vasodilatory effects of GSK1016790A are dependent on activation of TRPV4 channels. Moreover, GSK1016790A produced relaxation of resistance arteries and DVR in kidneys. Although the DVR experiments were performed in rats and may not represent the function of the vasa recta in mice, the lack of the capsaicin effect in rat DVR is not because arteriolar TRPV1 channels of this species have atypical capsaicin pharmacology[49]. The results of this study showed that GSK10167790A effects occurred at similar concentrations in both species. These results indicate a lack of functional TRPV1 channels in the DVR.

Taken together, these results also indicate that functional TRPV4 channels are widely distributed in the renal vasculature. The distribution of functional TRPV4 in the DVR may be therapeutically exploitable to target the "reflow phenomenon" in AKI or sepsis-associated vasoconstriction in the kidney. These ideas require experimental testing in future studies. Notably, vasorelaxant effects of GSK1016790A were observed in mesenteric and renal resistance arteries of *Trpv1-/-* mice, but not of *Trpv4-/-* mice. These results suggest that TRPV4 channel gating occurs independently of TRPV1 channels. Thus, endothelial TRPV4 and TRPV1 channels present novel therapeutic targets in the kidney, each of which can be independently targeted by TRP modulators.

The role of eNOS/NO in TRPV4-mediated vasodilation is a matter of debate. The present data suggest that eNOS plays an important role in TRPV4-mediated vasodilation of renal vessels. The involvement of NO in TRPV4 effects has been reported in a number of different arteries and species, including 1st order mouse mesenteric arteries[50], rat pulmonary arteries[51], and rodent aortas[52]. In contrast,

Sonkusare et al.[10] and Marrelli et al.[53] studied small mouse mesenteric arteries (similar to our study) and rat middle cerebral arteries, and they found that NO does not play a major role in TRPV4-dependent relaxation of these vessels. Differences in vessel type and species may account for the apparent differences in vascular TRPV4 signaling to produce vasodilation[52]. Lastly, the present results indicate that functional TRPV4 channels, but not TRPV1, are present in DVR providing medullary blood supply into the kidney.

4.3 K_V7.1 channels in renal arteries

The results of these studies indicate that K_V7.1 channels do not contribute to vascular contraction in mouse renal arteries. No functional role of K_V7.1 channels in R-L3 induced relaxations was observed. Thus, K_V7.1 channels are apparently not involved in controlling arterial tone by alpha1 adrenergic vasoconstrictors. In addition, R-L3 is not an appropriate pharmacological tool for studying the function of native vascular K_V7.1 channels in mice.

In conclusion, K_V7.1 channels do not contribute to vascular contraction in mouse renal arteries.TRPV4 channels are capable of promoting endothelial relaxation in renal resistance arteries, renal conduit arteries, and medullary vasa recta, whereas functional TRPV1 channels have a more narrow, discrete distribution, namely in the renal resistance vasculature. Because TRPV1 and TRPV4 have unique sites of vasoregulatory function in the kidney, targeted activation of TRPV1 or TRPV4 may provide a promising novel strategy for modulating regional blood flow in this organ.
For a complete description of the result and discussion see:

Chen L, Kaßmann M, Sendeski M, Tsvetkov D, Marko L, Michalick L, Riehle M, Liedtke WB, Kuebler WM, Harteneck C, Tepel M, Patzak A, and Gollasch M. Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. Acta Physiol (Oxf). 2015;213(2):481–91.

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Affidavit

I, Lan Chen, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic Functional TRPV1 and TRPV4 channels in the murine renal vasculature. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____

Date Signature

Declaration of any eventual publications

Lan Chen had the following share in the following publications:

Publication 1:

Chen L, Kaßmann M, Sendeski M, Tsvetkov D, Marko L, Michalick L, Riehle M, Liedtke WB, Kuebler WM, Harteneck C, Tepel M, Patzak A, and Gollasch M. Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. Acta Physiol (Oxf). 2015;213(2):481–91.

Contribution in detail:

Help in design of experimental protocols, preparation of vessels, isometric contraction force measurements in mouse arteries, kidney perfusion, analysis and interpretation of data, writing the first draft of the manuscript, interpretation of results, and literature searches.

Publication 2:

Chen L, Marko L, Kaßmann M, Zhu Y, Wu KY, and Gollasch M. Role of TRPV1 channels in ischemia/reperfusion-induced acute kidney injury. PlosOne. 2014;9(10):e109842.

Contribution in detail:

Help in design of experimental protocols, preparation of vessels, isometric contraction force measurements in mouse arteries, kidney perfusion, analysis and interpretation of data, writing the first draft of the manuscript, manuscript revisions, interpretation of results, and literature searches.

Publication 3:

Tsvetkov D, Kaßmann M, Tano JY, **Chen L**, Schleifenbaum J, Voelkl J, Lang F, Huang Y, and Gollasch M. Do KCNQ1 channels contribute to control of arterial vascular tone? British Journal of Pharmacology. 2017;174(2):150–162.

Contribution in detail:

Help in design of experimental protocols, preparation of vessels, contributions to experiments, writing the manuscript, and analysis and interpretation of data.

Signature, date and stamp of the supervising university teacher
Prof. Dr. med. Dr. rer. nat. Maik Gollasch

_____

Signature of doctoral student
Lan Chen

Selected publications

Publication #1: Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. (pages 25-35)

Chen L, Kaßmann M, Sendeski M, Tsvetkov D, Marko L, Michalick L, Riehle M, Liedtke W. B, Kuebler W. M, Harteneck C, Tepel M, Patzak A and Gollasch M. Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. Acta Physiol (Oxf). 2015;213(2):481-91.
http://dx.doi.org/10.1111/apha.12355

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Publication #2 : Role of TRPV1 channels in ischemia/reperfusion-induced acute kidney injury.(pages 36-45)

Chen L, Marko L, Kaßmann M, Zhu Y, Wu K.Y, Gollasch M. Role of TRPV1 channels in ischemia/reperfusion-induced acute kidney injury. PlosOne. 2014 ; 9(10):e109842. http://dx.doi.org/10.1371/journal.pone.0109842

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Publication #3: Do KCNQ1 channels contribute to control of arterial vascular tone? (pages 46-58)

Tsvetkov D, Kaßmann M, Tano J.Y, **Chen L,** Schleifenbaum J, Voelkl J, Lang F, Huang Y, and Gollasch M. Do KCNQ1 channels contribute to control of arterial vascular tone? British Journal of Pharmacology. 2017;174(2):150-162.
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Curriculum Vitae

Due to privacy settings, the CV will not be displayed in the online version of this document.

Complete list of publications

1. **Chen L**, Kaßmann M, Sendeski M, Tsvetkov D, Marko L, Michalick L, Riehle M, Liedtke W. B, Kuebler W. M, Harteneck C, Tepel M, Patzak A and Gollasch M. Functional TRPV1 and TRPV4 channels along different segments of the renal vasculature. Acta Physiol (Oxf). 2015;213(2):481-91.
2. **Chen L**, Marko L, Kaßmann M, Zhu Y, Wu K.Y, Gollasch M. Role of TRPV1 channels in ischemia/reperfusion-induced acute kidney injury. PlosOne. 2014 ; 9(10):e109842.
3. **Chen L**, Ling Y.S, Lin C.H and Guan T.J. Combined use of heparin and anisodamine reduces the risk of early thrombosis in native arteriovenous fistula. Vascular.2013; 21(6): 369–374.
4. **Chen L**, Ling YS, Lin CH, He JX, Guan TJ. High dose ESAs are associated with high iPTH levels in hemodialysis patients with end-stage kidney disease: a retrospective analysis. Front Public Health. 2015;18;3:258.
5. **Chen L,** Chen YY, Ling Y.S, Lin C.H, He JX and Guan T.J. Investigation of optimum N-terminal probrain natriuretic peptide level in patients on maintained hemodialysis. Renal failure. 2017;39(1):372-378.
6. Tsvetkov D, Kaßmann M, Tano J.Y, **Chen L**, Schleifenbaum J, Voelkl J, Lang F, Huang Y, and Gollasch M. Do KCNQ1 channels contribute to control of arterial vascular tone? British Journal of Pharmacology. 2017;174(2):150-162.
7. Ke SK, **Chen L**, Duan HB, Tu YR. Opposing actions of TRPV4 channel activation in the lung vasculature. Respir Physiol Neurobiol. 2015 Dec;219:43-50.

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