Tissue kallikrein deficiency and renovascular hypertension in the mouse

Violaine Griol-Charhbili1,5, Laurent Sabbah2,3,4, Juliana Colucci1, Marie-Pascale Vincent1, Véronique Baudrie1,2, Dominique Laude1,2, Jean-Luc Elghozi1,2,4, Patrick Bruneval1,2,4, Nicolas Picard1,2, Pierre Meneton1,2, François Alhenc-Gelas1,2,4 and Christine Richer1,4,5.

1INSERM U872, CRC, Paris, France; 2Paris Descartes University, Paris; 3INSERM U633, Hôpital Broussais, Paris; 4Asistance publique, Hôpitaux de Paris; 5Université Paris-Sud, Le Kremlin-Bicêtre, France.

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Correspondence:

Christine Richer-Giudicelli
INSERM U872,
15, rue de l’Ecole de Médecine, 75270 Paris, France
Tel: 33 1 44 07 90 38
Fax: 33 1 44 07 90 40
E-mail: christine.richer-giudicelli@u-psud.fr

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Abstract

The kallikrein kinin system (KKS) is involved in arterial and renal functions. It may have an antihypertensive effect in both essential and secondary forms of hypertension. The role of the KKS in the development of 2-kidney, 1-clip (2K1C) hypertension, a high-renin model, was investigated in mice rendered deficient in tissue kallikrein (TK) and kinins by TK gene inactivation (TK-/-) and in their wild type littermates (TK+/+). Four weeks after clipping the renal artery, blood flow was reduced in the clipped kidney (2K1C-TK+/+: -90%, 2K1C-TK-/-: -93% vs Sham-operated mice), and the kidney mass had also decreased (2K1C-TK+/+: -65%, 2K1C-TK-/-: -66%), whereas in the unclipped kidney, blood flow (2K1C-TK+/+: +19%, 2K1C-TK-/-: +17%) and kidney mass (2K1C-TK+/+: +32%, 2K1C-TK-/-: +30%) had both increased. The plasma renin concentration (2K1C-TK+/+: +78%, 2K1C-TK-/-: +65%) and renal renin content of the clipped kidney (2K1C-TK+/+: +58%, 2K1C-TK-/-: +65%) had increased significantly. There was no difference for these parameters between 2K1C-TK+/+ and 2K1C-TK-/- mice. Blood pressure (BP) monitored by telemetry and by plethysmography, rose immediately after clipping in both genotypes, and reached similar levels (2K1C-TK+/+: +24%, 2K1C-TK-/-: +21%). 2K1C-TK+/+ and 2K1C-TK-/- mice developed similar concentric left ventricular hypertrophy (+24% and +17% respectively), with normal cardiac function. These findings suggest that in the context of chronic unilateral reduction in renal blood flow, TK and kinins do not influence the trophicity of kidneys, the synthesis and secretion of renin, BP increase and cardiac remodelling due to renin angiotensin system activation.

Key Words

Kallikrein kinin system, renin-angiotensin system, renovascular hypertension, mice
Introduction

Tissue kallikrein (TK), the major kinin-forming enzyme in mammals, is synthesized in several organs including arteries and kidneys. TK is involved in the function of both capacitance and resistance arteries, and in vasomotor and trophic adaptation to flow (3, 6, 7, 9, 29, 33, 34). In the kidney, TK is also present in the distal tubule. Long-standing and more recent evidence suggest that TK is involved in the control of electrolyte transport mechanisms in the tubule, and in sodium and calcium homeostasis, via both kinin- and non kinin-mediated mechanisms (9, 33, 34).

Several arguments have suggested that TK is involved in the control of blood pressure (BP), and that a TK defect may be implicated in the onset of hypertension. For instance, urinary TK activity is inversely correlated with BP in man (27) and in strains of hypertensive rats (1, 26). It has been suggested that genetically high urinary kallikrein excretion may protect against essential hypertension in man (8). It has also been suggested that reduced expression of the TK gene (klk1) may contribute to salt-sensitive hypertension in Dahl rats (21). However, mice with a disrupted TK gene display normal BP (29), suggesting that TK does not play an important role in regulating BP under normal conditions. This finding does not exclude the possibility that TK may influence the development of secondary forms of hypertension. In rats with 2-kidney,1-clip renovascular hypertension, renal TK gene expression has been reported to be unaltered during the onset of hypertension, and then to decrease during the chronic phase of established hypertension, with a reduction in both urinary and vascular TK activity (15, 31). TK synthesis and secretion are reduced in the clipped kidney, but remain unaltered in the contralateral kidney (19). Whereas hypertension induced by clipping a renal artery is clearly renin- and angiotensin-dependent, observations referred to above, suggest that TK activity may protect against kidney dysfunction and excessive elevation of BP. In this context, we tested this hypothesis in TK deficient mice in which the gene encoding for TK
had been disrupted (29, 42). These mice exhibit extremely low levels of kinins in the kidney (29).

Materials and Methods

Mice. TK+/+ and TK-/- mice were generated in our laboratory as previously described (29, 42). The mouse mutants had been back-crossed in a C57BL/6 genetic background (Charles River, L’Arbresle, France) for over ten generations before obtaining the wild type TK+/+ and homozygous mutant TK-/- littermates used in our experiments by heterozygous crossing. Mice were housed in an air-conditioned room with a 12:12-hour light (8h-20h)-dark (20h-8h) cycle, and received standard mouse chow (AO4; Scientific Animal Food and Engineering, Augy, France) and tap water ad libitum. All the experimental procedures were performed in accordance with the European regulations for the care and use of laboratory animals (L 358-86/609/EEC).

Two-kidney, one-clip hypertension (2K1C). Adult male mice were anesthetized by isoflurane inhalation (3% in an oxygen stream). The left kidney was exposed through a small flank incision and externalized. The left renal artery was isolated from the renal vein and connective tissue. A U-shaped silver clip (3 x 2 x 1 mm with a 2-mm-long cleft, and 120µm internal gap, Excidel, SA) was placed near the aorta, around the renal artery for partial occlusion (44). Two groups of mice underwent clipping of the left renal artery: 2K1C-TK+/+ (n=31) and 2K1C-TK-/- (n=32). Control mice underwent a sham procedure (n=44, TK+/+: n=32 and TK-/-: n=12). Sham procedures were performed as described above (incision, isolation of the renal artery), except for the clipping of the left renal artery. In addition, 10 TK+/+ and 10 TK-/- mice underwent left renal artery clipping and were used for telemetric measurement of BP before and up to 24 days after surgery.
**Blood pressure.** Blood pressure was followed by two different methods. Firstly, systolic blood pressure (SBP) was measured by tail-cuff plethysmography in trained conscious mice (11, 29) using a BP2000 (Visitech, Bioseb, France). Measurement was performed four weeks after clipping in the animals used for non invasive and invasive hemodynamic determinations (Sham n=38, 2K1C-TK+/+ n=26, 2K1C-TK-/- n=26). An additional set of experiments was devoted to analye the evolution of SBP 3, 8, 15, 21 and 28 days after clipping (Sham n=6, 2K1C-TK+/+ n=5, 2K1C-TK-/- n=6). Blood pressure was also measured telemetrically. The mice were anesthetized with isoflurane (5% in an oxygen stream). The BP radiotelemetry probe (TA11PA-C10, Data Sciences International, St Paul, MN, USA) was implanted. The catheter of the telemeter was inserted into the left common carotid artery, and the body of the probe was positioned in the right flank (4). The mice received amoxicillin (20 mg/kg, i.p., Clamoxyl, Smith Kline and Beecham laboratories, Nanterre, France), and ketoprofen (5 mg/kg, i.p., Profenid, Aventis, Paris, France) just after surgery. After a two-week recovery period, each individual mouse cage was placed on the top of a radio-receiver (Model RPC-1) for monitoring BP, heart rate (HR), and spontaneous locomotor activity in conscious freely moving animal. The telemetry BP signal was digitized at 1000 Hz. The experimental data were collected continuously in real time, stored on the local hard disk and analysed using the Data Sciences acquisition system and the Dataquest ART analysis software. BP, HR and locomotor activity were recorded daily continuously over 4 hours (10 a.m. - 2 p.m.) under basal conditions before clipping, and up to 24 days after inducing renovascular hypertension.

**Cardiac function, renal and coronary blood flows.** These parameters were measured 4 weeks after clipping in mice (Sham, n=19; 2K1C-TK+/+, n=12 and 2K1C-TK-/-, n=14) anesthetized with pentobarbital (60 mg/kg, i.p.). Catheters were placed in the left femoral artery, and in the left ventricle (LV) via the right carotid artery. The maximum and minimum rates of rise of LV pressure (dP/dtmax and dP/dtmin) were continuously visualized and recorded on a PC computer using a MP100 system (Biopac systems, Cerom, Paris, France).
Renal (clipped and non-clipped kidneys) and coronary blood flows were determined according to the reference blood sample technique using the fluorescent microsphere method (38).

**Cardiac remodelling.** Trans-thoracic two-dimensional echocardiographic measurements were performed under light anesthesia (ketamine, 70 mg/kg and xylazine, 10 mg/kg ip) using a digital ultrasound device (Sequoia 512, Acuson Corp, Mountain View, CA, USA) equipped with a specifically designed 13-15 MHz short-focus linear array probe (15L8) (36, 39). Two-dimensional images were obtained with M-mode cursor positioned perpendicular to the inter-ventricular septum and the posterior wall of the LV at the tip of the mitral valve leaflets. End diastolic (EDD) and systolic (ESD) LV diameter, and the inter-ventricular septum (S) and posterior (P) wall thicknesses were measured in diastole using the American Society of Echocardiography leading edge method (39). From these parameters, the fractional shortening (FS, %) and LV mass (LVM, g) were calculated as: 

\[ FS = \left(\frac{EDD - ESD}{EDD}\right) \times 100 \]

\[ LVM = \left[\left(S + EDD + P\right)^3 - EDD^3\right] \times 1.055 \]

All measurements were averaged for 3 consecutive cardiac cycles, and analyzed by a single observer who was unaware of the status of the animals. At sacrifice four weeks after clipping, LV and RV weights were determined gravimetrically in all animals.

**Renin.** Four weeks after clipping, blood samples from the retro-orbital plexus were collected into heparinized Eppendorf tubes from the mice under ketamine-xylazine anesthesia after the echocardiographic measurements. The plasma renin concentration (PRC) was determined by radioimmunoassay of angiotensin I generated after incubating the plasma for 1 hour at pH 8.5 in the presence of an excess of rat angiotensinogen (28). Renal renin immunostaining was performed in Sham (TK+/+, n=14 and TK-/- n=9), 2K1C-TK+/+ (n=15) and 2K1C-TK-/- (n=17) mice as previously described (35). Sections (5µm) from formalin-fixed paraffin-embedded kidneys were deparaffinized, incubated in 1% hydrogen peroxide, and blocked with 3% normal goat serum in PBS pH7.6. The primary antibody (18)
(polyclonal rabbit antiserum) detects mouse salivary gland and renal renin, and detection was carried out using biotinylated anti-rabbit IgG, avidin peroxidase complex (Vectastain Elite ABC kit, vector laboratories) with 3,3’-diaminobenzidine (Sigma chemical Co) in 0.1% hydrogen peroxide. The intensity of immunostaining localized in the juxtaglomerular apparatus was analyzed by optical microscopy, and scored from 0 (no labeling) to 5 (strong labeling). Total renin staining per section was expressed as the mean labeling index (35).

**Urinary parameters.** Four weeks after clipping, randomly selected mice (Sham-TK+/+, n=10; Sham-TK-/-, n=9; 2K1C-TK+/+, n=13 and 2K1C-TK-/-, n=12) were housed individually in metabolic cages for 24-h urine collection for urinary sodium, potassium, chloride and calcium assays (Konelab v. 6.0.7). Urinary kallikrein activity was assessed by quantifying generation of p-nitroanilide (p-NA) with a spectrophotometer at 405 nm (Biorad 3550, Hercules, CA, USA) after incubating the urine with the substrate S 2266 (H-D-Val-Leu-Arg-pNA, Chromogenix-Instrumentation Laboratory, Milan, Italy) (22).

**Statistical analysis.** Results are expressed as mean ± SEM. The means were compared by one-way ANOVA followed by post hoc analysis using the JMP software System (JMP, SAS Institute Inc, Cary, NC). Telemetric data were analyzed by two-way ANOVA (Surgery/Genotype) for paired values. The level of statistical significance was fixed at p<0.05.

**Results**

Under basal conditions, before clipping, there was no difference between TK+/+ and TK-/- mice for any of the parameters evaluated, including renal and cardiac morphology and function, BP, HR, PRC, with the exception of the urinary kallikrein activity which was not detectable in TK-/- mice. Consequently, the Sham mice of both genotypes were pooled into a single Sham group.
Renal characteristics of 2K1C mice. In 2K1C-TK+/+ mice, four weeks after clipping, a major decrease in the left renal blood flow was observed (-90%, when expressed in ml/min: vs Sham, p<0.001, Figure 1A; -68%, when expressed per gram of tissue: vs Sham, p<0.001), whereas the right renal blood flow was increased (+19%, when expressed in ml/min: vs Sham, p<0.001, Figure 1A; -8%, when expressed per gram of tissue: vs Sham, NS). The sum of ipsilateral and contralateral renal blood flow (both kidneys) was not significantly different from that of the Sham animals (6.75±0.66 ml/min/g vs Sham 8.02±0.60, NS). The left-clipped kidney became atrophic (-65% vs Sham, p<0.001, Figure 1B and 1C), whereas the contralateral unclipped kidney developed hypertrophy (+32% vs Sham, p<0.001). Urinary Na+/K+ was unchanged (Table 1).

In 2K1C-TK-/- mice, clipping the left renal artery also led to marked fall in left renal blood flow (-93%, when expressed in ml/min: vs Sham, p<0.001) whereas the right renal blood flow increased (+17%, when expressed in ml/min: vs Sham, p<0.001, Figure 1A; -2%, when expressed per gram of tissue: vs Sham, NS). Total, right plus left, renal blood flow remained unchanged compared to Sham (7.37±1.04 vs Sham 8.02±0.60 ml/min/g, NS). The left kidney became atrophic (-66%, p<0.001 vs Sham), and the right kidney developed compensatory hypertrophy (+30%, p<0.001 vs Sham, NS vs 2K1C-TK+/+, Figure 1B). There was no significant difference between 2K1C-TK-/- and 2K1C-TK+/+ mice regarding ipsilateral, contralateral and total renal blood flow, ipsilateral and contralateral kidney weight, or urinary parameters (Figure 1, Table 1).

Histological analysis revealed no change in the contralateral kidney, whereas the clipped kidney displayed chronic ischemia in both genotypes (data not shown). The ratios of right kidney weight to left kidney weight did not significantly differ among the two clipped groups or among the two sham groups (data not shown).
Renal and Plasma renin content in 2K1C mice. Under basal conditions, before clipping, there was no difference between TK+/+ and TK-/- mice for PRC (2.7±0.2 vs 2.2±0.2 µg AngI/ml/h, NS). Four weeks after clipping, PRC had increased in the 2K1C-TK+/+ group (+78%, p<0.05 vs Sham, Figure 2). In TK-deficient mice, PRC had also increased to the same extent as in the 2K1C-TK+/+ mice (+65%, p<0.05 vs Sham and NS vs 2K1C-TK+/+, Figure 2).

In both 2K1C-TK+/+ and 2K1C-TK-/− groups, immunoreactive renin increased in the left kidney (+58% and +65% respectively, p<0.05 vs Sham, Figure 3), and decreased in the contralateral kidney (-42% and -40% respectively, p<0.05 vs Sham, Figure 3). There was no significant difference between the genotypes with regard to the renin content in the kidneys.

In 2K1C-TK+/+ mice, the urinary kallikrein activity was the same as in the Sham-TK+/+ group (2K1C-TK+/+: 0.21±0.02, Sham-TK+/+: 0.22±0.02 nKcat/µmol creatinine). As expected, no urinary kallikrein activity was detected in either of the TK-/- groups.

Blood pressure in 2K1C mice. Telemetric measurements showed that under basal conditions, there was no significant differences between the 24-h profiles of BP, HR and locomotor activity of Sham-TK+/+ and Sham-TK-/- mice under a 12:12-hour light/dark cycle (Figure 4).

In 2K1C-TK+/+ mice, hypertension developed immediately after clipping. SBP increased significantly the first day after clipping (+ 24% vs the day before clipping, p<0.05) (Figure 5).

In 2K1C-TK-/− mice, hypertension also occurred soon after clipping (+ 20% one day after clipping vs the day before clipping, p<0.05) (Figure 5), but did not significantly differ from that observed in 2K1C-TK+/+ mice. During the first week after clipping SBP of both 2K1C-TK+/+ and 2K1C-TK-/− mice was significantly higher than the corresponding basal value measured in each group before clipping. Thereafter, SBP remained elevated in all clipped mice but tended to decrease over time. There was no significant difference between genotypes. HR was not affected by clipping and there was no difference between genotypes (Figure 5). Telemetric measurements were also performed in 2 Sham operated mice. There
was no difference in SBP, HR and locomotor activity before and after surgery (data not shown).

SBP was also evaluated by plethysmography in another dedicated experiment with three groups of mice (Figure 6). The results document a lack of effect on blood pressure of sham procedure, a significant rise in SBP in clipped mice with no effect of genotype.

Cardiac remodelling in 2K1C mice. In both genotypes, a similar increase in septal wall thickness (roughly +20% vs Sham, p<0.001) and posterior wall thickness (roughly +14% vs Sham, p<0.001) developed, whereas end-diastolic diameter was not altered (Figure 7). The LV mass to body weight ratio obtained from echocardiographic measurements was also higher in both 2K1C groups than in the Sham animals. These results were confirmed by measuring gravimetrically the LV weight to body weight ratio at sacrifice in the same animals (2K1C-TK+/+: 4.145±0.161, +24%; 2K1C-TK-/-: 3.910±0.124, +17%, NS, both p<0.05 vs Sham: 3.350 ± 0.061 mg/g). The same results were obtained in the groups of mice used for SBP measurement by plethysmography at regular intervals after clipping (2K1C-TK+/+: 3.993±0.088, +13%; 2K1C-TK-/-: 4.013±0.154 +14%, NS both p<0.05 vs Sham :3.520±0.142). These data show that clipping the left renal artery had led to similar concentric LV hypertrophy in both genotypes. Histological analysis of LV confirmed the presence of moderate interstitial and pericoronary fibrosis in both 2K1C groups (not shown), but LV morphology did not differ in TK+/+ and TK-/- animals.

Cardiac function in 2K1C mice. Coronary blood flow expressed per g of tissue, tended to be lower than in Sham mice in the 2K1C-TK+/+ and 2K1C-TK-/- groups, but the three experimental groups did not differ statistically (Table 2). Left ventricular functional parameters evaluated four weeks after clipping non-invasively by echocardiography (FS) or invasively by dP/dtmax and dP/dtmin determination, showed no difference from Sham animals (Table 2). There was no significant difference between the genotypes.
Discussion

In this study, we observed that clipping a renal artery in mice led to activation of the renin-angiotensin system (RAS) without affecting TK urinary activity. Rapid-onset moderate hypertension developed with concentric LV hypertrophy. The lack of TK in TK-/- mice did not modify the degree of RAS activation, since PRC and renal renin labeling were the same in 2K1C-TK-/- and 2K1C-TK+/+ mice. Following clipping, the kinetics of the onset of hypertension in 2K1C-TK-/- mice was similar to that in their wild type littermates. No significant differences were observed between the two genotypes regarding cardiac and renal morphology and function at up to 4 weeks. These findings suggest that TK does not play any physiologically significant role in 2K1C renovascular hypertension in the mouse.

Under our experimental conditions, hypertension developed rapidly then BP reached a plateau. A moderate elevation of BP (10-20%) in this mouse experimental model has also been reported by other authors (2, 17, 23, 25, 32, 44), but in a few studies, a greater increase in BP (roughly 50%) has been observed (11, 20, 22, 24). It is now established that the elevation of BP is less marked in 2K1C mice than in 2K1C rats (24). In addition, glomerulosclerosis, which usually occurs in the unclipped kidney of rats exposed to high BP (16), was not observed in mice, probably as a consequence of the lower BP level induced in mice, and/or the known resistance of C57BL/6 mice to the development of glomerulosclerosis (30). Some studies of the kinetics of renovascular hypertension development in mice have shown that hypertension occurred very early after clipping, as in our study, and then the BP stabilized (14, 16, 44) or even decreased (17). In other studies, a sustained increase in BP over several weeks was observed (10, 11, 25). Different degrees of reduction in renal blood flow and activation of the RAS, and/or the use of different methods to measure BP could account for the minor discrepancies between these studies. In the present
study, we evaluated BP by two different methods. Both documented the lack of effect of TK gene inactivation.

2K1C renovascular hypertension is typically a model of renin-dependent hypertension, but, it has been suggested that the KKS may have an antihypertensive effect in this model. The study was based on the hypothesis that before renal atrophy occurs, kallikreins and kinins, present in the ipsilateral kidney may have influenced renal hemodynamics, and protected against renal ischemic damage in the early phase of renovascular disease, thus resulting in delayed hypertension. This is the reason why we followed BP on the first days after clipping; Also, in the contralateral kidney that undergoes compensatory hypertrophy kallikrein synthesis remains unaltered (19) and could have influenced renal function and blood pressure. Finally kallikrein is present in capacitance and resistance arteries and is involved in the control of vascular tone (7, 29). The development of renovascular hypertension has been reported to be accelerated in B2-deficient (B2−/−) mice (25), and hypertension to be aggravated (11), despite similar plasma renin activity and tissue gene expression of RAS components. In contrast, Duka et al. have reported that 2K1C B2−/− mice displayed the same increase in BP as their wild type littermates (14).

In our study, hypertension developed with similar kinetics and magnitude in TK +/+ and TK−/− mice after clipping. We especially focused on the early period after clipping, where renin secretion is activated and trophicity of the kidney is not yet altered, especially in the distal tubule where kallikrein is synthetized. Kallikrein deficiency did not potentiate the hypertensive effect of renin secretion. Cardiac hypertrophy was the same in both genotype groups, confirming that cardiac afterload has been similar during the follow-up period. Some studies have suggested that kinins have a direct inhibitory action on LV hypertrophy, the development of which was not related to their hemodynamic effects (12, 13, 43, 45). However, no exacerbation of cardiac hypertrophy was reported in 2K1C mice lacking the B1 or the B2 receptor (11, 14, 25). As in these studies, we found that the LV hypertrophy which
developed after clipping was not influenced by the absence of TK and kinins. It is interesting to note that the pathogenesis of hypertensive remodelling seems to be different from post-ischemic heart remodelling, as far as TK and kinin involvement is concerned. TK deficiency indeed exaggerates cardiac remodelling and decreases survival post myocardial infarction (36, 37). The difference is however not surprising as the development of cardiac remodelling is strongly influenced by underlying myocardial disease and peripheral hemodynamic status, which are very different among the two models. Renal renin synthesis and secretion are causally linked to hypertension in 2K1C mice. These parameters were not altered in TK-/- mice. The reduction in renal blood flow in the clipped kidney was very similar in wild-type and TK-deficient mice. These observations do not support the hypothesis that TK or kinins have a role in renal renin secretion and activation (5, 40, 41), at least under conditions of strong hemodynamic stimulation. Finally, the observation that ipsilateral and contralateral kidney morphology was not influenced by TK deficiency suggests that TK and kinins play no role in bilateral renal remodelling secondary to chronic unilateral ischemia.

**Perspectives and Significance**

The study shows that TK deficiency does not play any detectable role in 2K1C renovascular hypertension in mice. While TK and kinins influence cardiac remodelling in other experimental settings, they do not seem to exert any counter-regulatory influence on cardiac hypertrophy in 2K1C hypertension. Kallikrein remains abundant in the contralateral kidney, but does not play any role in compensatory hypertrophy. These negative findings were obtained in an experimental model in which BP elevation and cardiac hypertrophy are secondary to a major stimulation of renin secretion and high angiotensin II production. In fact, they should not be extrapolated to other forms of hypertension involving different pathogenic mechanisms, as supported by the observation of a hypertensive effect of TK
deficiency during chronic aldosterone administration, a situation where, contrary to 2K1C hypertension, kallikrein synthesis is increased and renin secretion is suppressed (unpublished results).

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References


Figure legends

Figure 1: (A) Renal blood flow (mean ± SEM, n=8-12), (B) Kidney weight/body weight ratio, (mean ± SEM, n= 26-38), (C) Kidneys at sacrifice, four weeks after clipping the left renal artery or sham surgery. L: left kidney, R: right kidney.*p<0.01, **p<0.001 vs Sham.

Figure 2: Plasma renin concentration four weeks after clipping the left renal artery or sham surgery in the three experimental groups. Data are means ± SEM, n=10-36. *p<0.05 vs Sham.

Figure 3: A. Left and right renal renin labeling, four weeks after clipping the left renal artery or sham surgery, n=15-23/group. *p<0.05 vs Sham. B. Representative immunostaining of renin in a 2K1C-TK+/+ mice. L: left kidney, R: right kidney.

Figure 4: Profiles over 24h in systolic blood pressure (A. SBP), heart rate (B. HR) and activity (C.) under a 12:12-hour light (8h-20h)/dark (20h-8h) schedule in TK+/+ (n=10) and TK-/- (n=10) mice instrumented with a telemetric system under basal condition.

Figure 5: (A) Systolic blood pressure and (B) heart rate recorded daily between 10 a.m. and 2 p.m. in conscious mice (telemetry) before (0), and after left renal artery clipping in TK+/+ and TK-/- mice. Data are means ± SEM, bpm: beats/min. n=8-10/group. *p<0.01 vs corresponding basal condition before clipping (0), NS between genotype.

Figure 6: Systolic blood pressure measured in trained conscious mice before (0) and 3,8,15,21 and 28 days after clipping in TK+/+ (n=5) and TK-/- (n=6) mice (plethysmography). *p<0.05 vs Sham (n=6).
Figure 7: Morphological cardiac parameters measured or calculated by echocardiography in the three groups four weeks after left renal artery clipping. Data are means ± SEM, n=26-38/group. *p<0.01, **p<0.001 vs Sham.
Table 1: Biological parameters in the three groups measured four weeks after left renal artery clipping or sham surgery.

<table>
<thead>
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<th>SHAM</th>
<th>2K1C-TK+/+</th>
<th>2K1C-TK-/-</th>
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<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>13</td>
<td>12</td>
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<tr>
<td>Body weight (g)</td>
<td>33 ± 1</td>
<td>32 ± 1</td>
<td>31 ± 1</td>
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<td>Diuresis (ml/24h)</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.2</td>
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<td>Calcium (mol/mol creatinine)</td>
<td>0.26 ± 0.02</td>
<td>0.28 ± 0.04</td>
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<td>Chloride (mol/mol creatinine)</td>
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<td>34.1 ± 1.9</td>
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<td>Potassium (mol/mol creatinine)</td>
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<td>Sodium (mol/mol creatinine)</td>
<td>26.1 ± 1.4</td>
<td>25.7 ± 2.4</td>
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</table>

Values are means ± SEM; n, number of mice.
Results are not statistically different for sham, 2K1C-TK+/+ and 2K1C-TK-/-.
Table 2: Cardiac functional parameters in the three groups measured four weeks after left renal artery clipping or sham surgery evaluated by left ventricular catheterization or echocardiography.

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>2K1C-TK+/+</th>
<th>2K1C-TK/-/</th>
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<tr>
<td>Coronary Blood Flow(ml/min/g)</td>
<td>6.4 ± 0.7</td>
<td>5.5 ± 0.6</td>
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<td>FS (%)</td>
<td>46 ± 1</td>
<td>46 ± 2</td>
<td>49 ± 1</td>
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<td>dP/dtmax (mmHg/s)</td>
<td>2179 ± 77</td>
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<td>dP/dtmin (mmHg/s)</td>
<td>-2490 ± 70</td>
<td>-2472 ± 95</td>
<td>-2441 ± 42</td>
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</tbody>
</table>

Values are means ± SEM. Results are not statistically different for sham, 2K1C-TK+/+ and 2K1C-TK/-/.
FS: fractional shortening, dP/dt_max and dP/dt_min, maximum and minimum first degree differential of the left ventricular pressure.
**Figure 1**

**A** Renal blood flow

![Renal blood flow graph](image)

**B** Kidney weight / body weight

![Kidney weight graph](image)

**C** Kidneys at sacrifice

![Kidney images](image)
Plasma Renin Concentration

Figure 2

[Bar graph showing the comparison of Plasma Renin Concentration between Sham, 2K1C-TK+/+, and 2K1C-TK-/- with statistical significance marked by asterisks.]
**Figure 3**

A

![Bar chart showing index of renal renin labeling for Sham, 2K1C-TK^{+/+}, and 2K1C-TK^{-/-} groups, with left and right kidneys indicated. * denotes statistical significance.](image)

B

![Images of left and right kidney sections labeled Left and Right.](image)
**Systolic Blood Pressure over 24H**

- TK\(-/\)  
- TK\(+/+\)

**Heart Rate over 24H**

**Locomotor activity over 24H**

Figure 4
Figure 5

A  Systolic Blood pressure (telemetry)

B  Heart Rate (telemetry)
Systolic Blood pressure before (0) and 3, 8, 15, 21 and 28 days after clipping or sham surgery

Figure 6

SHAM  2K1C TK+/+  2K1C TK/−
Figure 7