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INTEGRATIVE PHYSIOLOGY

No apparent role for T-type Ca^{2+} channels in renal autoregulation

Rasmus Hassing Frandsen[1] · Max Salomonsson[1] · Pernille B. L. Hansen[2] ·
Lars J. Jensen[3] · Thomas Hartig Braunstein[1] · Niels-Henrik Holstein-Rathlou[1] ·
Charlotte Mehlin Sorensen[1]

Abstract Renal autoregulation protects glomerular capillaries against increases in renal perfusion pressure (RPP). In the mesentery, both L- and T-type calcium channels are involved in autoregulation. L-type calcium channels participate in renal autoregulation, but the role of T-type channels is not fully elucidated due to lack of selective pharmacological inhibitors. The role of T- and L-type calcium channels in the response to acute increases in RPP in T-type channel knockout mice (Ca$_V$3.1) and normo- and hypertensive rats was examined. Changes in afferent arteriolar diameter in the kidneys from wild-type and Ca$_V$3.1 knockout mice were assessed. Autoregulation of renal blood flow was examined during acute increases in RPP in normo- and hypertensive rats under pharmacological blockade of T- and L-type calcium channels using mibefradil (0.1 μM) and nifedipine (1 μM). In contrast to the results from previous pharmacological studies, genetic deletion of T-type channels Ca$_V$3.1 did not affect renal autoregulation. Pharmacological blockade of T-type channels using concentrations of mibefradil which specifically blocks T-type channels also had no effect in wild-type or knockout mice. Blockade of L-type channels significantly attenuated renal autoregulation in both strains. These findings are supported by in vivo studies where blockade of T-type channels had no effect on changes in the renal vascular resistance after acute increases in RPP in normo- and hypertensive rats. These findings show that genetic deletion of T-type channels Ca$_V$3.1 or treatment with low concentrations of mibefradil does not affect renal autoregulation. Thus, T-type calcium channels are not involved in renal autoregulation in response to acute increases in RPP.

Keywords Renal blood flow · Autoregulation · Calcium channel · Renal vascular resistance

Introduction

Renal blood flow (RBF) and glomerular filtration rate (GFR) are subjected to active autoregulation [47]. Renal autoregulation serves both a regulatory and a protective purpose [29]. The former is illustrated by the need for stable electrolyte (e.g., Na$^+$) handling by the nephron despite short-term variations in renal perfusion pressure (RPP). The importance of the latter is indicated by the renal microvascular and glomerular deterioration during hypertension [21]. The dual autoregulation of RBF and GFR indicates that the tone of the afferent arteriole is the main effector in this mechanism [2, 52], but preglomerular vessels such as the arcuate and interlobular arteries also contribute significantly to the autoregulatory response [4, 6]. In other vascular beds, autoregulation is mainly executed by the myogenic and the metabolic mechanisms. In the kidney, an additional mechanism, the tubuloglomerular feedback (TGF) mechanism, contributes to autoregulation of RBF and GFR. The normal characteristics of renal autoregulation are changed in hypertension as the lower limit of autoregulation of RBF is reset to a higher perfusion pressure (e.g., in spontaneously hypertensive rats (SHRs)) [24].

✉ Charlotte Mehlin Sorensen
  cmehlin@sund.ku.dk

1 Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

2 Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense C, Denmark

3 Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

As in most vascular beds, voltage-gated Ca^{2+} channels play a dominant role in regulation of renal vascular resistance (RVR). These channels can be divided into high voltage-activated channels (L-type, P/Q-type, N-type, and R-type) and low voltage-activated channels (T-type) [8]. L-type ($Ca_v1.2$) channels expressed in vascular smooth muscle cells of most arterioles significantly affect arterial blood pressure via its effect on arteriolar tone and thus total peripheral resistance [38]. In the rat renal microcirculation, both L-type ($Ca_v1.2$) and T-type channels ($Ca_v3.1$ and 3.2) are expressed in preglomerular vessels including arcuate and interlobular arteries, afferent arterioles, and juxtamedullary efferent arterioles [16]. Also, $Ca_v3.1$ and 3.2 are found in murine cortical efferent arterioles [42]. L-type channels contribute mainly to afferent arteriolar tone [3, 5], whereas T-type channels seem to regulate both afferent and efferent arteriolar tones [11, 41, 42]. For decades, it has been known that $Ca_v1.2$ calcium channel blockers (CCBs) attenuate autoregulation of RBF [37, 40] and GFR [37]. This finding is in line with the observation that the preglomerular vasculature is the main effector in the renal autoregulation [2, 52]. The possible involvement of T-type channels in the autoregulation of RBF and GFR is less elucidated, but in mesenteric arteries, T-type channels are involved in the autoregulation [1].

Some authors report that treatment with T-type CCBs attenuates renal autoregulation [11, 14]. This was found in both isolated kidneys and in vivo in normotensive rats. Unfortunately, pharmacological blockers of these channels, such as mibefradil, pimozide, or $NiCl_2$, are unspecific and also inhibit L-type channels in a concentration-dependent manner [10, 36, 54] although low concentrations of mibefradil (100 nM) are suggested to inhibit T-type channels without significant effects on L-type channels [33]. Available results regarding the effect of T-type Ca^{2+} channels on renal autoregulation might therefore reflect a combined effect of L- and T-type inhibition. Accordingly, the possible role of T-type channels in renal autoregulation needs further elucidation. An alternative approach is the use of genetically modified mice lacking a functional T-type channel. Both $Ca_v3.1$ and $Ca_v3.2$ knockout mice are currently available [9, 25]. These mice have changes in renal hemodynamic characteristics compared to wild-type mice. $Ca_v3.1$ contributes to regulation of renal vascular resistance in vivo, whereas $Ca_v3.2$ contributes to dilation of efferent arterioles [53]. The effect of these changes has never been examined in renal autoregulation but autoregulation in the mesenteric vascular bed is significantly attenuated in $Ca_v3.1$ knockout mice [1].

The aim of the present study was to examine the importance of T-type calcium channels in the renal autoregulation. To achieve this we used mice lacking functional $Ca_v3.1$ channels. Based on in vivo data from $Ca_v3.1$ and 3.2 knockout mice, genetic deletion of $Ca_v3.1$ was hypothesized to reduce the autoregulatory constriction of the afferent arteriole in response to acute increases in RPP. Administration of CCBs targeting both $Ca_v3.1$ and 3.2 were hypothesized to lead to increased RBF after acute RPP increases in vivo. The aim was achieved by investigating afferent arteriolar diameter changes in response to acute RPP increases before and after blocking L- and T-type channels in $Ca_v3.1$ knockout mice and in wild-type C57Bl/6J mice, utilizing the in vitro blood-perfused juxtamedullary nephron technique. We also assessed autoregulation of RBF in anesthetized normotensive Sprague-Dawley (SD) rats and SHRs by evaluating RBF responses to acute increases in RPP under pharmacological blockade of renal L- and/or T-type Ca^{2+} channels. The concentrations of mibefradil used in this study have been shown to mainly block T-type calcium channels [32, 33].

Methods

For the in vitro blood-perfused juxtamedullary nephron technique, six $Ca_v3.1^{-/-}$ mice (30.8±1.0 g) bred on a C57Bl/6J background and nine commercially available C57Bl/6J mice (33.1±0.7 g; Taconic, Denmark) were used. Mesenteric arterioles from the $Ca_v3.1^{-/-}$ mice were used in another study [1] where origin and genotyping of the mice is described in detail. The $Ca_v3.1^{-/-}$ mice were originally developed in HS Shin's laboratory [25]. The mice were age matched (age 4 to 8 months). Due to the relatively high age of the mice, an age-matched analysis was performed in order to establish whether there was an age-dependent change of the renal myogenic response as has been shown in mesenteric arteries [15]. Fifteen male SD rats (Taconic, Denmark) were used as blood donors.

The in vivo experiments were performed on 35 weight-matched male SD rats (316±6 g; Taconic, Denmark) and 18 male SHRs (294±7 g; Taconic, USA).

The animal experiments were performed in accordance with the guidelines given by the Danish National Animal Experiments Inspectorate by which all protocols were approved. Animals were held in the animal facility at University of Copenhagen with a 12:12 h light/dark cycle with free access to drinking water and standard diet. All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Copenhagen at which the studies were conducted.

All chemicals were obtained from Sigma-Aldrich, Denmark, unless otherwise stated.

Immunostaining

Immunofluorescence microscopy of $Ca_v3.1$ expression in the mouse kidney vessels was performed according to a recent publication on mesenteric arterioles [1]. In brief, renal tissue was fixed in 2 % paraformaldehyde dissolved in phosphate-

buffered saline (PBS) for 1 h and then cryo-protected in 30 % sucrose. The tissue was embedded in Tissue-Tek and snap-frozen in liquid nitrogen. Sections of f 5-μm thickness were cut and hydrated 3×5 min in PBS. Unspecific staining was blocked using 5 % donkey serum. The primary $Ca_V3.1$ antibody (1:1500 in PBS; gift from Dr. LL Cribbs, Loyola University, Ill.) was added for 90 min. For peptide pre-absorption experiments, primary antibody (1:1500) was incubated for 1 h with 40 μg/mL of its immunizing peptide, according to [1]. After incubation, sections were washed in high-salt PBS followed by normal PBS and incubated with an Alexa 488-labeled secondary antibody, 4′,6-diamidino-2-phenylindole (DAPI), and rhodamine-phalloidin (not shown). Mouse sections were imaged using a Leica DM RB epifluorescence microscope with a ×40/1.0–0.5 objective. For rat tissue, 10-μm cryosections were fixed in 2 % paraformaldehyde and boiled in PBS and the primary antibody (same as for mouse tissue) incubated in 2.5 % skim milk in a concentration of 1:1500. The secondary antibody was incubated in 4 % BSA/PBS. Nuclei were stained with DAPI. Rat sections were imaged using a Zeiss LSM 700 with a ×63/1.4 objective.

Mouse in vitro blood-perfused juxtamedullary nephron technique

Blood collection from rat donors

Blood was collected from isoflurane-anesthetized rats. A catheter placed in the carotid artery was used to collect blood in a heparinized syringe. The blood was then centrifuged, plasma was removed and the buffy coat discarded. Red blood cells (RBCs) were washed twice in 0.9 % NaCl. Plasma was filtered (0.2 μm, Advantec, Tokyo, Japan) and recombined with RBCs and Tyrode buffer (NaCl: [136.9 mM]; NaH_2PO_4: [0.42 mM]; $NaHCO_3$: [11.9 mM]; KCl: [2.7 mM]; $MgCl_2$: [2.2 mM]; D-glucose: [5.6 mM]; $CaCl_2$: [1.8 mM]) containing 5 % bovine serum albumin (ICPbio International Ltd, Auckland, New Zealand) to a hematocrit of 25 %.

Preparation of kidney

Experiments were conducted based on the blood-perfused juxtamedullary nephron technique outlined by Casellas and Navar [7] adapted to mice [18]. Briefly, mice were sacrificed immediately before use by cervical dislocation and the intestine was removed for other experiments [1]. A cannula was instantly inserted into the descending aorta to perfuse the kidney. The cannula system includes a 27-gauge blunted needle for introduction into the renal artery and two PE-10 lines for blood perfusion and measurement of perfusion pressure. The kidney was perfused with Tyrode buffer (described above). The kidneys were excised, and the cannula was advanced into the right renal artery. The left kidney was snap-frozen in liquid

nitrogen for later immunostaining. A longitudinal slice was made along the kidney to expose the papilla without damaging it. The papilla was carefully checked for any remaining coagulated blood. If red lines were visible in the papilla, the kidney was discarded. The papilla was reflected back to reveal the inner cortical surface. Venous tissue on the cortical surface was cut open to gain access to the renal vasculature. Ligatures (Ethilon, New Jersey, USA; 10–0 sutures) were tied around larger arteries to restrict perfusion to the juxtamedullary afferent arterioles of the inner cortical surface. After removal of connective tissue, the Tyrode buffer was replaced with blood from the donor rat. The preparation was viewed and recorded using an Olympus BX50WI microscope with a PixelFly digital 12-bit CCD camera using the CamWare software (PCO, Kelheim, Germany). Renal perfusion pressure readings were acquired with a PowerLab/8SP data acquisition system (ADInstruments, Colorado Springs, CO, USA). During the experiment, the kidney was superfused with 37 °C Tyrode buffer containing 1 % albumin.

Experimental protocol for the mouse in vitro blood-perfused juxtamedullary nephron technique

An afferent arteriole was chosen based on intact glomerular filtration and adequate blood flow. Measurements of afferent diameter was made >100 μm upstream from the glomerulus in order to avoid the juxtaglomerular part which is mainly influenced by TGF. After initiation of blood perfusion, an equilibration period of 15 min was allowed. RPP was set to 75 mmHg and increased in steps of 20 up to 155 mmHg. Each pressure step lasted 3 min. After the first set of pressure steps, the superfusion fluid was changed to one containing 10 μM nifedipine. After equilibration for 10 min, the pressure steps were repeated. Hereafter, a washout period of 15 min was allowed and superfusion was changed to contain [0.1 μM] mibefradil. After 10-min equilibration, the stepwise increase in perfusion pressure was repeated.

Surgical preparation for in vivo rat experiments

Anesthesia was induced with 8 % sevoflurane delivered in 35 % O_2 and 65 % N_2 and maintained at ~2–4 % sevoflurane. A polyethylene (PE)-50 catheter connected to a pressure transducer (Statham P23-dB) was inserted in the left carotid artery for blood pressure measurements. Two PE-10 catheters were placed in the right external jugular vein for infusion of the muscle relaxant Nimbex® (0.85 mg/mL, cisatracurium besylate, GlaxoSmithKline) and saline (20 μL/min). A tracheostomy was performed, and rats were placed on a servo-controlled heating table to maintain physiological body temperature and connected to a small animal respirator (Type 7025, Ugo Basile, Italy; rate=70 strokes/min, tidal volume=2–3 mL). Midline and

transverse subcostal incisions were performed. The left kidney was exposed and superfused with isotonic saline at 37 °C. The left common iliac artery was exposed and cannulated with a PE-10 catheter, which was advanced through the abdominal aorta into the left renal artery for infusion of the test agents in order to reduce systemic effects of the drugs. The catheter was perfused with isotonic saline at a rate of 10 μL/min. A PE-10 tube, connected to a PE-50 tube, was introduced into the left ureter to ensure free urine flow. The left renal artery was stripped from fat and connective tissue, and an ultrasonic flow probe (Transonic 1 PRB) was placed around it for measurement of RBF. RBF and RPP were recorded online with a PowerLab/8SP system (ADInstruments) for later offline analysis. The celiac trunk, the superior mesenteric artery, and the abdominal aorta caudally to the renal artery were exposed, and 4–0 silk ligatures were placed around them for later RPP manipulation. After surgery, a 30-min resting period was allowed, before the experimental protocol was initiated. Upon termination of the experimental protocol, the right kidney was removed and snap-frozen in liquid nitrogen for later immunostaining. The animal was then euthanized by decapitation.

Experimental protocol for in vivo rat experiments

In SD rats, the renal autoregulatory responses to increased RPP were tested during intrarenal infusion of saline, mibefradil [0.1 μM], nifedipine [1 μM], or mibefradil [0.1 μM]+nifedipine [1 μM]. In SHRs, the response was tested under infusion of saline, mibefradil [0.1 μM], or nifedipine [1 μM]. As we were not able to maintain an adequate RPP in the nifedipine-treated SHR, we did not test the effect of mibefradil+nifedipine. Infusion rate in the renal catheter was increased from 10 to 40 μL/min during drug administration. The concentrations of drugs listed above are estimated plasma concentrations calculated assuming an average renal plasma flow of 3 mL/min and a hematocrit of 40 % [44]. Mibefradil has been shown to mainly block T-type CCs at the concentration used in this study [32, 33]. In a previous study, 0.1 μM mibefradil induced only minor changes in RBF and RPP in anesthetized SD rats [50]. The nifedipine concentration was kept at 1 μM in order to maintain an adequate RPP.

A baseline period of 5 min saline perfusion initiated the experiment. Hereafter, saline infusion was continued, or mibefradil, nifedipine, or a combination thereof was infused for an additional 5-min control period. Then, RPP was raised in two steps of app. 25 mmHg by constriction of the intestinal arteries and the abdominal aorta caudally of the renal artery as previously described [45]. It has been shown that intestinal ischemia does not affect renal nerve activity, RBF or GFR [27].

Data analysis

Mouse in vitro blood-perfused juxtamedullary nephron technique

Afferent diameter was measured offline using the software ImageJ (NIH, Bethesda, MD). The luminal edges of the arteriole were tracked and the diameter measured manually every 10 s throughout the experiment. In vitro results are presented as the mean of the last minute of every pressure step (μm)± standard error of the mean (SEM).

In vivo rat experiments

The mean RBF and RPP during the last minute prior to intrarenal infusion of saline or CCBs are defined as baseline values. Likewise, control RBF and RPP are defined as the mean values during the minute preceding the first RPP increase. RVR was calculated as RPP/RBF, and the mean values of RBF and RPP during the 5-min pressure step was used to create autoregulation curves of RVR. Linear regression was performed on individual autoregulation curves to obtain the overall slope.

Statistics

All statistical analyses were performed using the SigmaPlot software (SyStat Software Inc., San Jose, CA). Initial effects of mibefradil and nifedipine treatment in vivo were tested using two-tailed paired Student's t test. When comparing control RBF and RVR between groups, one-way ANOVA was used. When comparing changes in RVR within groups, one-way repeated measures (RM) ANOVA was used. Bodyweight was compared using two-tailed non-paired Student's t test. Changes in afferent arteriole diameter within and between groups were analyzed using two-way RM ANOVA. A P value <0.05 was considered significant. ANOVAs were followed by Student-Newman-Keuls test. If normality test failed, ANOVA on ranks was followed by Dunn's test. All values are presented as means±SEM.

Results

Immunostaining

Staining of $Ca_V3.1$ was detected in vascular smooth muscle cells of afferent arterioles from rats and mice (Fig. 1a, c) and in larger renal arteries (not shown). The green fluorescent staining in mouse glomerular arterioles (Fig. 1c) was suppressed by pre-absorption with the immunizing peptide (Fig. 1d), confirming specificity of the $Ca_V3.1$ antibody as shown in previous studies [1].
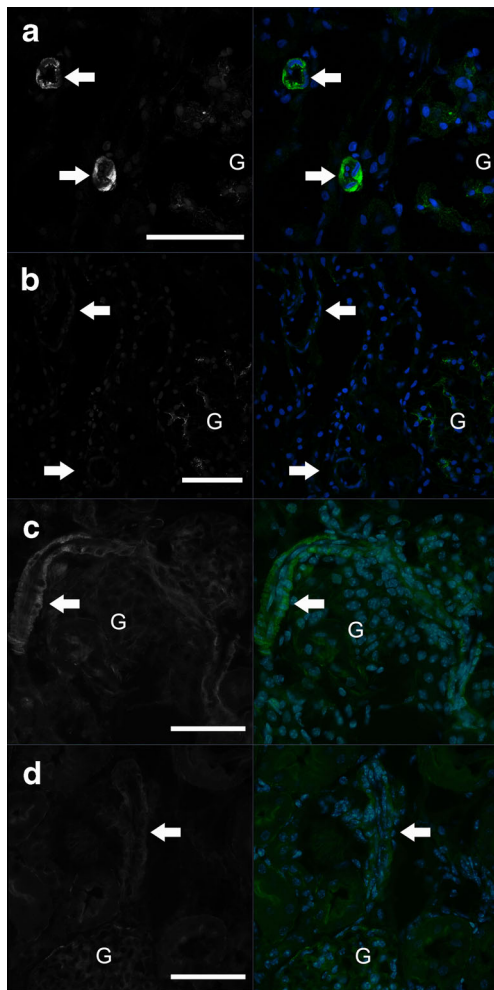
Fig. 1 Ca$_V$3.1 expression in rodent kidneys. **a** Rat kidney. **b** A section of the same rat kidney but with the secondary antibody alone. **c** Mouse kidney. **d** A mouse kidney control where the primary antibody had been pre-incubated with its peptide antigen. Please note that in **a** and **b**, there is a slight crosstalk between the channels resulting in weak nuclear staining also in the 488-nm channel of the Ca$_V$3.1 staining. *Arrows* pointing to arterioles and *G* shows the position of glomeruli. Exposure settings were unchanged between **a** and **b** and between **c** and **d**. Note that **a** and **b** were imaged with a confocal microscope, whereas **c** and **d** were imaged on a fluorescence microscope. All *scale bars* are 50 μm

Autoregulation in afferent arterioles

The mice used in these experiments varied in age from 4–8 months. To examine if the renal autoregulatory response changes significantly with increased age, the results from wild-type (WT) mice were analyzed in relation to age. When RPP was increased to 155 mmHg in kidneys from young (app. 4 month) and old (app. 8 month) mice, the afferent arteriolar diameter decreased to 79.9±8.7 and 79.3±4.5 % of the initial diameter, respectively. This finding indicates that the renal autoregulatory efficiency is not altered between the ages 4 and 8 months.

Afferent arteriolar diameter

Baseline afferent arteriolar diameter measured at 75 mmHg did not differ significantly between WT ($n=9$) and the T-type Ca$_V$3.1 KO ($n=6$) mice averaging 16.6±1.2 and 19.5±2.2 μm, respectively. Increasing RPP from 75 to 155 mmHg in steps of 20 mmHg significantly reduced afferent arteriolar diameter in both WT and Ca$_V$3.1 KO mice (Fig. 2a, b; $P<0.01$). The slope of the autoregulation curves was −0.043 ±0.009 μm/mmHg in WT and −0.051±0.015 μm/mmHg in Ca$_V$3.1 KO which was not significantly (NS) different. This demonstrates a significant afferent arteriolar vasoconstriction in response to acute increases in RPP in mice lacking Ca$_V$3.1 similar to WT mice.

After superfusion with nifedipine, afferent arteriolar diameter in WT was 19.5±1.2 μm at 75 mmHg (NS vs. control diameter). In Ca$_V$3.1 KO, it was 22.3±3.0 μm at an RPP of 75 mmHg (NS vs. control diameter in Ca$_V$3.1 KO mice). Increases in RPP from 75 to 155 mmHg did not significantly change afferent arteriolar diameter indicating that inhibition of L-type calcium channels attenuates pressure-induced afferent
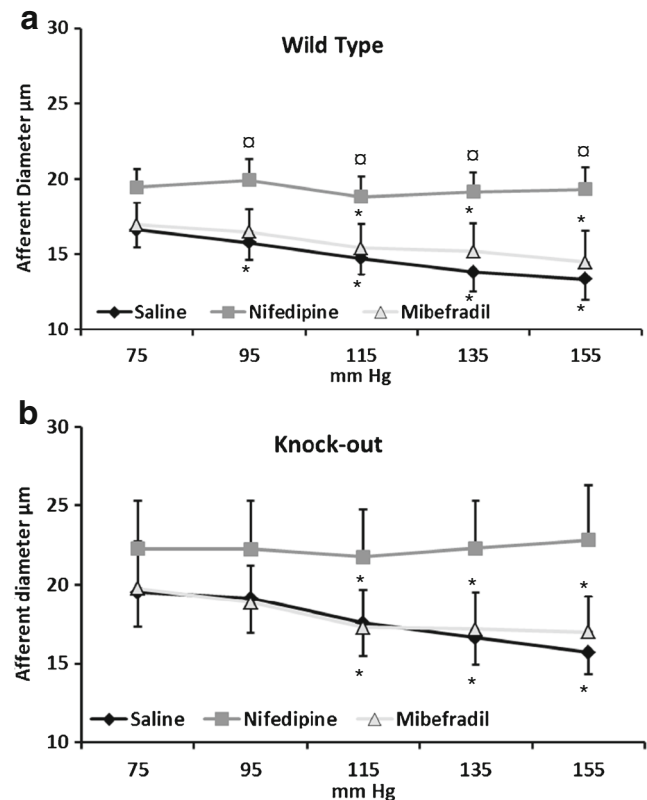
Fig. 2 Changes in afferent arteriolar diameter in response to step increases in RPP during superfusion with saline (*black line*; *diamond*), mibefradil (*light gray*; *triangle*), or nifedipine (*dark gray*; *square*). **a** Wild-type mice ($n=9$). **b** Ca$_V$3.1 knockout mice ($n=6$). *$P<0.05$ vs. 75 mmHg. $^\square P<0.05$ vs. saline at same RPP

arteriolar vasoconstriction (Fig. 2a, b). The slope of the curves after addition of nifedipine was -0.008 ± 0.003 and 0.006 ± 0.017 μm/mmHg, respectively, which was significantly different from the control slope in both strains ($P<0.05$).

Superfusion with mibefradil to inhibit T-type Ca^{2+} channels returned afferent arteriolar diameter to 16.9 ± 1.5 μm in WT and 19.6 ± 3.0 μm in $Ca_V3.1$ KO (Fig 2a, b) at 75 mmHg. Concomitant increases in RPP up to 155 mmHg significantly reduced afferent arteriolar diameter in both strains and revealed a slope of -0.044 ± 0.008 and -0.036 ± 0.012 μm/mmHg, respectively (Fig. 2a, b), which was similar to the control slopes. These results show that the afferent arteriole maintains its autoregulatory properties after inhibition of both $Ca_V3.1$ and $Ca_V3.2$ T-type calcium channels (mibefradil half maximal inhibitory concentration (IC_{50}) is in the range 69–140 nM; [26, 31, 35]).

Autoregulation in normotensive rats

Table 1 shows the physiological data from the SD rats before (baseline) and after (control) treatment. Baseline blood pressure and RBF was comparable between groups. Baseline RVR was significantly lower in the nifedipine+mibefradil group.

Nifedipine treatment increased RBF and decreased RVR significantly. Treatment with nifedipine+mibefradil tended to increase RBF ($P=0.06$) and decreased RVR significantly. Nifedipine treatment did not cause RPP to fall below the lower limit of autoregulation previously established in normotensive rats [51].

Figure 3 shows changes in RVR found in normotensive rats during acute increases in RPP. Control RVR was not different between groups although the value for nifedipine+mibefradil tended to be lower ($P=0.052$). In both groups treated with nifedipine, it was not possible to increase RPP above 130 mmHg in the second step. The slopes of the RVR curve in these groups (0.154 ± 0.061 and 0.103 ± 0.017) were significantly less steep ($P<0.05$) than the slopes of the control (0.284 ± 0.038) and mibefradil-treated (0.287 ± 0.035) groups.

RVR increased in parallel to RPP elevations except in the group treated with nifedipine+mibefradil. This finding indicates that nifedipine treatment, alone or in combination with mibefradil, attenuates the effect of increased RPP on RVR seen in both the saline and mibefradil groups. The slope of the RVR curves was similar in the saline and mibefradil groups indicating that mibefradil treatment alone did not affect autoregulation of RBF.

Autoregulation in hypertensive rats

Physiological data from the SHR are shown in Table 2. Baseline blood pressure, RBF, and RVR was comparable between groups. After CCB treatment, RPP was significantly reduced in the nifedipine group while RVR did not change significantly. Mibefradil had no effect on RPP, RBF, or RVR.

In all groups, RVR increased as RPP increased (Fig. 4). The slope of the RVR curves in SHR was not different between the CCB-treated groups (Mib 0.183 ± 0.038 and Nif 0.208 ± 0.060) and the saline group (0.248 ± 0.036). However, the last RPP increase could not be completed in the nifedipine group as the rats were not able to maintain an RPP higher than 147 mmHg.

Discussion

The present study was performed to investigate the role of renal vascular T-type Ca^{2+} channels in the autoregulation of renal blood flow. We show that the autoregulatory response in vitro in afferent arterioles from $Ca_V3.1$ knockout mice was not significantly different from that seen in WT mice suggesting that $Ca_V3.1$ has no significant effect on afferent arteriolar autoregulation in the pressure range tested. We also show in vivo that in normotensive and hypertensive rats, treatment with 0.1 μM of the putative T-type channel blocker mibefradil does not affect the renal autoregulation of RBF compared to saline-treated rats. Renal administration of nifedipine in

Table 1 Physiological status of normotensive Sprague-Dawley rats and effect of treatment on RPP, RBF, and RVR

Group	Saline $N=9$	Mibefradil $N=7$	Nifedipine $N=12$	Nifedipine+mibefradil $N=7$
Baseline RPP (mmHg)	98±2	101±2	101±2	97±1
Control RPP (mmHg)	98±2	99±2	102±1	97±1
Baseline RBF (mL/min)	6.1±0.5	5.8±0.7	7.2±0.9	9.0±0.7
Control RBF (mL/min)	6.0±0.5	5.9±0.8	7.7±0.9*	9.5±0.8
Baseline RVR (mmHg/mL/min)	17.1±1.5	18.6±1.8	17.2±2.6	11.0±0.8[#]
Control RVR (mmHg/mL/min)	17.2±1.5	18.4±1.9	15.8±2.1*	10.5±0.8*

Baseline values=values before treatment, control values=values after treatment

*$P<0.05$ vs. baseline; [#] $P<0.05$ vs. saline-treated group

Fig. 3 Changes in RVR in response to acute step increases in RPP in normotensive rats treated with saline (*black line*; *diamond*, *n*=9), mibefradil (*light gray*; *triangle*, *n*=7), nifedipine (*dark gray*; *square*, *n*=12), or nifedipine and mibefradil combined (*gray*: *circle*, *n*=7). Values are mean±SEM. *P<0.05 vs. control RVR (at ~100 mmHg; ANOVA on ranks)
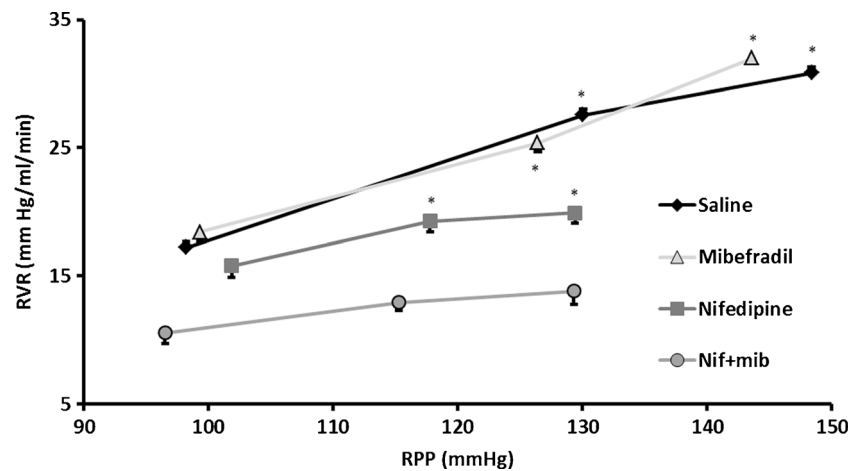
normotensive rats led to smaller increases in RVR induced by increasing RPP than in control and mibefradil-treated rats, which is compatible with a significantly impaired autoregulation.

The role of T-type calcium channels in renal autoregulation has been difficult to clarify. Lack of specific pharmacological inhibitors has added to the confusion. Genetically modified mice models lacking $Ca_V3.1$ or $Ca_V3.2$ have recently been developed and are useful tools in this regard. We show that in mice lacking $Ca_V3.1$, acute increases in RPP induced the same change in afferent arteriolar diameter as in WT mice suggesting that $Ca_V3.1$ has no effect on afferent arteriolar constriction during autoregulation of RBF. Furthermore, in afferent arterioles from WT and KO kidneys, treatment with 0.1 μM mibefradil was without effect. This concentration of mibefradil is suggested to inhibit T-type channels without significant effects on L-type channels [33]. This was confirmed for the first time in the present study as mibefradil had no effect in Cav3.1 KO mice. Furthermore, the lack of effect of mibefradil in $Ca_V3.1$ KO kidneys is interesting since

mibefradil inhibits $Ca_V3.2$ with an IC_{50} of 0.069 μM [31]. Thus, despite the fact that activation of $Ca_V3.2$ channels has been shown to induce vasodilation in renal efferent arterioles [17, 42], the present results indicate that $Ca_V3.2$ channels are not involved in renal autoregulation.

Further support for a limited role for T-type channels in renal hemodynamics has been obtained by Smirnov and co-workers who were not able to detect any T-type channel current in freshly isolated myocytes from rat afferent or efferent arterioles [48]. They could detect such currents in myocytes from the rat tail artery using the same approach. However, there is electrophysiological evidence of T-type calcium currents in the rat interlobar and arcuate arteries [13]. The same study found a heterogeneity of Ca^{2+} currents, with some myocytes displaying either T-type currents or L-type currents, and the rest displaying a mixture of currents. Arcuate and interlobular arteries have been shown to be involved in renal autoregulation [4, 6]; therefore, functionally active T-type Ca^{2+} channels are present in the preglomerular vasculature and could potentially play a role in renal autoregulation. However, the present results clearly show that this is not the case.

In contrast to our findings in the WT mouse kidneys perfused with mibefradil, afferent arteriolar diameter measurements obtained from the isolated rat kidneys after acute pressure increases during superfusion with another putative T-type channel blocker, pimozide, showed significantly reduced autoregulatory ability [11]. It should be noted that the concentration of pimozide used in that study was above the concentration known to inhibit L-type Ca^{2+} channels in vitro [10]. Other results obtained in vivo in $Ca_V3.1$ and $Ca_V3.2$ knockout mice [53] showed that lack of $Ca_V3.1$ resulted in increased baseline renal plasma flow and lack of $Ca_V3.2$ induced increases in GFR. We found a tendency to a larger diameter in afferent arterioles from $Ca_V3.1$ knockout mice compared to arterioles from wild-type mice consistent with an increased renal plasma flow in vivo. In contrast to the maintained autoregulation found after mibefradil treatment, we observed that

Table 2 Physiological status of hypertensive SHR and effect of treatment on RPP, RBF, and RVR

Group	Saline $N=7$	Mibefradil $N=6$	Nifedipine $N=5$
Baseline RPP (mmHg)	127±1	133±3	130±1
Control RPP (mmHg)	129±1	135±3	121±2*
Baseline RBF (mL/min)	4.1±0.2	5.1±0.7	4.5±0.4
Control RBF (mL/min)	4.1±0.3	5.1±0.7	4.6±0.6
Baseline RVR (mmHg/ml/min)	31.1±1.8	29.4±5.3	30.0±2.7
Control RVR (mmHg/ml/min)	32.2±2.1	29.5±5.1	27.9±3.4

Baseline values=values before treatment, control values=values after treatment

*P<0.05 vs. baseline

Fig. 4 Changes in RVR in response to acute step increases in RPP in hypertensive rats treated with saline (*black line*; *diamond*, *n=7*), mibefradil (*light gray*; *triangle*, *n=6*), or nifedipine (*dark gray*; *square*, *n=5*). Values are mean±SEM. *P<0.05 vs. control RVR (at ~100 mmHg)
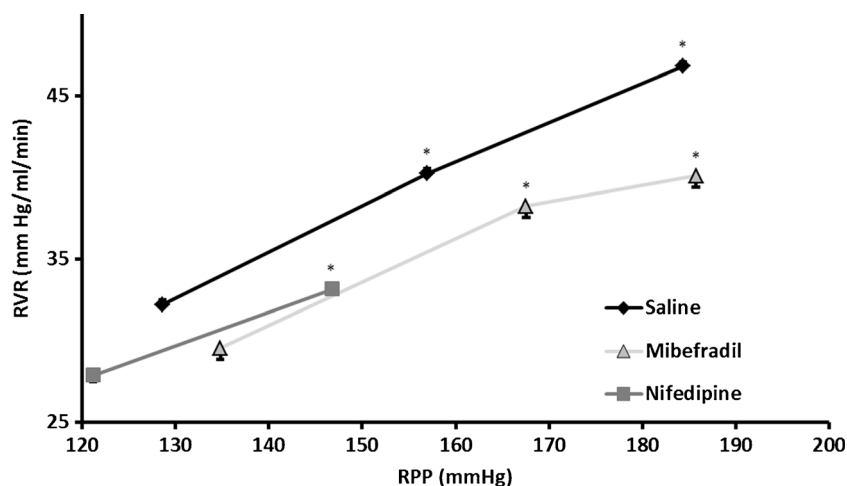
nifedipine reduced the afferent arteriolar constriction induced by acute pressure increases in both WT and $Ca_V3.1$ KO mice. This has previously been shown in WT mice and rats [19, 49] supporting the in vivo experiments. Thus, while L-type Ca^{2+} channels are central in the autoregulation of RBF, in contrast to previously published results, we found that T-type channels do not seem to play a major role during acute autoregulation.

Mibefradil treatment in normo- and hypertensive rats did not change control mean arterial pressure (MAP) or RBF significantly, indicating that T-type Ca^{2+} channels are not activated during baseline conditions in anesthetized rats. The chosen concentration of mibefradil has previously been used in vivo and found effective in reducing the vasoconstriction following closure of several renal vascular K+ channels [50]. This shows that the concentration used is affecting the T-type channels. These results are in contrast to results obtained in normotensive dogs where mibefradil infusion, albeit in a higher concentration than used in our experiments, slightly increased RBF but had no effect on GFR [22]. It has been shown that higher concentrations of mibefradil in addition to blocking of T-type channels also inhibit L-type channels [20, 34]. However, in the present study, the results are obtained after a short infusion time and using a low concentration of mibefradil shown to inhibit primarily T-type Ca^{2+} channels [32, 33]. Our data on the afferent arteriolar diameter also confirms that the chosen mibefradil concentration preferentially inhibits T-type Ca^{2+} channels but not L-type channels.

Infusion of nifedipine had a significant effect on renal hemodynamics in both normotensive and hypertensive rats, increasing RBF and decreasing RVR as shown previously [55]. In hypertensive SHR, the effect of nifedipine was most significant on MAP as observed before [46] underlining the antihypertensive effect. This is in agreement with the observation that the membrane potential of vascular smooth muscle cells in SHRs is more depolarized than in normotensive rats [30].

As the membrane potential of afferent arteriolar vascular smooth muscle cells is in the range of the activation potential for L-type calcium channels, small alterations in membrane potential could have large effects on the opening probability of these channels and thus on the effect of nifedipine [12, 28]. Furthermore, an upregulation of L-type calcium channels has been shown in mesenteric and skeletal arteries from SHR [43].

After 2–4-day treatment with mibefradil, Griffin et al. [14] showed that autoregulation of RBF in response to acute pressure increases was abolished in SD rats. The rats received mibefradil (0.06 %) in their chow, and the treatment did not change MAP or RBF. In contrast, we did not find any support for a role for T-type channels in the renal vasoconstriction associated with autoregulation of RBF during pressure increases. We found that both normotensive and hypertensive control rats responded to acute increases in RPP with a significant increase in RVR. The same effect was seen after treatment with mibefradil although there was a tendency towards a decreased RVR in mibefradil-treated SHRs. The slope of the curves obtained in control rats and mibefradil-treated rats was similar in both normotensive and hypertensive strains suggesting that T-type channels play no major role in the acute renal autoregulation.

Treatment of normotensive rats with nifedipine significantly reduced the increase in RVR seen after acute RPP increases indicating that nifedipine reduces the autoregulatory capacity. This has been shown by others in response to increases in RPP [14, 39]. The slope of the curve was significantly different from the slope found in the saline and mibefradil groups. In hypertensive rats, the effect of nifedipine on MAP was more pronounced than in normotensive rats [23], and a second pressure increase was not possible. Combining nifedipine and mibefradil in normotensive rats reduced initial RVR and abolished the increase in RVR found in the saline and mibefradil groups. Also, the slope of the RVR curve was

significantly different from the slopes obtained in the control and mibefradil groups. The effect of nifedipine and mibefradil combined was similar to the effect seen with nifedipine alone and supports the idea that L- and T-type channel blockers have no additive effect [11].

In summary, we have shown that deletion of $Ca_V3.1$ or acute pharmacological inhibition of T-type calcium channels does not significantly affect renal autoregulation. Deletion of $Ca_V3.1$ did not affect the autoregulatory response assessed as pressure induced vasoconstriction in afferent arterioles. In support of this, autoregulation of RBF in normo- and hypertensive rats in response to acute pressure increases was not affected by treatment of mibefradil. In contrast, nifedipine reduced autoregulation of RBF in normotensive rats which was also supported by measurements of pressure-induced changes in afferent arteriolar diameter. Thus, we conclude that renal vascular T-type channels have no major role in autoregulation of rodent renal blood flow.

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