Functional Importance of L- and P/Q-Type Voltage-Gated Calcium Channels in Human Renal Vasculature

Pernille B. Hansen, Christian B. Poulsen, Steen Walter, Niels Marcussen, Leanne L. Cribbs, Ole Skøtt, Boye L. Jensen

Abstract—Calcium channel blockers are widely used for treatment of hypertension, because they decrease peripheral vascular resistance through inhibition of voltage-gated calcium channels. Animal studies of renal vasculature have shown expression of several types of calcium channels that are involved in kidney function. It was hypothesized that human renal vascular excitation-contraction coupling involves different subtypes of channels. In human renal artery and dissected intrarenal blood vessels from nephrectomies, PCR analysis showed expression of L-type (Ca_{L}, 1.2), P/Q-type (Ca_{P/Q}, 2.1), and T-type subtype (Ca_{T}, 3.1 and Ca_{T}, 3.2) voltage-gated calcium channels (Ca_{S}s), and quantitative PCR showed highest expression of L-type channels in renal arteries and variable expression between patients of subtypes of calcium channels in intrarenal vessels. Immunohistochemical labeling of kidney sections revealed signals for Ca_{L}, 2.1 and Ca_{T}, 3.1 associated with smooth muscle cells of preglomerular and postglomerular vessels. In human intrarenal arteries, depolarization with potassium induced a contraction inhibited by the L-type antagonist nifedipine, EC_{50} 1.2\times 10^{-5} \text{ mol/L}. The T-type antagonist mibefradil inhibited the potassium-induced constriction with large variations between patients. Interestingly, the P/Q-type antagonist, ω-agatoxin IVA, inhibited significantly the contraction with 24% at 10^{-5} \text{ mol/L}. In conclusion L-, P/Q, and T-type channels are expressed in human renal blood vessels, and L- and P/Q-type channels are of functional importance for the depolarization-induced vasoconstriction. The contribution of P/Q-type channels to contraction in the human vasculature is a novel mechanism for the regulation of renal blood flow and suggests that clinical treatment with calcium blockers might affect vascular reactivity also through P/Q-type channel inhibition. (Hypertension. 2011;58:00-00.) ● Online Data Supplement

Key Words: excitation-contraction coupling ■ renal hemodynamics ■ human ■ kidney ■ vascular biology ■ calcium channel

Calcium antagonists are widely used as pharmacological treatment for arterial hypertension and act primarily through vasodilation in the resistance vessels. Despite the existence of several subtypes of voltage-gated calcium channels (Ca_{S}s), the physiological importance of different subtypes of calcium channels for vascular smooth muscle function in humans is unknown.

The family of Ca_{S}s is divided into high-voltage activated channels (including L- and P/Q-type Ca_{S}s), and low-voltage activated channels, to which the T-type Ca_{T} belongs. Genes encoding mRNAs for Ca_{S} α_{1}-subunits have been cloned, and the L-type channels include Ca_{L}, 1.1 to 1.4, P/Q-type includes Ca_{P/Q}, 2.1, and T-type channels include 3 different Ca_{S}s, 3.1 to 3.3. In animal studies, vascular smooth muscle cells from renal preglomerular vessels express Ca_{L}, 1.2, Ca_{P/Q}, 2.1, Ca_{T}, 3.1, and Ca_{T}, 3.2. Expression of P/Q-type channels in aorta and preglomerular and postglomerular smooth muscle cells was surprising, because this channel is typically associated with neurons and the most abundant calcium channel in the brain with 2 splice variants, Ca_{L}, 2.1a and b. Postglomerular vessels from rats showed a regional heterogeneity in the expression pattern of Ca_{S}s with L- (Ca_{L}, 1.2) and T-type (Ca_{T}, 3.1 and 3.2) subunits expressed in efferent arterioles from juxtamedullary glomeruli, and only T-type-channels were observed in mouse cortical efferent arterioles. In rodents, excitation-contraction coupling in renal resistance vessels involves several types of voltage-gated calcium channels, and the activation mechanisms that induce vasoconstriction in afferent and efferent arterioles in the renal cortex are different. Depolarization and Ca_{S} activity are involved in the pathway that leads to vasoconstriction in preglomerular vasculature. In postglomerular efferent arterioles, calcium influx pathways are not dependent on depolarization and are resistant to L-type calcium channel antagonists and sensitive to T-type antagonists. In vivo data confirm the heterogeneity between afferent and efferent arterioles, and an
important contribution from T-type channels has been suggested for efferent arterioles.\textsuperscript{13–15}

Several recent clinical studies have suggested that T-type blockers have additional beneficial effects on renal function and blood pressure compared with traditional L-type blockers.\textsuperscript{16–18} In a changeover study, benidipine (L- and T-type antagonist) caused a larger reduction in blood pressure and proteinuria compared with L-type treatment using amlodipine.\textsuperscript{10} The observation of superior renoprotective effects with a combined L- and T-type treatment is in agreement with demonstration of a vasoconstrictor effect of T-type channels on efferent arterioles in animal studies.

Because several types of Cav are involved in changes in preglomerular and postglomerular vessel diameters in rodents, inhibition of 1 or several Cav\textsubscript{s} will affect regulation of renal blood flow, glomerular ultrafiltration pressure, and medullary blood flow. These effects influence glomerular filtration rate and salt and water homeostasis and thereby blood pressure. Furthermore, the kidney vascular segments are involved in several pathological conditions, such as diabetes mellitus and hypertension. Despite this significant clinical finding that indicates T-type channels in postglomerular vessels in humans, there is no information available regarding the expression patterns and functional significance of Cav\textsubscript{s} subtypes in the human renovascular bed. It is, therefore, relevant to investigate the significance of Cav\textsubscript{s} subtypes in human renal blood vessels.

The purpose of the present study was to elucidate the molecular expression pattern of Cav\textsubscript{s} and their functional significance in human renal preglomerular arteries. Focus was on L-, P/Q-, and T-type channels. Expression and location of Cav\textsubscript{s} were determined by PCR and immunostaining. The functional importance was investigated by measurement of force development in a myograph setting. Viability of the vascular smooth muscle and endothelial cells was tested by demonstrating contraction to phenylephrine (10\textsuperscript{–5} mol/L) and relaxation to acetylcholine (10\textsuperscript{–6} mol/L), respectively. For details please see the online Data Supplement.

**Methods**

**Animals**

The experimental protocol was approved by the Danish Animal Experiments Inspectorate under the Danish Ministry of Justice, and animal care followed the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals. Male and female mouse (C57BL/6) intrarenal arteries (segmental arteries) were isolated by microdissection. Furthermore, mRNA from several types of Cav subtypes in human renal blood vessels.

**Human Material**

The use of human material was approved by the Danish Ethical Committee and in agreement with declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46. Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. Human renal blood vessels were taken from patients who underwent nephrectomy. Only the part of the kidney consisting of normal tissue was used. Human renal and intrarenal arteries (interlobar and arcuate arteries) were isolated by microdissection.

**PCR Analysis**

RT-PCR and real-time PCR were performed on microdissected mouse intrarenal arteries (segmental arteries) and human renal and intrarenal arteries. For details please see the online Data Supplement at http://hyper.ahajournals.org.

**Immunohistochemistry**

For Cav\textsubscript{2.1} immunohistochemistry, human kidney sections were incubated with primary antibody rabbit anti-Cav\textsubscript{2.1} (Alomone ACC-001). For Cav\textsubscript{3.1} immunohistochemistry, sections of mouse and human kidneys were incubated overnight using primary rabbit anti-Cav\textsubscript{3.1} antibody.\textsuperscript{19} For details please see the online Data Supplement.

**Isometric Force Measurements in Intrarenal Arteries of Humans and Mice**

Human and mouse intrarenal arteries were suspended in physiological saline solution in a Halphen-Mulvany wire myograph, and isometric force development was measured. Viability of the vascular smooth muscle and endothelial cells was tested by demonstrating contraction to phenylephrine (10\textsuperscript{–5} mol/L) and relaxation to acetylcholine (10\textsuperscript{–6} mol/L), respectively. For details please see the online Data Supplement.

**Protocols**

In intrarenal arteries, the contraction induced by high potassium solution at 70 mmol/L (EC\textsubscript{75} human) or 50 mmol/L (EC\textsubscript{75} mouse) was tested in the presence of the \(\alpha\)-adrenoceptor antagonist phentolamine (10\textsuperscript{–5} mol/L) to exclude potential involvement of nerve-mediated responses. The effect of the L-type antagonist nifedipine (10\textsuperscript{–9} to 10\textsuperscript{–8} mol/L) and the T-type antagonist mibefradil (10\textsuperscript{–9} to 10\textsuperscript{–8} mol/L) on high potassium solution-elicited constriction was tested. The involvement of P/Q-type calcium channels was tested by a concentration-response relationship using toxin \(\omega\)-agatoxin IVA (10\textsuperscript{–9} to 10\textsuperscript{–8} mol/L, Alomine Laboratory, Jerusalem, Israel). For details please see the online Data Supplement.
amplification products. Positive controls were brain cDNAs, and amplification yielded products of the expected size. Using template from renal segmental arteries from mice (Figure 1C), we observed amplification products of the expected size for CaV1.2, CaV2.1a, CaV3.1, and CaV3.2.

We then examined the relative expression of the different calcium channels compared with CaV1.2 in human renal arteries and intrarenal arteries. In human renal arteries, CaV1.2 was the most abundant in all patients with the expression of CaV1.2 being the most abundant, followed by CaV2.1a, CaV3.1, and CaV3.2.

Figure 1. PCR analysis for voltage-gated calcium channel (CaV) in human and mice renal blood vessels. A, RT-PCR amplification of cDNA from 4 human renal arteries (R. artery; numbers refer to patient No.), CaV1.2, CaV2.1a, CaV3.1, and CaV3.2. Positive control was brain, and negative controls were brain-RT (omission of reverse transcriptase during reverse transcription), and H2O (water instead of cDNA in the PCR). B, As A but using cDNA from human intrarenal arteries as template. C, Expression of CaV1.2, CaV2.1a, CaV3.1, and CaV3.2 in murine intrarenal arteries (n=4). Positive control was whole kidney and negative controls were kidney-RT, omission of reverse transcriptase, and H2O.

Isometric Force Measurements in Intrarenal Arteries of Humans and Mice
The functional involvement of different types of calcium channels was tested in isolated human intrarenal blood vessels after depolarization. For details on vascular variability with the entire renal vasculature, including larger arteries (Figure 3A), glomerular arterioles (Figure 3C), and vasa recta (Figure 3D). Smooth muscle cells from all types of blood vessels were stained. Furthermore, mice arteries and arterioles also stained positive for CaV3.1 (Figure 3E), with no labeling in the negative control (Figure 3B and 3F). Also, labeling for CaV2.1 protein was observed in arteries and arterioles of human sections (Figure 4). Interestingly, arteries displayed immunopositive labeling for CaV2.1 in both smooth muscle cells and endothelial cells (Figure 4A and 4B). Cortical glomerular arterioles were also positive for CaV2.1 (Figure 4D). Figure 4E and 4F shows distinct staining of medullary vasa recta. Omission of primary antibody (negative control) yielded no staining (Figure 4C).

Isometric Force Measurements in Intrarenal Arteries of Humans and Mice
The functional involvement of different types of calcium channels was tested in isolated human intrarenal blood vessels after depolarization. For details on vascular variability
between patients, please see the online Data Supplement. Potassium concentration-dependently contracted human blood vessels with an EC_{75} of 70 mmol/L, and administration of the L-type antagonist nifedipine concentration-dependently inhibited contraction (EC_{50} of 1.2 \times 10^{-8} \text{ mmol/L}), with a first significant inhibition (from 100.0% to 5.7 \pm 9.6%) at 10^{-7} \text{ mmol/L} (Figure 5A). Full inhibition was obtained in all of the patients at 10^{-6} \text{ mmol/L} of nifedipine. In mice, nifedipine also concentration-dependently inhibited the potassium-induced contraction with a significant inhibition at 10^{-8} \text{ mmol/L} (Figure 5B).

Next we examined whether the T-type Cav{s} are involved in depolarization-induced contraction. Mibefradil had a very variable effect on blood vessels from different patients with a significant inhibition of the potassium-induced contraction at 10^{-5} \text{ mmol/L} to 40.6\pm6.0% contraction with an EC_{50} of 6.8\times10^{-7} \text{ mmol/L} (Figure 5C). In mice, a less variable response was observed, and the first significant inhibition was obtained at a concentration of 10^{-6} \text{ mmol/L} (Figure 5D).

The functional effect of the P/Q-type channels was tested by application of the antagonist ω-agatoxin IVA. The antagonist dose-dependently inhibited the contraction with significant inhibition from 100.0% to 75.8\pm2.9% at 10^{-9} \text{ mmol/L (Figure 5E)}. ω-Agatoxin IVA had no significant effect in mice intrarenal vessels (Figure 5F).

**Discussion**

The present study establishes by combined molecular identification, immunohistochemical localization, and in vitro pharmacological characterization that L-, P/Q-, and T-type calcium channels are expressed, and L- and P/Q-type channels are of functional importance in human renal preglomerular arteries. Also, postglomerular vessels express P/Q- and T-type channels, and particularly P/Q-type channels were associated with the endothelium in postglomerular vasa recta. Similar results have been obtained in vasculature of rodents. The involvement of different types of calcium channels in human renal vascular contractility has been unclear, but the present study shows for the first time that depolarization consistently constricts the blood vessels not only through an L-type channel–dependent activation but also with the involvement of the P/Q type. No significant effect of T-type blockade on contraction was observed.

Immunohistochemical localization and mRNA detection corroborate novel evidence for the coexistence of P/Q-type,
Figure 5. Depolarization-induced contraction of human and mouse intrarenal arteries. A, Effect of increasing concentrations of nifedipine (10⁻⁹ to 10⁻⁶ mol/L) on potassium-induced contraction in human intrarenal (IR) arteries mounted in a myograph. Data shown are individual patients including means (n=6). Numbers refer to patient No. B, Dose-response effect of nifedipine in murine intrarenal (IR) arteries on potassium-induced contraction. Data are mean±SEM (n=6). C, Effect of T-type voltage-gated calcium channel (Cav) inhibition by mibefradil on potassium-induced contraction in human intrarenal blood vessels. The response was variable between individual patients (numbers refer to patient No.). Data shown are individual patients including means (n=7). D, Dose-response effect of mibefradil in murine intrarenal arteries on potassium-induced contraction. Data are mean±SEM (n=6). E, Effect of increasing concentrations of ω-agatoxin IVA (10⁻¹⁰ to 10⁻⁶ mol/L) on potassium-induced contraction in human intrarenal arteries. Data shown are individual patients including means (n=6). F, Dose-response effect of ω-agatoxin IVA in murine intrarenal arteries on potassium-induced contraction. Data are mean±SEM (n=6). *P<0.05 vs potassium alone.

T-type, and L-type Cav₃s in identical vascular segments of the human kidney in agreement with rodent data.⁴⁻⁸ The dissection technique allowed us to isolate mRNA from intrarenal arteries, and the expression level of the channels was variable between patients. However, all of the patients expressed several types of calcium channels. The variability could be attributed to individual genetic or (patho)physiological differences, but also the different primary diseases and variable pharmacological regimens could be important. Hypertensive rats have increased expression of L-type channels in vascular smooth muscle cells, and increased blood pressure and membrane depolarization promote channel expression at the cell membrane.²⁰ Whether this is true for humans need to be investigated.

Immunohistochemistry data show that the channels are expressed in vascular smooth muscle cells and suggest an expression of P/Q-type channels in endothelial cells in both large arteries and vasa recta. In a recent study of calcium current in rat vasa recta pericytes, P/Q-type antagonist had no effect on calcium currents,⁴¹ in contrast to rat preglomerular myocytes.⁴ However, endothelial cells were not investigated.

Cav₃s of the Cav₃.2 and Cav₃.1 types have been reported previously in endothelial cells in rats,²²⁻²³ albeit with unclear function. The antibody specific for the T-type subunit Cav₃.2 did not apply to human tissue, and we did not observe a Cav₃.1 labeling of human renal endothelial cells.

In agreement with the molecular data, functional data from the present study provide new evidence for the involvement of several types of channels in the excitation-contraction mechanism in human renal vasculature. Depolarization led to contraction that was sensitive to the L-type antagonist nifedipine in all of the tested patients, which confirms the exquisite voltage dependence of preglomerular contraction known from many species. Also, the EC₅₀ observed in the present study (EC₅₀ of 1.2×10⁻⁵ mol/L) is in agreement with the reported human EC₅₀ for L-type channels (EC₅₀ of 1 to 10×10⁻⁵ mol/L). Surprisingly, also P/Q-type channels contributed to the potassium-induced contraction, because the specific P/Q-type antagonist ω-agatoxin IVA inhibited the contraction by ≈24%. ω-Agatoxin IVA is a selective P/Q-type antagonist that blocks P-type channels at concentrations
<10 nmol/L, whereas the Q channels are inhibited at higher concentrations,24 and the observed effect at low concentrations of ω-agatoxin IVA in the present study is, therefore, probably attributed to a P-type current. This effect indicates a new player in the contraction mechanism in human renal arteries. In agreement, Ca_{2.1}-mediated currents have been observed in human umbilical arteries and rat pregglomerular myocytes.7,25 The biophysical properties vary between calcium currents. Compared with L-type currents, P/Q-type Ca^{2+} currents are slower, have a Ca^{2+}-independent rate of inactivation, and have inhibitory modulation by G proteins. The latter feature is involved in hormonal regulation of channel properties;26 thus, the presence of P/Q-type currents in the human vasculature could give rise to novel mechanisms for hormonal influence on human intrarenal vascular reactivity. In rodents, P/Q-type calcium channels contribute significantly to the increase in intracellular [Ca^{2+}] and contraction after depolarization of renal vasculature.4 Mice deficient in P/Q-type Ca_{α} display a phenotype with pronounced neurological deficits, but abnormalities of cardiovascular function were not investigated.27 In humans, mutations in the gene encoding the Ca_{α,2.1} subunit are associated with episodic ataxia type 2, familial hemiplegic migraine, and spinocerebellar ataxia type 6.28,29 Whether mutations in the Ca_{α,2.1} subunit gene are involved in the pathogenesis of renal and vascular disorders is unknown.

The T-type blocker mibefradil selectively inhibits T-type currents at low concentrations, with an EC_{50} of ~10 nmol/L.30,31 In the present study, mibefradil blocked K^{+}-induced contraction at 10^{-9} mol/L in some patients, whereas in others the contraction was not affected until 10^{-6} mol/L, a concentration also inhibiting L-type channels. Our data show a large variation between patients questioning whether T-type channels play a role for contraction of larger renal vasculature, and with a mean EC_{50} of 6.8×10^{-7} mol/L, no significant T-type channel effect was observed. In agreement, T-type channels have been shown to have their dominant effects in smaller human and rat arterioles compared with larger arteries.32 Also in rodents, T-type channels are most important for the contraction of smaller blood vessels,33 suggesting that T-type channels might also be of greater significance in human renal arterioles compared with arteries.

Calcium blockers are widely used in conditions such as hypertension34,35 because they possess the ability to decrease peripheral vascular resistance, affect renal tubules leading to natriuresis, and affect cardiac function. The antagonists differ in their selectivity toward L-, T-, and P/Q-type calcium channels.36 L-type calcium channel blockers, such as the dihydropyridines verapamil and diltiazem, have been reported to significantly inhibit T-type and P-type calcium currents when used in concentrations that are not maximal for the L-type blockade.36,37 Amlodipine inhibits L- and P/Q-type calcium channels equally well in oocytes, whereas nifedipine is selective toward L-type channels. Such differences may contribute to in vivo differences of the calcium channel blockers in vascular beds. The present demonstration of functional P/Q-type Ca_{α} in the human renal vasculature therefore suggests that certain conventional calcium channel blockers also exert effects on P-type Ca_{α}.

Our evidence for T-type channels in human renal blood vessels is in agreement with the notion that T-type blockers have specific and beneficial intrarenal effects compared with L-type blockers.16–18 In a crossover study, benidipine (L- and T-type antagonist) caused a larger reduction in blood pressure and proteinuria compared with L-type treatment using amlodipine,16 and benidipine favorably affects renal function in patients with essential hypertension compared with amlodipine.18 These studies suggest a clinical benefit of benidipine over amlodipine as an antihypertensive drug because of a renoprotective effect. The observation of superior renoprotective effects with a combined L- and T-type treatment is in agreement with the previous demonstration of a differential expression of calcium channels in rodent kidney with a vasostrictor effect of T-type channels on efferent arterioles.3,8 This information is not yet available from the human kidneys for technical reasons. A study on human glomerular arterioles would elucidate this issue to be of great interest, because these vascular resistance vessels determine renal blood flow rate and glomerular filtration rate and affect blood pressure.

**Perspectives**

In conclusion, L-, P/Q-, and T-type Ca_{α}s are present in human renal blood vessels, and L- and P/Q-type channels are of functional importance for the depolarization-induced vasconstriction. These novel observations are relevant for the understanding of regulation of human renal blood flow and glomerular filtration rate and should be considered when choosing calcium blockers for clinical treatment. The functional importance of P/Q-type channels in the human vasculature could give rise to novel mechanisms for the regulation of renal blood flow and suggests that clinical treatment with calcium blockers might affect vascular reactivity through P/Q-type channel inhibition.

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**Disclosures**

None.

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FUNCTIONAL IMPORTANCE OF L- AND P/Q-TYPE VOLTAGE GATED CALCIUM CHANNELS IN HUMAN RENAL VASCULATURE

Pernille B. Hansen¹, Christian B. Poulsen¹, Steen Walter², Niels Marcussen³, Leanne L. Cribbs⁴, Ole Skøtt¹, Boye L. Jensen¹,

¹Cardiovascular and Renal Research, University of Southern Denmark, DK-5000 Odense, Denmark.
²Department of Urology, Odense University Hospital, DK-5000 Odense, Denmark.
³Department of Pathology, University of Southern Denmark, DK-5000 Odense, Denmark.
⁴Cardiovascular Institute, Loyola University Medical Center, Maywood, Il, USA.

Short title: Calcium channels in human renal vasculature

Correspondence

Pernille B. Hansen, Ph. D.
Dept. of Physiology and Pharmacology, University of Southern Denmark
Winslowparken 21, 3. DK-5000, Odense C, DENMARK
Phone: +45 6550 3717 (direct), Fax: +45 6613 3479
E-mail: pbhansen@health.sdu.dk
Expanded materials and methods

Animals
Male and female mice (C57BL/6) had free access to rodent chow (Altromin, Lage, Germany) and tap water. Mouse intra-renal arteries (segmental arteries) were isolated by microdissection under stereomicroscope for functional studies.

Human Material
Human renal blood vessels were taken from patients, who underwent nephrectomy for renal cancer or cysts. Only the part of the kidney consisting of normal tissue was used. All patients gave their informed written consent to participate in the study. Kidneys were extirpated and immediately transported to the Institute of Pathology. Human renal and intra-renal arteries (interlobar and arcuate arteries) were isolated by microdissection under stereomicroscope within one hour of receipt of the material.

PCR-analysis:
Reverse Transcription PCR and real-time PCR was performed: RNA was isolated from microdissected mouse intra-renal arteries (segmental arteries) and human renal and intra-renal arteries by TRIzol reagent from Invitrogen (Carlsbad, CA, USA) and reverse transcribed using Superscript and oligo (dT). PCR consisted of 35 cycles and each cycle included incubation at 95ºC for 20 seconds, 60ºC for 20 seconds and 72ºC for 20 seconds. Specific primers were used for each receptor subtype.

Real-time PCR: Quantitative three-step real-time PCR was performed on a Mx3000 real time PCR instrument (Stratagene) using 2X IQ SYBR Green Supermix (BIO-RAD) according to the instructions from the manufacturer. All measurements were performed in duplicate. Real-time PCR consisted of 40 cycles and each cycle included incubation at 95ºC for 20 seconds, 60ºC for 20 seconds and 72ºC for 20 seconds. Specific primers were used for each receptor subtype.

Mouse primers: Ca.1.2(Genbank NM009781 sense 5’-TGC CTA CGG ACT TCT CTT CC-3’ and antisense 5’-GCT CCT TTC CCT CCT AGA GC-3’ covering 150bp), Ca.2.1(Genbank NM 007578 sense 5’-GCC CTT CGA GTG TTC AAC-3’ and antisense(Ca.2.1a, P-type channels)5’-CTC AGG TTG ATG AAG TTA TTC C-3’ covering 185bp, antisense(Ca.2.1b, Q-type channels) 5’-CAG GTT GAT GAA GTT ATT CGG-3’ covering 189bp), Ca.3.1(Genbank NM 009783 sense 5’-GAA CGT GAG GCC AAG AGT-3’ and antisense 5’-GCT GAG GCC AAG AGT-3’ covering 221bp), Ca.3.2(Genbank NM 021415 sense 5’-GCT CTC CCC CGT CTA CTT CG -3’ antisense 5’-AGA TAC TTT GCG CAC GAC CAG G-3’ covering 247bp). GAPDH, sense 5’-TGA TGG CAT GGA CTG TGG-3’ antisense 5’-CAG CAA TGC ATC CTG CAC-3’ covering 104bp.

Human primers: Ca.1.2(Genbank NM 199460 sense 5’- GCA ACG GCT GGA ACC TAC TA-3’ and antisense 5’-CGA TGG CAT TGG TGG AGA TGA TGG TG-3’ covering 1982), Ca.2.1(Genbank NM 001127222 sense 5’-CAA CCA CAC CGT CGT ACA AG-3’ and antisense 5’-AAA GTA GCG CAG GTT CAG GA-3’ covering 202bp), Ca.3.1(Genbank NM 018896 sense 5’-CTT CGA TGG TGT CAT TGT GG-3’ and antisense 5’-TAA GCA GCA TGC AGA AGG TG-3’ covering 227bp), Ca.3.2(Genbank NM 021098 sense 5’- ATC ATG CTC AAC TGC GTG AC-3’ and antisense 5’-TTG TGT CCG TCC AAC GAG TA-3’ covering 248bp). GAPDH, sense 5’- CGA GAT CCC TCC AAA ATC AA-3’ antisense 5’-GTC TTC TGG GTG GCA GTG AT-3’ covering 323bp.
Negative controls included water and RNA where no reverse transcriptase was added to the reaction (-RT). Positive control was whole kidney tissue (mouse) and human brain for the human part of the study (BD – Diagnostic Systems, USA). The efficiency of the all primers used was between 95-100%.

**Immunohistochemistry**

For Ca\textsubscript{2.1} immunohistochemistry, human kidneys were embedded in paraffin and cut in 4 µm slices. Then permeabilized using ethanol in decreasing concentrations (99% -> 70%) followed by 3% goat serum and incubation with primary antibody rabbit anti-Ca\textsubscript{2.1} diluted 1:50 (Alomone ACC-001) over night. For Ca\textsubscript{3.1} immunohistochemistry, paraffin embedded sections of mouse and human kidneys were treated with 0.2% triton X followed by 0.5% hydrogen peroxide for 5 minutes and 3% bovine serum albumin. Next the sections were incubated overnight using primary rabbit anti-Ca\textsubscript{V3.1} antibody diluted 1:500. Secondary antibody goat anti-rabbit IgG, horseradish peroxidase labeled (DAKO) or Alexa 568 conjugated goat anti-rabbit (Molecular Probes) diluted 1:1000 were applied. Horseradish peroxidase labeled sections were stained with diaminobenzidine (DAB\textsuperscript{-} substrate-chromogen system, DAKO) and counter stained with hematoxylin.

**Isometric force measurements in intra-renal arteries of human and mice**

Human intra-renal arteries consisting of interlobar and arcuate arteries and renal arteries were removed under stereomicroscope and stored in the following solution, in mmol/L: NaCl 103, KCl 5.4, NaHCO\textsubscript{3} 4.0, NaH\textsubscript{2}PO\textsubscript{4} 1.5, MgSO\textsubscript{4} 0.8, glucose 5.1, Na-pyruvate 0.9, Na-isethionic acid 30, HEPES 5.6 and in ml/L: MEM vitamin solution 10 (Sigma M6895), MEM essential amino acid solution 20 (Sigma M5550), and MEM nonessential amino acid solution 10 (Sigma M7145) at 4\textdegree C until further use. Mice were killed by cervical dislocation and the kidneys were placed in ice cold physiological salt solution (PSS), in mmol/L: NaCl 115, NaHCO\textsubscript{3} 25, MgSO\textsubscript{4} 1.2, K\textsubscript{2}HPO\textsubscript{4} 2.5, CaCl\textsubscript{2} 1.3, glucose 5.5, and HEPES 10 equilibrated with 5% CO\textsubscript{2} in air at pH 7.4. Human and mouse intra-renal arteries were suspended in PSS in a Halpern-Mulvany wire myograph (model 610, Danish Myo Technology A/S, Aarhus, Denmark) and isometric force development was measured (PowerLab, ADInstruments, Colorado Springs, CO, USA). Two rings per artery were incubated at 37\textdegree C in PSS. Then, the rings were normalized according the manufacturer’s protocol and allowed to equilibrate for 30 minutes. Viability of the vascular smooth muscle and endothelial cells was tested by demonstrating contraction to phenylephrine (10\textsuperscript{-6} mol/L) and relaxation to acetylcholine (10\textsuperscript{-6} mol/L), respectively. This was repeated by the end of the experiment. Blood vessels developing a contraction larger than 2 mN in response to phenylephrine were included in the study.

**Protocols: human arteries.** In intra-renal arteries the contraction induced by high potassium solution (HPS) 70 mM (EC75) was tested in the presence of the alpha-adrenoceptor antagonist phentolamin (PHE) 10\textsuperscript{-6} mol/L to exclude potential involvement of nerve mediated responses. The effect of the L-type antagonist nifedipine (10\textsuperscript{-9}-10\textsuperscript{-6} mol/L, Sigma-Aldrich) on HPS-elicited constriction was tested. Each concentration of nifedipine was applied for 5 minutes in the presence of PHE followed by 5 minute HPS administration; this was repeated for each concentration. Furthermore, concentration-response curves were obtained for the T-type antagonist mibefradil (Sigma-Aldrich) in the concentration range 10\textsuperscript{-10} to 10\textsuperscript{-5} mol/L. The involvement of P/Q-type calcium channels was tested by a concentration- response relationship using toxin \textomega-agatoxin IVA (10\textsuperscript{-10} -10\textsuperscript{-8} mol/L, Alomine Lab. Jerusalem, Israel).
Protocols: mouse arteries. Concentration-response curves were obtained for nifedipine, mibefradil ω-agatoxin IVA as described for human material using 50 mmol/L potassium (EC75) instead of 70 mmol/L.

Statistical Analysis

Data are presented as means ± SEM; n represents the number of experiments with different mice or humans. Myograph data were normalized to consecutive applications of high potassium as several administrations of potassium led to increased potassium induced responses. Significance of changes was calculated by one-way analysis of variance (ANOVA) with Bonferroni reduction for multiple comparisons. P<0.05 was considered significant.
Tables and supporting information

The study included 21 patients (15 males and 6 females). The primary diagnoses were either tumor or cysts (Table S1). The median age was 63 (range 28-81 years) and median blood pressure was 134/77 mmHg (range 146 ± 4/81 ± 1 mmHg (Table S1). None of the patients were treated with calcium blockers before donation of kidney material.

Table S1. Characteristics of the patient cohort that was examined. BP: Systolic/diastolic blood pressure before surgery. Renal cell carcinomas (RCC)

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<th>Pt. No.</th>
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<th>BP mmHg</th>
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<td>133/83</td>
<td>RCC</td>
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<tr>
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<td>185/99</td>
<td>RCC</td>
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Figures and supporting information

*Isometric force measurements in intra-renal arteries of human*

The variability in the vascular responses between patients used for the functional studies is shown in figure S1. Phenylephrine (PE) contracted all vessels by at least 2 mN and acetylcholine decreased the contraction by a mean of 27%.

**Figure S1**

Effect of phenylephrine (10⁻⁶ mol/L) and acetylcholine (10⁻⁶ mol/L) on human intra-renal (IR) arteries mounted in a myograph. Data shown are individual patients (mean of two vascular rings) including means for all patients. Numbers refer to patient no.