Published in final edited form as:

Pflugers Arch. 2018 June ; 470(6): 969–978. doi:10.1007/s00424-018-2119-y.

Lack of connexin 40 decreases the calcium sensitivity of renin secreting juxtaglomerular cells

Dominik Steppan[1], **Lisa Geis**[2], **Lin Pan**[3], **Kenneth Gross**[4], **Charlotte Wagner**[1], **Armin Kurtz**[1]

[1]Institute of Physiology, University of Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany

[2]Clinic for Nephrology, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, D-93053 Regensburg, Germany

[3]Department of Pathology, Brigham and Women's Hospital, 652 NRB, 77 Ave Louis Pasteur, Boston, MA 02115

[4]Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Elm and Carlton Sts, Buffalo, New York 14263-0001

Abstract

The so called calcium paradoxon of renin describes the phenomenon that exocytosis of renin from juxtaglomerular cells of the kidney is stimulated by lowering of the extracellular calcium concentration. The yet poorly understood effect of extracellular calcium on renin secretion appears to depend on the function of the gap junction protein connexin 40 (Cx40) in renin producing cells.

This study aimed to elucidate the role of Cx40 for the calcium dependency of renin secretion in more detail by investigating if Cx40 function is really essential for the influence of extracellular calcium on renin secretion, if and how Cx40 affects intracellular calcium dynamics in renin secreting cells and if Cx40 mediated gap junctional coupling of renin secreting cells with the mesangial cell area is relevant for the influence of extracellular calcium on renin secretion.

Renin secretion was studied in isolated perfused mouse kidneys. Calcium measurements were performed in renin producing cells of micro-dissected glomeruli. The ultrastructure of renin secreting cells was examined by electron microscopy.

We found that Cx40 was not essential for stimulation of renin secretion by lowering of the extracellular calcium concentration. Instead Cx40 increased the sensitivity of renin secretion response towards lowering of the extracellular calcium concentration. In line, the sensitivity and dynamics of intracellular calcium in response to lowering of extracellular calcium were dampened when renin secreting cells lacked Cx40. Disruption of gap junctional coupling of renin secreting cells by selective deletion of Cx40 from mesangial cells, however, did not change the stimulation of renin secretion by lowering of the extracellular calcium concentration. Deletion of Cx40 from renin cells but not from mesangial cells was associated with a shift of renin expression from perivascular cells of afferent arterioles to extraglomerular mesangial cells.

Corresponding Author: Dominik Steppan, Dominik.Steppan@ur.de.

Our findings suggest that Cx40 is not directly involved in the regulation of renin secretion by extracellular calcium. Instead it appears that in renin secreting cells of the kidney lacking Cx40, intracellular calcium dynamics and therefore also renin secretion are desensitized towards changes of extracellular calcium. Whether the dampened calcium response of renin secreting cells lacking Cx40 function results from a direct involvement of Cx40 in intracellular calcium regulation or from the cell type shift of renin expression from perivascular to mesangial cells remains to be clarified. In any case, Cx40 mediated gap junctional coupling between renin and mesangial cells is not relevant for the calcium paradoxon of renin secretion.

Keywords

Connexin 40; renin; calcium juxtaglomerular cells; calcium paradoxon

Introduction

The protease renin is the key regulator of the renin angiotensin aldosterone system (RAAS). It is produced, stored and secreted by pericyte like cells (32) of the juxtaglomerular areas in the kidney (14). Renin is released from juxtaglomerular cells by compound exocytosis (33) mainly triggered by the cyclic-AMP signaling pathway (3). Also calcium appears to be involved in the regulation of control of renin secretion, albeit in a rather atypical way which was coined by the term calcium paradoxon of renin secretion (14). In this context, renin secretion is inhibited by commonly known calcium mobilizing hormones such as angiotensin II or endothelins (3). In addition, renin secretion is markedly stimulated by lowering of the extracellular concentration of calcium (1, 10, 14, 26, 31, 36). The physiological role of extracellular calcium on renin secretion is central to the baroreceptor control (14) of renin secretion (30). Recent studies reported the absence of the effect of perfusion pressure and of extracellular calcium on renin secretion in kidneys lacking Cx40, suggesting a link between the calcium paradoxon of renin secretion and the function of the gap junction protein connexin 40 (Cx40) (12, 38). Cx40 is considered to be the most relevant gap junction protein of renin secreting cells (15, 17, 22, 40). Renin secreting juxtaglomerular cells of the kidney form gap junctions among each other and with their neighbored endothelial and mesangial cells (9, 16, 19, 20, 35). It has not yet been unraveled how Cx40 could be involved in the atypical regulation of renin secretion by calcium. So far it is known that gap junctional coupling between renin and endothelial cells is not essential for the effect of extracellular calcium on renin secretion (39) and that defective Cx40 function in renin cells is associated with a shift of renin expression from perivascular cells of afferent arterioles to juxtaglomerular mesangial cells (23).

Regarding the possible involvement of Cx40 in the regulation of renin secretion, it is conceivable that a defect of Cx40 leads to an altered calcium handling in renin secreting cells, which finally results in an altered dynamic of renin secretion towards changes of the extracellular calcium concentration. Such altered calcium dynamics in Cx40 defective renin secreting cells could directly result from an altered calcium regulation of the cells per se or more indirectly result from a disturbed calcium transmission between coupled renin cells and extraglomerular mesangial cells (35, 37). In principle, it is also possible that the shift of

renin expression from perivascular to mesangial cells, which occurs in Cx40 defective renin cells, is a major cause for the apparent calcium insensitivity of renin secretion. In this study, we have therefore investigated if and how deletion of Cx40 alters the calcium handling of renin secreting cells and if intact Cx40 coupling with mesangial cells is relevant for the function of renin secreting cells.

Materials and Methods

Animals

All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the local ethics committee. Animals had free access to standard rodent chow (0.6% NaCl; Ssniff, Germany) and tap water. Male and female 8 to 12-week-old modified mice and wildtype littermates derived from heterozygous breeding pairs were used in this study. For the induction of Cre-mediated recombination in the respective genotypes, animals received chow containing 400 mg tamoxifen citrate per kilogram (TD.07262, Harlan Laboratories, Netherlands) for 4 weeks followed by a 3-week period with the regular standard chow (NaCl 0.6 %; Ssniff, Germany). All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals, published by the NIH, and approved by the local ethics committee.

***Cre^{ER} Cx40$^{fl/fl}$* mice:** Mice with a floxed *Cx40* gene (Cx40$^{fl/fl}$) (4) and mice with tamoxifen-inducible Cre recombinase (Cre^{ER}) (8) were mated to generate mice that were homozygous for the floxed *Cx40* gene (Cx40^{flfl}) carrying one allele of Cre^{ER}. Corresponding littermates exhibiting no Cre recombinase Cx40^{flfl} served as controls.

***PDGFRß$^{+/Cre}$Cx40$^{fl/fl}$* mice:** Mice with inducible Cx40 deletion in mesangial cells were generated by mating *Cx40$^{fl/fl}$* mice (4) with a mouse line that expresses tamoxifen-inducible Cre recombinase under the control of the beta-type platelet-derived growth factor receptor promotor (11) (*Pdgfrb-CreERT2* mice, referred to as *PDGFRß$^{+/Cre}$* mice in this work). Generation of the PDGFRß-CreERT2 transgene (Ralf H. Adams) is unpublished, and a full characterization of this line will be published elsewhere.

***RenGFP BAC* mice:** A homologous recombination was used to introduce a green fluorescent protein (GFP) cassette into exon one of the renin gene, which contained a 240 kb bacterial artificial chromosome (BAC) to create a construct that has GFP expression controlled by the renin regulatory region (RenGFP BAC). Thus all cells actively expressing the renin gene could be identified by GFP fluorescence (13).

***RenGFP BAC Cre^{ER} Cx40$^{fl/fl}$* mice:** RenGFP BAC mice with tamoxifen inducible Cx40 deletion were generated by crossbreeding the aforementioned RenGFP BAC mice with Cre^{ER} Cx40$^{fl/fl}$ mice.

Isolated perfused mouse kidney

The isolated perfused mouse kidney model has been described in detail elsewhere (5, 38). All animals were anesthetized with an intraperitoneal injection of Ketamin (50 mg/kg body weight, Curamed, Karlsruhe, Germany) and Xylazin (80 mg/kg body weight, Ratiopharm, Ulm, Germany), followed by cannulation of the abdominal aorta and excision of the right kidney, which was placed in a thermostated moistening chamber, and perfused at constant pressure. Using an electronic feedback control, perfusion pressure could be changed and held constant at desired values (40 and 90 mm Hg). Venous effluent of the cannulated renal vein was collected for determination of both renin activity and venous blood flow. The basic perfusion medium consisted of a modified Krebs–Henseleit solution supplemented with 6 g/100 ml bovine serum albumin and with freshly washed human red blood cells (10% hematocrit). For determination of renin secretion rates, samples of the venous effluent were taken in intervals of 2 min during each experimental period.

Renin secretion measurement

Renin concentration in plasma samples was measured on the basis of the generation of angiotensin-I after the addition of plasma from bilaterally nephrectomized male rats as excess renin substrate. The generated angiotensin-I (ng angiotensin-I/hour per mL) was determined by angiotensin I (Plasma Renin Activity) ELISA kit (IBL International GmbH, Hamburg, Germany). Renin secretion was modulated by variation of perfusion pressure and adding defined concentrations of isoproterenol and EGTA (all from Sigma, Deisenhofen, Germany) to the perfusate.

Glomeruli isolation and calcium measurements

Calcium measurement was performed on isolated single glomeruli. For this purpose, 7 week old RenGFP BAC mice and RenGFP BAC TamCre-Cx40 fl/fl mice were killed by cervical dislocation. The kidneys were excised and the kidney cortex was isolated and minced, followed by a 20 minute digestion with a modified Ringer-solution containing Collagenase Type 2 (Worthington, Lakewood NJ, USA), Trypsin Inhibitor Type II-S and glycine (all from Sigma, Deisenhofen Germany). After digestion, single glomeruli with attached renin producing cells were identified by GFP-fluorescence using a Stereo Discovery V8 microscope, a HXP 120V lamp and the filter set 38 HE (all from Carl Zeiss GmbH, Oberkochen, Germany) and collected for calcium measurements. Prior to measurements, single Glomeruli were placed in a perfusion bath, secured with a manipulator and loaded with fura-2-AM (5μM, 30min at 37°C). Washout was performed by constant perfusion (3ml/min) with modified Ringer-solution (5mM HEPES, 145 mM NaCl, 1,6mM K_2HPO_4, 0,4mM KH_2PO_4, 5mM Glucose, 1mM $MgCl_2$, 2,5mM $CaCl_2$, pH 7,4). Solutions containing EGTA were made by adding the respective amount of EGTA and readjusting the pH to 7,4 with NaOH. Calcium-measurements were performed by placing regions of interest (ROI) directly on the GFP fluorescence of renin producing cells (Fig.1). Fura-2 emission ratios of this regions were detected at 340nm and 380nm excitation (340/380 ratio) using a VisiFluor Calcium Imaging System and analyzed using the Visiview Software (all from Visitron Systems, Puchheim, Germany).

Immunohistochemistry

Kidneys were perfusion-fixed with 3 % paraformaldehyde/PBS, dehydrated in a graduated methanol series, and embedded in paraffin. Sagittal sections (5 μm) were blocked with 10 % horse serum/1 % BSA in PBS before the primary antibodies (chicken anti-renin: Davids Biotech.Regensburg,Germany, rabbit anti-Cx40: Alpha Diagnostic Intl. Inc. USA, mouse anti-αSM-actin: Abcam Cambridge,UK) were applied. After three washes, sections were stained with Cy2, Cy5 and tetramethylrhodamine B isothiocyanate, secondary antibodies. Slices were mounted with DakoCytomation Glycergel mounting medium and were viewed with an Axiovert Microscope.

Transmission electron microscopy

Kidneys were fixated with constant pressure (90 mm Hg) for 3 min by perfusion with phosphate-buffered saline (PBS) buffer containing 2% glutaraldehyde. The kidney was cut in half and stored at 4 °C in 2% glutaraldehyde/PBS until embedment for TEM. Then the kidney tissue was cut in 1-mm^3-wide blocks and embedded in epoxyde resin (epoxy embedment kit, Fluka, Neu-Ulm, Germany) using an automatic microwave (Leica EM AMV, Leica, Germany). The embedded tissue was cut into 70-nm-thick serial slices using an ultramicrotome (EM UC7, Leica, Wetzlar, Germany), which were then placed on copper grids coated with pioloform. The serial slices were contrasted using a 4% uranyl acetate solution and a 0.5% lead citrate solution. For the acquisition of the images of a juxtaglomerular cell, a transmission electron microscope (Phillips CM12 TEM, Fei & Co, Eindhoven, Netherlands) with a LaB_5 cathode and an acceleration voltage of 120 keV was used. The digitalization was carried out with a TEM-1000 slow-scan CCD camera and the program EM-Menu 4.0 (both from TVIPS-Tietz GmbH, Gauting, Germany).

Statistics

Values are given as means ± SE. Differences between experimental groups were analyzed by ANOVA and Bonferroni's adjustment for multiple comparisons. Otherwise, student's *t*-test was used to test significance of difference between two groups. $P < 0.05$ was considered statistically significant.

Results

Renin secretion was determined from isolated mouse kidneys perfused at a constant pressure of 90mmH (unless otherwise noted) with a perfusate containing a total calcium concentration of 2.5 mMol/L. The catecholamine agonist isoproterenol (10nM) stimulated renin secretion about 4-fold both in wt and in Cx40 deficient kidneys (Fig.2). Repeated additions of EGTA at final concentration of 3 mMol/L caused rapid, reversible and reproducible increases of renin secretion. The amplitude of stimulation was markedly higher in wildtype than in Cx40 kidneys (Fig.2).

Cx40 is not essential for the stimulation of renin secretion by decreased extracellular calcium

The effect of 3.0 mMol/L EGTA on renin secretion was quantified from 16 kidneys of both genotypes which were collected over two years. It becomes evident from Fig.3 (a)

that EGTA markedly stimulated renin secretion from all wt-kidneys. Also renin secretion from the majority of Cx40 deficient kidneys responded to EGTA, however, with a weaker response than in wt kidneys (Fig.3, b). On average, EGTA enhanced renin secretion 4.5 fold in wt kidneys and 1.8-fold in Cx40 deficient kidneys (Fig.3, c).

Lack of Cx40 causes a shift of renin expression from juxtaglomerular perivascular to mesangial cells

The different response of renin secretion to EGTA from wildtype and Cx40 deficient kidneys was also seen by electronmicroscopical analysis of renin cells. In wildtype kidneys, renin cells containing big granules were located in the walls of afferent arterioles (Fig.4, a). Perfusion of the kidneys with isoproterenol and EGTA led to intracellular fusion of individual granules in combination with a decrease of electron density of the granules. In accordance with our previous observations (12, 23) renin producing cells in Cx40 deficient kidneys were mainly found outside the vessel walls in the extracellular mesangial cell field of the glomerular tuft (Fig.4, b). The cells contained numerous electron dense vesicles and the granules appeared smaller than in wildtype kidneys. More prominently, there were no signs of granule fusion and change of vesicular electron density upon perfusion of the kidneys with isoproterenol and EGTA (Fig.4, b).

Lack of Cx40 decreases the dynamics of intracellular calcium and of renin secretion in response to changes of the extracellular calcium concentration

Addition of graded concentrations of EGTA to the perfusate at final concentrations from 0.25 to 3.0 mMol/L led to graded stimulations of renin secretion. In wt kidneys renin secretion began to increase with 0.5 mMol EGTA which corresponds to a total EGTA unbound calcium concentration of 2.0 mM. In Cx40 deficient kidneys the threshold EGTA concentration required to stimulate renin secretion was 1.0 mM, what corresponds to a total EGTA unbound calcium concentration of 1.5 mM (Fig.5).

We next determined the effect of graded concentrations of extracellular EGTA on the intracellular calcium concentration in renin producing cells. For this purpose fura-2 measurements were performed in renin producing cells of isolated glomeruli, which were microdissected from the kidneys of BAC-RenGFP mice (13). Renin producing cells of these mice express an endogenous GFP label and can therefore be identified noninvasively. As shown in Fig. 6, graded concentrations of EGTA to the extracellular buffer led to time dependent changes of the intracellular calcium concentration. Whilst EGTA at lower concentration only led to smaller transient decreases of intracellular calcium (not shown), concentrations of EGTA of 1mM and higher led to rather rapid and substantial decreases of intracellular calcium (Fig.6, a, b, c). The decline of intracellular calcium in response to EGTA was clearly delayed in Cx40ko vs wt kidneys.

Removal of EGTA from the extracellular solution led to a rapid recovery of the intracellular calcium concentration of renin producing cells. The recovery of intracellular calcium occurred faster in wildtype renin cells compared with Cx40 deficient renin cells (Fig.7).

Calcium clearance from the cytosol after a calcium transient is lower in Cx40 deficient renin cells

To examine if Cx40 deficient renin producing cells show more general alterations of intracellular calcium regulation we examined the effect of a short pulse of angiotensin II (1nM) which is already known to mobilize calcium in juxtaglomerular cells (21). As shown in Fig. 8, ANGII induced a calcium transient followed by a sustained elevation of the intracellular calcium concentration. The initial calcium peak in response to ANGII was rather similar for normal and for Cx40 deficient renin secreting cells (Fig. 8). Assuming that cytosolic calcium levels after the calcium transient should reach again basal values, the data suggest that in Cx40 defective renin cells, an apparently longer time interval is required for reaching basal values again. This would mean that the cytosolic calcium clearance rate is lower in Cx40 defective than in normal renin cells.

Role of renin cell – mesangial cell coupling for the control of renin secretion

We finally addressed the possibility that the gap junctional coupling of renin secreting cells with the extraglomerular mesangium might be of relevance for the intracellular calcium regulation and of consequence for the response of renin secretion to changes of extracellular calcium. For this purpose we generated mice with a tamoxifen inducible deletion of Cx40 in mesangial cells. This was achieved with PDGFRß-CreERT Cx40 fl/fl mice, because PDGFRß is expressed in intra- and extraglomerular mesangial cells (2) but not in renin producing cells (25). As shown in Fig. 10, glomeruli of PDGFRß-CreERT2 Cx40 fl/fl mice showed markedly reduced Cx40 immunoreactivity in the intra- and extraglomerular mesangium, whilst Cx40 immunoreactivity in renin producing cells was maintained. Notably, the characteristic perivascular positioning of renin producing cells was not changed by deletion of Cx40 from mesangial cells (Fig.9, a, b).

Renin secretion from these kidneys behaved very similar to wildtype kidneys in the way that renin secretion was stimulated by EGTA and also by a fall of the perfusion pressure (Fig. 10). In parallel experiments, we also examined kidneys with inducible global deletion of Cx40. Renin secretion from those kidneys was neither stimulated by EGTA nor by a reduction of the perfusion pressure from 90 to 40 mmHg. (Fig. 10).

Discussion

This study aimed to investigate the response of renin secreting cells of the kidney to changes of the extracellular calcium concentration in dependency on the function of Cx40. Although we studied kidneys with inducible global deletion of Cx40, we believe that an altered behavior of renin secretion can be attributed to the lack of Cx40 in renin cells, because renin cell specific deletion of Cx40 produces a very similar renin phenotype as does global deletion of Cx40 (39).

Reduced calcium clearing from the cytosol as a reason for decreased calcium sensitivity of renin secretion

We observed that in Cx40 deficient kidneys on the one hand the intracellular concentration of calcium in renin cells and on the other hand renin secretion response to decreases of

the extracellular calcium are markedly attenuated when compared with normal renin cells. Conversely, the increase of cytosolic calcium induced by intracellular calcium release was not altered but the return of the cytosolic calcium concentration after the calcium transient was again delayed. This observation supports the conclusion that the calcium clearance from the cytosol of renin secreting cells is impaired if their Cx40 function is defective.

Possible reasons for the dampened calcium dynamics of Cx40 defective renin secreting cells

A decrease of cytosolic calcium concentration could in principle result from a calcium spreading into a gap junctional syncytium (6) and from the activity of Ca-ATPases (34). Since renin cells are part of a prominent GJ coupled syncytium comprising extra- and intracellular mesangial cells (28, 29, 40), it was obvious to consider the contribution of this coupling for calcium and pressure regulated renin secretion. Apparently this coupling is not essential in view of the normal response of renin secretion to drops of extracellular calcium and blood pressure in kidneys lacking Cx40 in mesangial cells. Previously, we already discovered that Cx40 gap junctional coupling between renin secreting cells and endothelial cells is not essential for the effects of pressure and of extracellular calcium on renin secretion (39). Therefore, the link between Cx40 and calcium sensitivity should reside within in the renin secreting cells themselves (39). One possible explanation could be the involvement of Cx40 hemichannels. It is known that connexin hemichannels open at low extracellular calcium concentrations (7, 27). However, if we assume that Cx40 hemichannels are calcium permeable, opening of the hemichannels should increase rather than decrease cytosolic calcium concentration, as long as the extracellular concentration of calcium is higher than the intracellular concentration. Therefore, a more likely explanation for a delayed intracellular calcium clearance is a lowered activity of the plasma membrane Ca-ATPase, from which four isoforms exist (34). According to our current knowledge, it is unknown if a more direct functional link between connexin proteins and specific PMCA isoforms exists. If this were the case, this link should surpass simple structural interactions because the calcium sensitivity of renin secretion remains reduced when single point mutated Cx40 proteins are inserted into the plasma membrane (18, 24).

Cellular shift of renin expression as a possible explanation for the reduced calcium sensitivity of renin secreting cells lacking Cx40

Finally, we wish to discuss the possibility that an altered PMCA-activity of Cx40 defective renin cells could be causally related to a shift of the renin expressing cell type. As already described (12, 23) and also confirmed in this study, renin expression in Cx40 renin cells shifts from perivascular cells to the extraglomerular cell field. The mechanisms underlying this shift of renin expression are not yet understood. It occurs only if Cx40 is defective in renin cells themselves (39) but not if Cx40 is defective only in neighbored endothelial cells (39) or in neighbored mesangial cells, as found in this study. One may infer from these findings that Cx40 mediated gap junctional coupling with endothelial or with mesangial cells is not the key mechanism that restricts renin expression to (peri)vascular cells of afferent arterioles. We cannot exclude that extraglomerular mesangial cells, to which renin expression shifts in the absence of Cx40, have a lower PMCA activity than (peri)vascular cells per se. Although we are aware of this remaining uncertainty, we think that the reduced

calcium sensitivity of renin secretion from Cx40 deficient kidneys can be attributed to an altered calcium handling of Cx40 deficient renin cells. In view of the strong calcium dependency of the effect of renal perfusion pressure on renin secretion, we furthermore think that the altered calcium handling of Cx40 defective renin cells contributes to the impaired pressure control of renin secretion from Cx40 defective kidneys.

Acknowledgements

The expert technical assistance provided by Ramona Steppan and by Robert Götz is gratefully acknowledged. The authors work was financially supported by the German Research Foundation (DFG, SFB 699) and by Roswell Park Cancer Institute and National Cancer Institute (NCI) grant P30CA016056.

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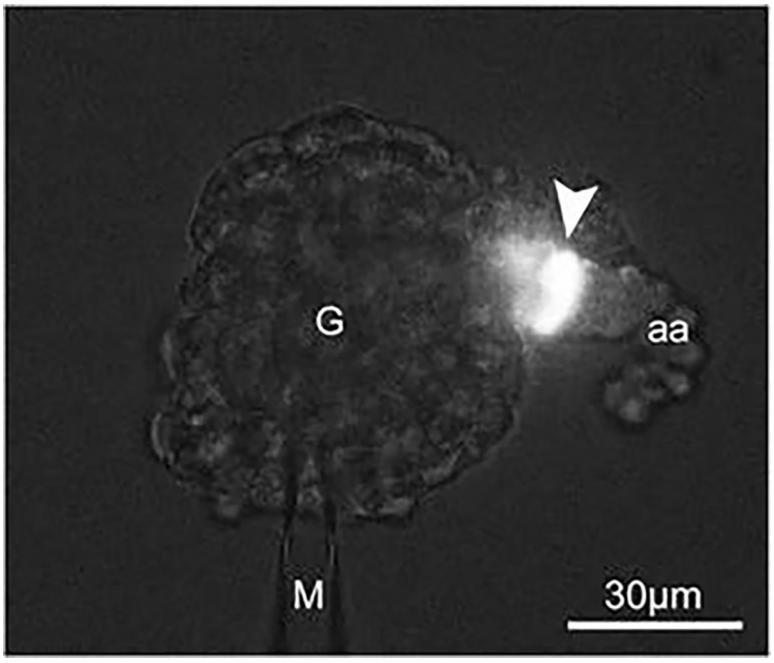

Fig.1.

Endogeneous GFP fluorescence (arrow) of a glomerulus microdissected from a RenGFP BAC mouse kidney. Flourescent cells indicate renin expressing cells. G: glomerulus, aa: afferent arteriole, M: manipulator

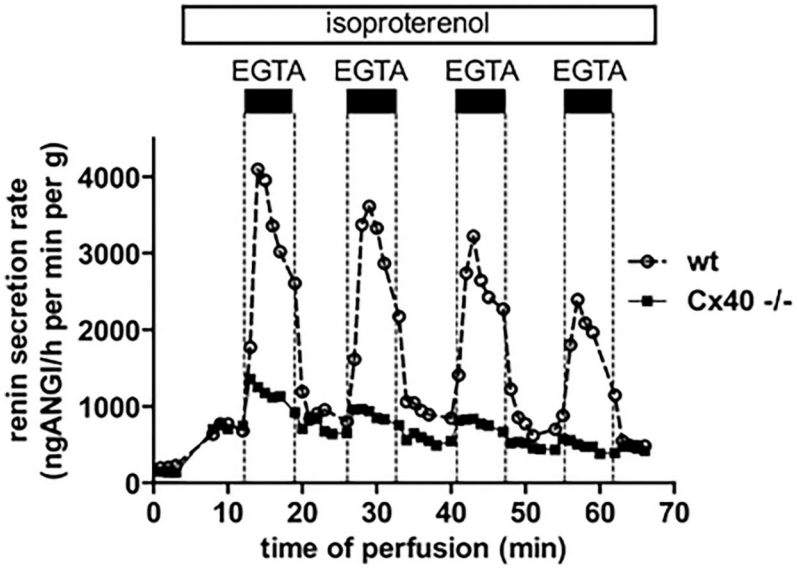

Fig.2.

Effects of repeated additions of EGTA (final concentration 3mM) to the perfusate of isolated perfused kidneys from wildtype (wt) and a Cx40 deficient (Cx40 −/−) mouse on renin secretion

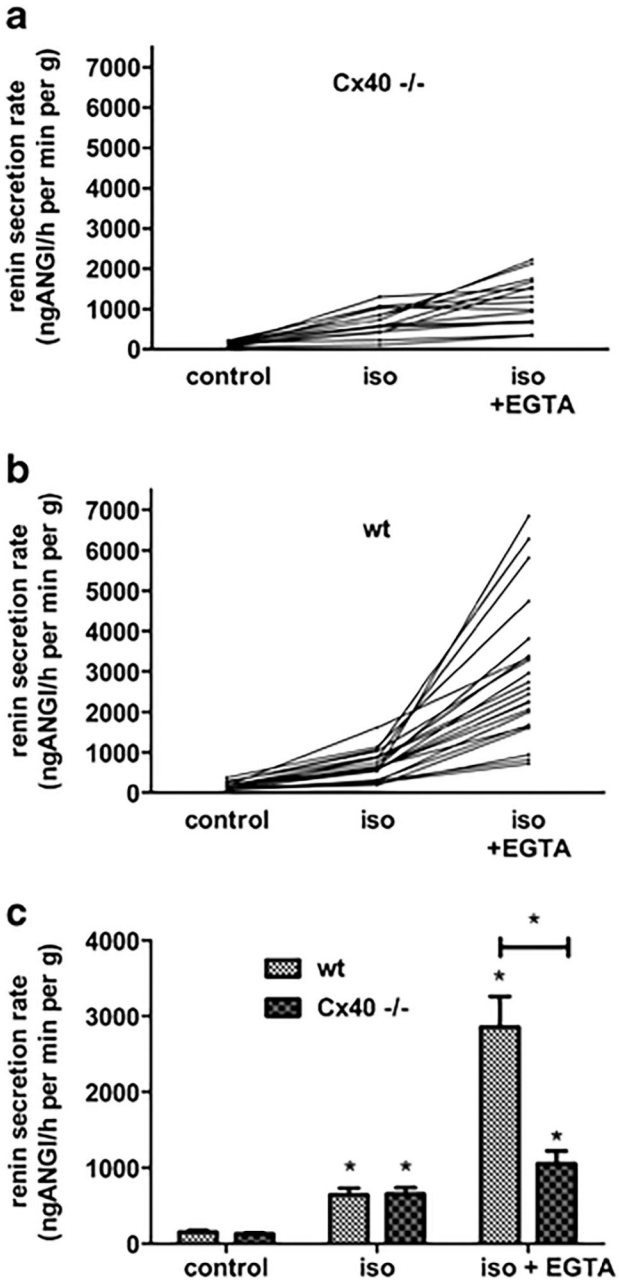

Fig.3.

Effects of isoproterenol (iso, 10nM) and of EGTA (3mM) on renin secretion from individual wildtype (a) and Cx40 −/− kidneys (b). Lower panel shows the statistical analysis of the effects (c). Data are from 16 kidneys of each genotype and are presented as means ±SEM. Asterisks indicate $p<0.05$

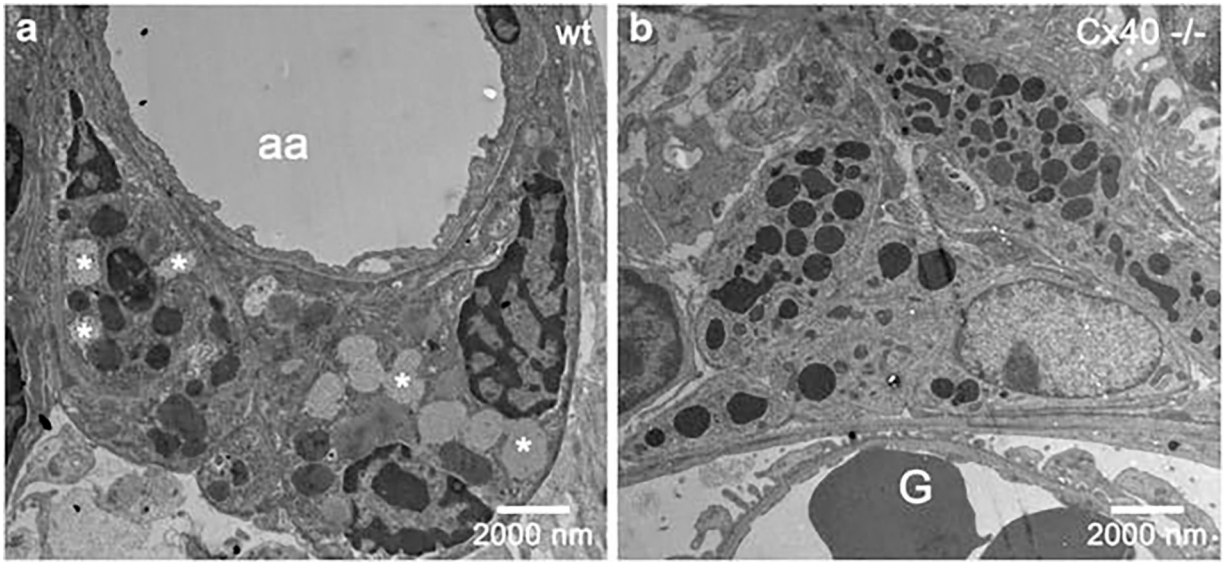

Fig.4.

Electronmicroscopical photomicrographs of renin cells from a wildtype (a) and a Cx40 −/− kidney (b) after 15 minutes of perfusion with isoproterenol (10nM) and EGTA (3mM). In wildtype kidneys renin cells are found in the walls of afferent arterioles (aa) and renin storage vesicles (asterisks) show reduced electron density and signs of intracellular vesicle fusion. In Cx40 −/− kidneys cells containing electron dense renin storage granules are mainly located in the extracellular glomerular tuft with closer contact to the glomerular capsule. The granules show homogeneous electron density and no signs of vesicle fusion. G: glomerulus

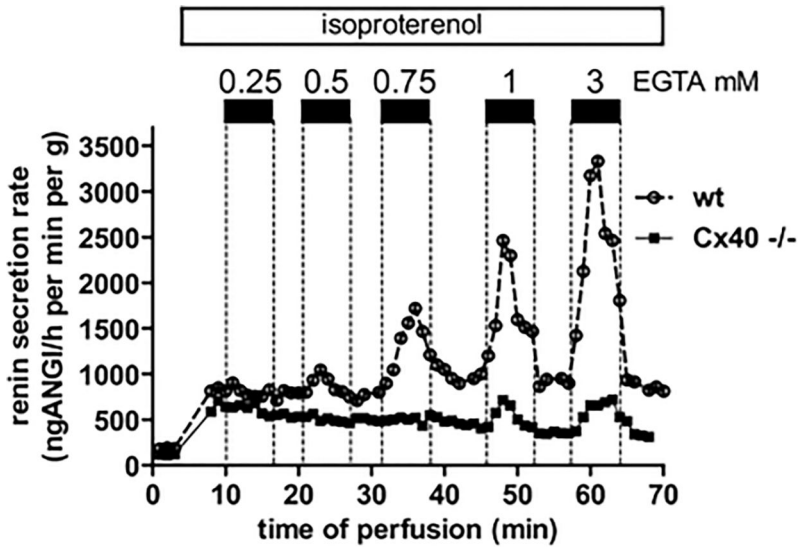

Fig.5.

Effects of graded concentrations of EGTA (0.25 to 3 mM) in the perfusate on renin secretion from kidneys isolated from a wildtype (wt) and a Cx40 deficient (Cx40 −/−) mouse

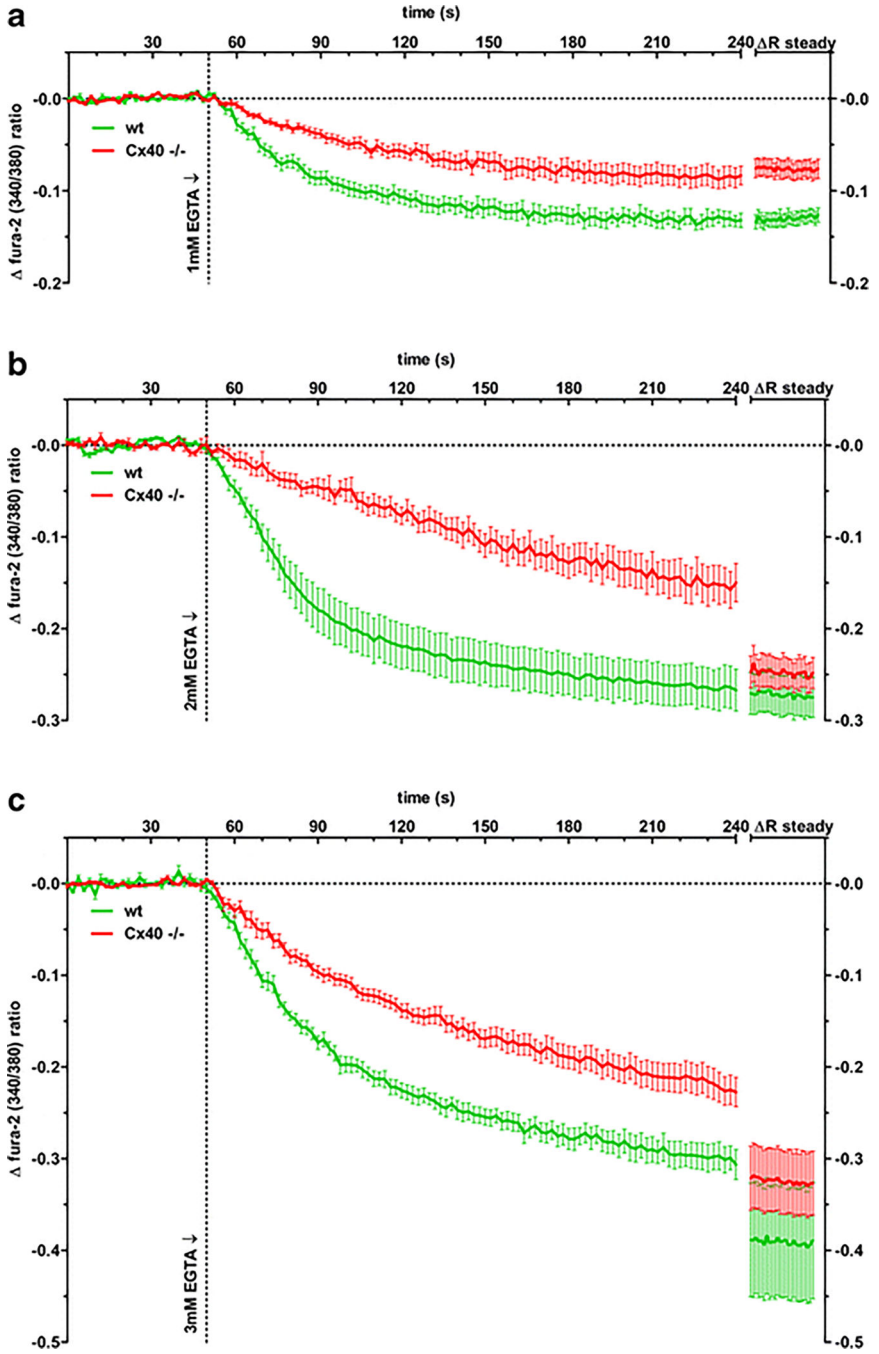

Fig.6.

Effects of extracellular EGTA (1–3 mM) on the time course of the fura 340/380 ratio of renin expressing cells in microdissected glomeruli from wildtype (wt) and Cx40 deficient (Cx40 −/−) kidneys. Data are means ± SEM of 16–19 cells for each EGTA concentration and each genotype. ΔR steady indicates steady state values measured approx. 900 sec after addition of EGTA

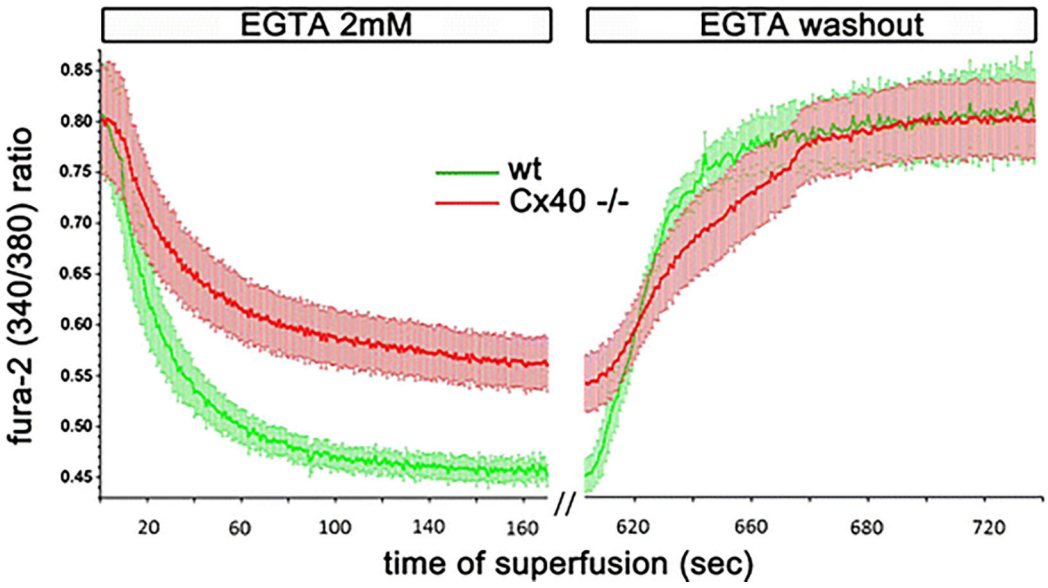

Fig.7.

Time course of fura-2 (340/380) ratio of renin expressing cells in microdissected glomeruli from wildtype (wt) and Cx40 deficient (Cx40 −/−) kidneys after addition and removal of EGTA (final concetration 2mM) to/from the perfusate. Data are means ±SEM of 9 renin cells of each genotype

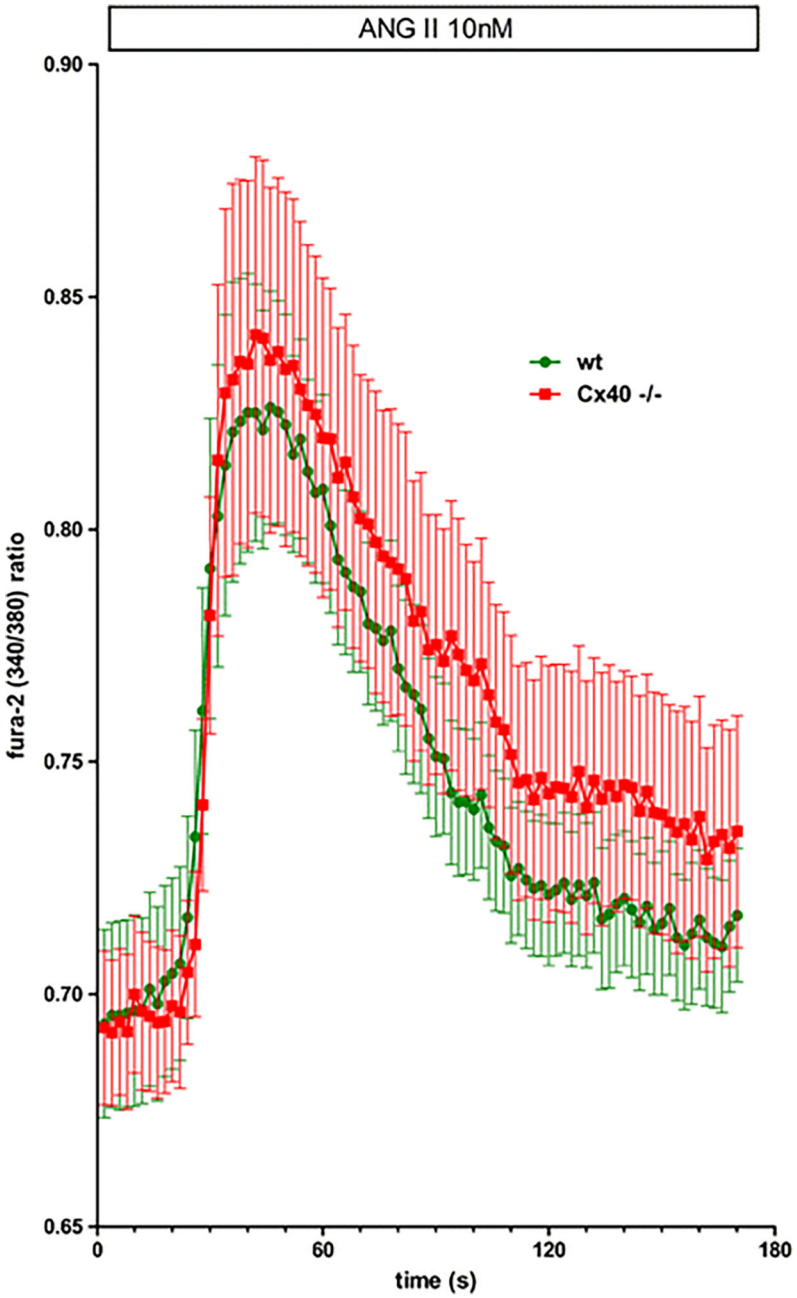

Fig.8.

Time course of fura-2 (340/380) ratio of renin expressing cells in microdissected glomeruli from wildtype (wt) and Cx40 deficient (Cx40−/−) kidneys after addition of angiotensin II (10nM). Data are means ±SEM of 13 (wildtype) and 15 (Cx40 −/−) renin cells.

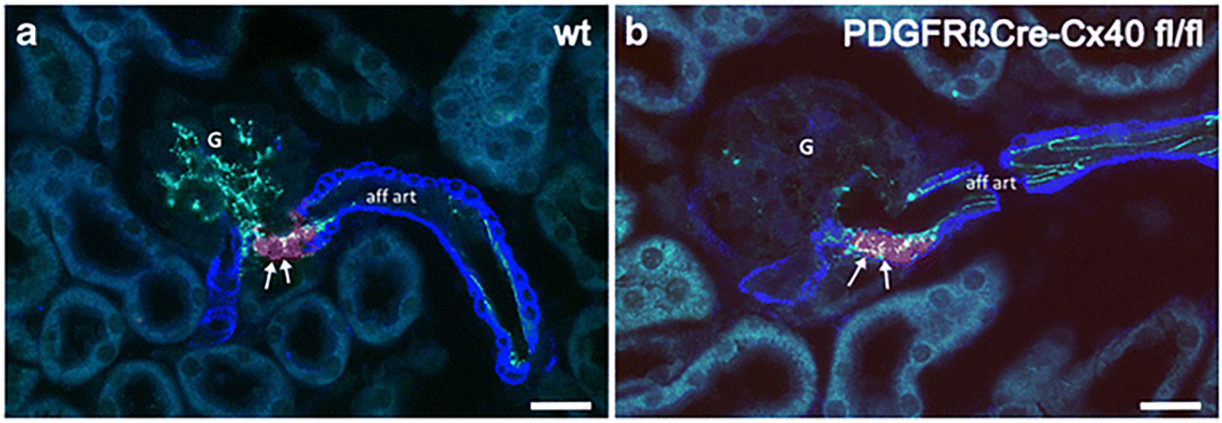

Fig.9.

Immunohistochemistry of renin (red), α-smooth muscle actin (blue) and connexin 40 (green) on kidney sections from a wildtype mouse (a) and a PDGFRßCre-Cx40fl/fl mouse (b). Note the absence of Cx40 immunoreactivity in intra- and extraglomerular mesangial cells of PDGFRßCre-Cx40fl/fl kidneys. Coexpression of renin and Cx40 in juxtaglomerular cells is maintained in both genotypes. Note also to the typical juxtaglomerular position of renin cells both in the wildtype and in the PDGFRßCre-Cx40fl/fl kidney. G: glomerulus; aff.art.: afferent arteriole; scale bar = 20μm

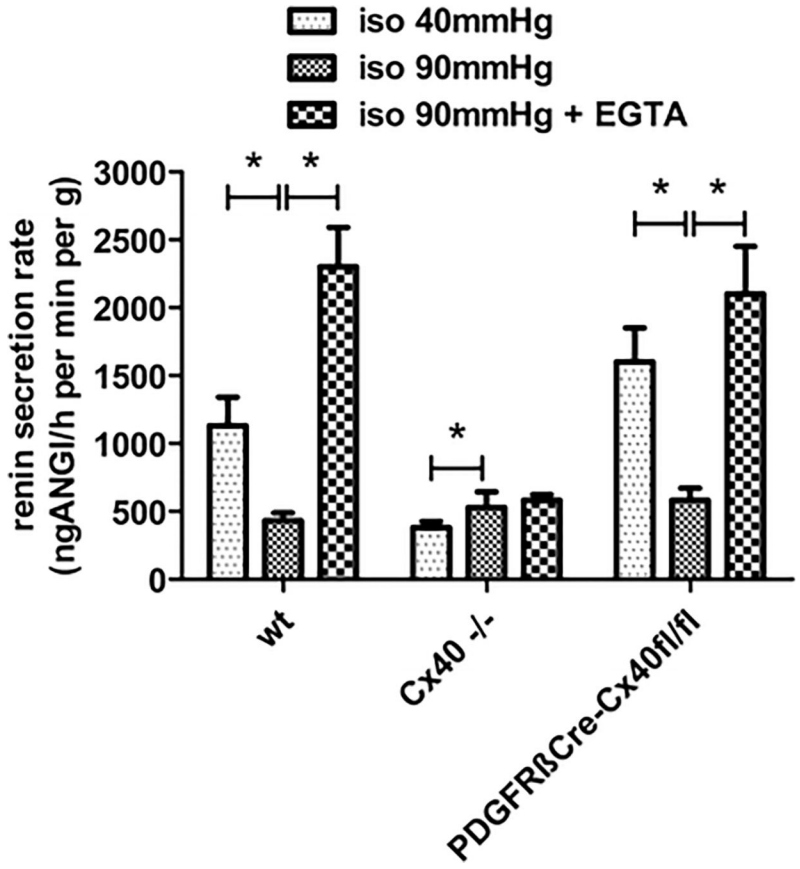

Fig.10.

Effects of lowering the perfusion pressure from 90 to 40 mmHg and of addition of EGTA (final concentration 3mM) at 90mmHg on renin secretion in the presence of isoproterenol (10nM) from kidneys isolated from wildtype (wt) mice, Cx40 deficient (Cx40 −/−) mice and from PDGFRßCre-Cx40fl/fl mice. Data are means ± SEM of five kidneys of each genotype. Asterisks indicate $p<0.05$.