Lack of Connexin 40 Causes Displacement of Renin-Producing Cells from Afferent Arterioles to the Extraglomerular Mesangium

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In the adult kidney, renin-producing cells are typically located in the walls of afferent arterioles at the transition into the glomerular capillary network. The mechanisms that are responsible for restricting renin expression to the juxtaglomerular position are largely unknown. This study showed that in mice that lack connexin 40 (Cx40), the predominant connexin of renin-producing cells, renin-positive cells are absent in the vessel walls and instead are found in cells of the extraglomerular mesangium, glomerular tuft, and periglomerular interstitium. Blocking macula densa transport function by acute administration of loop diuretics strongly enhances renin secretion *in vivo* and in isolated perfused kidneys of wild-type mice. This effect of loop diuretics is markedly attenuated *in vivo* and even blunted *in vitro* in Cx40-deficient mice. Even after prolonged stimulation of renin secretion by severe sodium depletion, renin expression is not seen in juxtaglomerular cells or in cells of more proximal parts of the arterial vessel wall as occurs normally. Instead, renin remains restricted to the extra-/periglomerular interstitium in Cx40-deficient mice. In contrast to the striking displacement of renin-expressing cells in the adult kidney, renin expression in the vessels of the developing kidney was found to be normal. This is the first evidence to indicate that cell-to-cell communication *via* gap junctions is essential for the correct juxtaglomerular positioning and recruitment of renin-producing cells. Moreover, these findings support the notion that gap junctions are relevant for the macula densa signaling to renin-producing cells.

J Am Soc Nephrol 18: 1103–1111, 2007. doi: 10.1681/ASN.2006090953

The aspartyl-protease renin is the regulating key enzyme of the renin-angiotensin-aldosterone system, which controls BP and extracellular volume. Renin is predominantly produced by the kidneys. Renin-producing cells of the kidneys show a high degree of plasticity. The cells are commonly considered to be a special subset of transformed vascular smooth muscle cells (VSMC) that are related to myofibroblasts or pericytes (1). During angiogenesis and vasculogenesis of the kidneys, renin-producing cells cover most of the arterial vascular tree (1–6). During vessel maturation, renin expression in proximal parts of the arteriole is progressively silenced so that in the adult kidney, it is condensed in juxtaglomerular cells of the ultimate part of the afferent arterioles (1). In this segment of the arteriole, renin-producing cells largely replace the typical VSMC. Because of the high number of renin storage vesicles, the cells achieve a cobble-stone like "epithelioid" appearance. Chronic stimulation of the renin-angiotensin-aldosterone system by extracellular volume depletion or increased sympathetic nerve activity is associated with re-emergence of renin expression in cells in the walls of larger renal arteries in a pattern that is not fully predictable (7–12). The cellular mechanisms that are responsible for initiating or terminating renin expression during vasculogenesis, for directing renin expression to the glomerular vascular pole in the adult kidney, and for restarting renin expression in some but not all cells of larger vessels are not yet understood. Although local humoral as well as biomechanical factors have been considered in this context, no clear concept has emerged.

In this study, we considered the role of cell-to-cell communication as a novel determinant of focal renal renin expression. Previous work established that cell-to-cell communication through gap junctions is important for the positioning and differentiation of resident cells (13–19). Within the juxtaglomerular apparatus (JGA), renin-producing cells not only form numerous gap junctions among each other but also are connected to extraglomerular cells and to endothelial cells of the afferent arteriole (20–22). The gap junctions of renin-producing cells are likely formed by connexin 40 (Cx40), which is expressed with high density in these cells (23–26). The prominent expression of Cx40 in the JGA is conspicuous, because with the exception of the electrical conduction system of the heart, Cx40 is almost exclusively expressed in the endothelium, where it contributes to propagation of vasodilation (27–31). In the (juxta)glomerular region, however, Cx40 is expressed by both endothelial and nonendothelial cells such as renin-producing and intra- and

Received September 3, 2006. Accepted January 18, 2007.

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1804-1103

extraglomerular mesangial cells (23–26). Other typical endothelial connexins, such as Cx43, are found in larger kidney vessels but not in the juxtaglomerular endothelial and renin-producing cells (23–26). In this study, we used mice that lacked Cx40 to explore the possibility that gap junctional coupling may be required for the differentiation and positioning of renin-producing cells during renal angiogenesis and vasculogenesis, for the condensation of renin to the JGA of the adult kidney, and for the recruitment of renin-expressing cells during prolonged challenges. Moreover, it was of interest for us to assess the role of Cx40 for the signaling of macula densa cells to renin-producing cells, a process that is considered to involve gap junctional function (32).

Materials and Methods

All animal experiments were conducted according to the National Institutes of Health guidelines for the care and use of animals in research. Kidneys were sampled from eight 12- to 20-wk-old male homozygous Cx40−/− mice (33), from three fetuses (days 17 to 19), and from three pups (postnatal day 1). Age-matched wild-type (wt) mice served as controls. In addition, five adult male Cx40+/− mice were examined. Furthermore, five adult male mice of the Cx40+/+ and Cx40−/− strains were pretreated with a low-salt diet (0.02% wt/wt) for 1 wk. In addition, these mice received the angiotensin-converting enzyme inhibitor (ACE) enalapril (10 mg/kg) *via* the drinking water for the last 3 d of the dietary treatment. The genetic background of Cx40-deficient and wt mice was considered identical because Cx40-deficient mice were backcrossed for seven generations on a C57Bl/6 background. The genotype of the mice was verified by PCR as described previously (34).

For assessment of the macula densa control of renin secretion *in vivo*, Cx40−/− and Cx40+/+ mice of either gender (25 to 30 g body wt) were used (Cx40+/+ seven male and seven female mice; Cx40−/− eight male and eight female mice). For determination of plasma renin concentration (PRC), blood samples were taken from the tail vein. Ten days after baseline blood collection, all mice received a single injection of furosemide (40 mg/kg body wt; Dimazon, Intervet, Germany) and a blood sample was taken 60 min thereafter from the tail vein.

Immunohistochemistry for Renin and α-Smooth Muscle Actin

The expression of renin and α-smooth muscle actin (α-SMA) was localized by immunohistochemistry. In brief, kidneys were fixed in methyl-Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) as described previously (35). Immunolabeling was performed on 5-μm paraffin sections. After blocking with 10% horse serum and 1% BSA in PBS, sections were incubated with anti-renin or anti–α-SMA antibodies (Beckman Coulter, Immunotech, Marseilles, France) overnight at 4°C, followed by incubation with a fluorescence secondary antibody.

Confocal Microscopy

Sections were analyzed with a confocal microscope (LSM 510; Zeiss, Göttingen, Germany) using sequential scanning (Plan Apochromat 63×/1.4 oil objective, excitation at 488 and 543 nm, emission at 505 to 530 and 560 to 615 nm, respectively).

Bromodeoxyuridine Incorporation

Bromodeoxyuridine (BrdU; Roche, Mannheim, Germany) incorporation into nuclei was determined as described previously (36). In brief, BrdU dissolved in sterile isotonic saline (10 mg/ml) was injected twice daily (0.1 mg BrdU/g body wt per d, intraperitoneally) starting on the day before the enalapril treatment period. After a total of 7 d, the mice were anesthetized with an intraperitoneal injection of 100 mg/kg 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid and 80 mg/kg ketamine-HCl and fixed by vascular perfusion with 4% paraformaldehyde. After the removal of kidneys and intestine and the embedding in paraffin, 5-μm sections were incubated in 4 M HCl for 30 min, neutralized by 0.1 M sodium borate (pH 8.5), and finally incubated in 0.1% trypsin in PBS. After blocking with 2% BSA and 0.1% TritonX-100 in PBS, sections were incubated with anti-BrdU antibody (1:1000) overnight, followed by incubation with a fluorescence secondary antibody.

Isolated Perfused Mouse Kidney

Male Cx40−/− and age-matched Cx40+/+ mice were used as kidney donors. The isolated perfused mouse kidney model was described in detail previously (37). Briefly, the mice were anesthetized with an intraperitoneal injection of 12 mg/kg xylazine (Rompun; Bayer, Wuppertal, Germany) and 80 mg/kg ketamine-HCl (Curamed, Karlsruhe, Germany); the abdominal aorta was cannulated; and the right kidney was excised, placed in a thermostated moistening chamber, and perfused at constant pressure (90 mmHg). Finally, the renal vein was cannulated and the venous effluent was collected for determination of renin activity and venous blood flow.

The basic perfusion medium consisted of a modified Krebs-Henseleit solution supplemented with 6 g/100 ml BSA and with freshly washed human red blood cells (10% hematocrit). Stock solutions of isoproterenol were dissolved in freshly prepared perfusate; stock solution of bumetanide was made in DMSO. All drugs were infused into the arterial limb of the perfusion circuit.

For the determination of renin secretion rates, three samples of the venous effluent were taken in intervals of 2 min during each experimental period. Renin activity in the venous effluent was determined by RIA (Byk & DiaSorin Diagnostics, Dietzenbach, Germany) as described previously. Renin secretion rates were calculated as the product of the renin activity and the venous flow rate (ml/min × g kidney weight).

Determination of PRC

For determination of PRC, the blood samples that were taken from the tail vein were centrifuged and the plasma was incubated for 1.5 h at 37°C with plasma from bilaterally nephrectomized male rats as renin substrate. The generated angiotensin I (ng/ml per h) was determined by RIA (Byk & DiaSorin Diagnostics).

Electron Microscopy

Kidneys were perfusion-fixed and embedded in Epon according to standard procedures. One-micrometer sections of several blocks including a series of 200 1-μm sections were cut with a diamond knife, stained with methylene blue (38), and studied by light microscopy. Ultrathin sections were prepared from selected areas and studied by a Philips 307 transmission electron microscope (Eindhoven, The Netherlands).

Three-Dimensional Reconstruction

Serial sections of kidney specimen were fixed and stained for renin and for α-SMA as described in the previous section. A three-dimensional (3-D) reconstruction of renin immunoreactivity and of α-SMA immunoreactivity was performed using the Amira 3.1 visualization program (Mercury Systems, Merignac, France).

Results

In adult kidneys of Cx40+/+ mice, renin-expressing cells were restricted to the vascular poles of the glomeruli. Renin-producing cells were integrated into the regular wall of the afferent arterioles in continuation of the vessel lined by VSMC (Figure 1, A and B). The latter cells were identified by the expression of α-SMA (Figure 1, A and B). Renin-producing cells at the very end of the afferent arterioles showed the typical epithelioid appearance (juxtaglomerular epithelioid cells) and expressed no α-SMA. Mixed-phenotype cells that expressed both renin and α-SMA were found to be located between the typical SMC and juxtaglomerular epithelioid cells (Figure 1, A and B). The distribution of renin-expressing cells in Cx40+/− kidneys was very similar to that in kidneys of wt mice (data not shown).

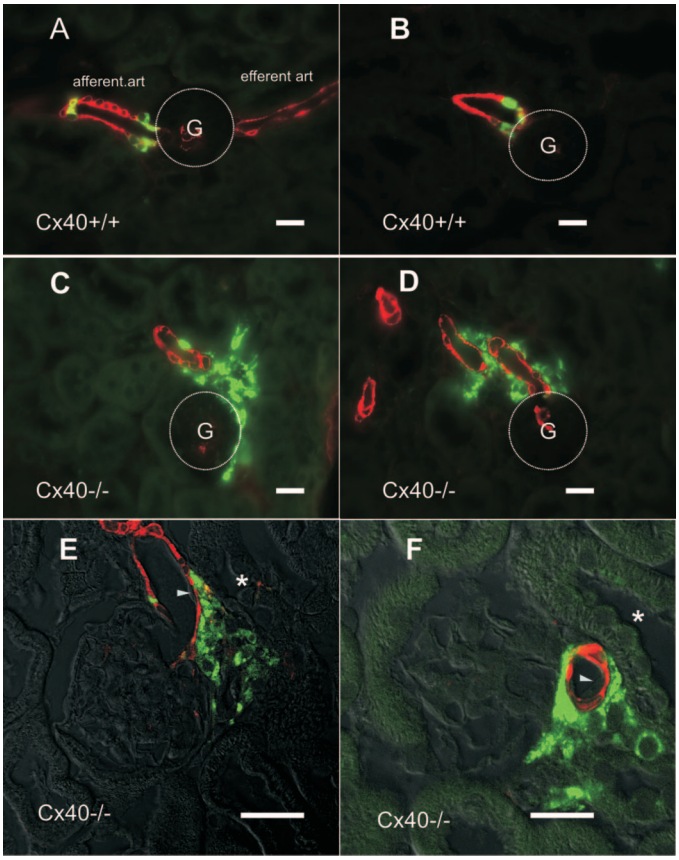

Figure 1. Immunohistochemistry for renin (green) and α-smooth muscle actin (α-SMA; red) on kidney sections of wild-type (wt; A and B) and of connexin 40 deficient (Cx40−/−; C and D) mice. In wt mice, renin immunoreactivity was found in the walls of afferent arterioles close to the glomeruli (dotted, G). Renin-positive cells had a regular form, appeared thicker, and were negative for α-SMA at the very end of the afferent arterioles. In Cx40−/− kidneys, the number of renin-positive cells in the juxtaglomerular region was clearly increased. The cells had a more irregular shape, and they were not integrated into the walls of the afferent arterioles. Impression was further corroborated by a confocal analysis (E and F) of kidney sections from Cx40−/− mice, showing that renin-positive cells were separated from endothelial cells by a layer of α-SMA–positive smooth muscle cells. *, Macula densa; arrowheads, endothelial cells. Bars = 20 μm.

In contrast, the number of renin-expressing cells in adult kidneys of Cx40-deficient mice was seemingly increased when compared with wt controls. The number of renin-producing cells in individual periglomerular regions of Cx40−/− kidneys was variable, ranging from 1 to 20 cells per glomerular region. Renin-positive cells in Cx40−/− kidneys were restricted to the juxtaglomerular/periglomerular interstitium (Figure 1, C and D). As confirmed by confocal microscopy, renin-producing cells in the juxtaglomerular region were not integrated into the wall of afferent arterioles but instead surrounded the vessel wall that consisted of endothelial cells and α-SMA–positive VSMC (Figure 1, E and F). Moreover, renin-producing cells extended into the region of the extraglomerular mesangium (EGM) and into the periglomerular interstitial space between tubules and around glomeruli. The cells did not show the classic epithelioid appearance but rather appeared mesenchyme-like and irregularly shaped. The cytosol of these cells showed renin immunoreactive granulation. All renin-positive cells stained negative for α-SMA. No renin-producing cells were associated with larger kidney vessels (Figure 1, C and D).

Electron microscopic analysis confirmed that walls of afferent arterioles were free of granular cells (Figure 2C). Furthermore, VSMC were found inside the glomerular stalk of Cx40−/− kidneys, whereas they normally terminate at the entrance into the JGA. VSMC were also observed in the intraglomerular segment of the efferent arteriole that is normally surrounded by mesangial cells and mesangial cell processes. In contrast to wt kidneys, the majority of extraglomerular mesangial cells in Cx40−/− mice contained granules as they are normally seen in renin-producing cells of the afferent arteriole (Figure 2, A through D). For example, tracing of an entire glomerulus in a series of 1-μm sections revealed no cells in the EGM without granules (Figure 2B). In addition, granular cells were found in the glomerular stalk to a variable extent. Occasionally, the intraglomerular segment of the efferent arteriole deep within the glomerular tuft also appeared granulated.

In other sites, EGM cells that looked fairly normal with elongated processes and without any granules were found (Figure 2E). The interstitial spaces between these cells seemed wider and were filled with a translucent amorphous matrix. Gap junctions were not encountered. Within the intraglomerular mesangium, foci of mesangiolysis that may be the result of mesangial cell proliferation and matrix production, leading to local mesangial expansions, were noted. The resulting narrowing of associated capillaries and their incorporation into the proliferating mesangium may cause obstruction and eventual degeneration.

In Cx40+/+ mice that received a low-sodium (0.02% wt/wt) diet and the ACEI ramipril, a clear increase of renin-expressing cells appeared in the walls of the preglomerular vessels (Figure 3, A and B). Cells that expressed both α-SMA and renin could be seen with increased frequency. In contrast, no expression of renin was found in the walls of the arterioles or larger vessels of Cx40−/− kidneys (Figure 3, C and D). Instead, the number of renin-expressing periglomerular cells seemed to be in-

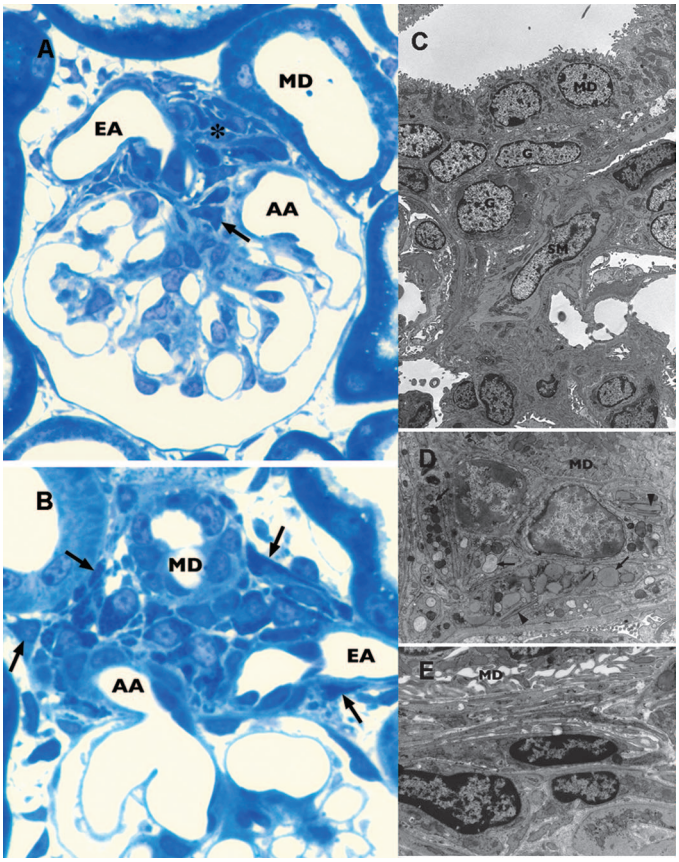

Figure 2. (A) High-resolution light microscopy longitudinal section (1 μm) through the juxtaglomerular apparatus (JGA) and glomerulus. Granular cells are abundant and are encountered at abnormal sites in the extraglomerular mesangium (EGM; *) and the glomerular stalk (arrow). (B) Oblique section through the JGA showing the abundance of granular cells in the EGM and as disseminated cells everywhere in the surroundings of the JGA (arrows). (C) Transmission electron microscopy longitudinal sections through the JGA. Granular cells (G) are seen within the EGM; a vascular smooth muscle cell (SM) extends into the glomerular stalk. (D) Transmission electron microscopy longitudinal sections through the JGA. Beneath the macula densa (MD), a group of EGM cells that contain abundant granules of variable electron density are seen (arrows); also protogranules (arrowheads) are regularly encountered. (E) Transmission electron microscopy longitudinal sections through the JGA. Beneath the macula densa are a group of EGM cells and processes that look fairly normal. Gap junctions have not been encountered between the EGM cells. AA, afferent arteriole; EA, efferent arteriole. Magnifications: ×500 in A; ×625 in B; ×1800 in C; ×5700 in D; ×4500 in E.

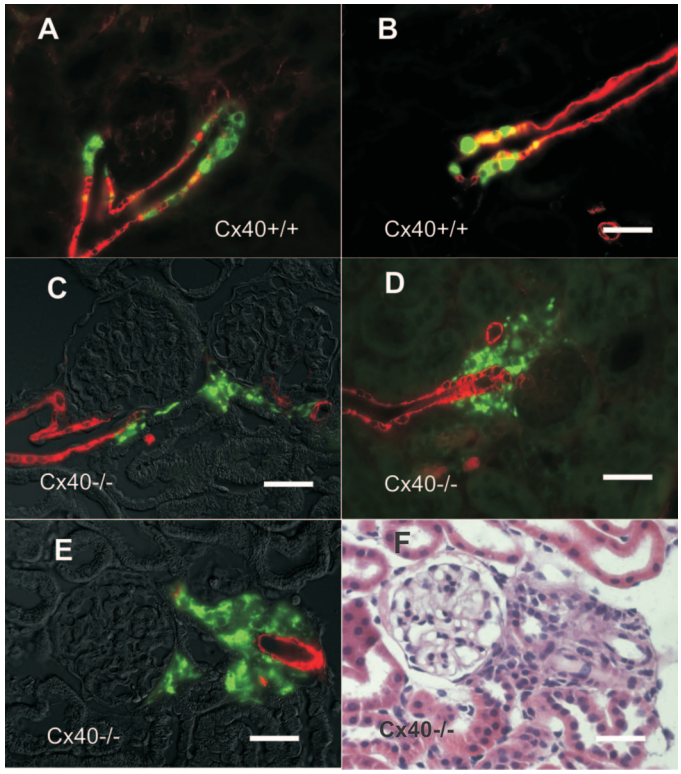

Figure 3. (A through D) Immunohistochemistry for renin (green) and α-SMA (red) on kidney sections of wt (A and B) and of Cx40−/− (C and D) mice treated with a combination of low salt and the angiotensin-converting enzyme inhibitor (ACEI) ramipril. In the kidneys of wt mice, renin expression was increased in the walls of the preglomerular vessels. Colocalization of renin and of α-SMA (yellow) was increased. In kidneys from Cx40−/− mice, again no renin immunoreactivity became visible in the vessel walls. Instead, renin expression spread out into the periglomerular-peritubular space. (E and F) Consecutive kidney sections of Cx40−/− mice treated with low salt and the ACEI stained with renin/α-SMA (E) and with hematoxylin-eosin (F). Bars = 20 μm.

creased. Cx40+/− behaved like Cx40+/+ kidneys (data not shown).

When the number of renin-expressing cells in the periglomerular region was particularly high, a clear expansion of the periglomerular cell mass could be readily detected by light microscopy (Figure 3, E and F). Otherwise the histology of the kidneys was normal, except for the appearance of sclerotic glomeruli. To determine whether expansion of periglomerular cell mass was associated with proliferation of interstitial cells, we determined the number of cells that underwent an S phase during treatment with low salt and the ACEI. Using the BrdU incorporation method, we found single BrdU incorporating nuclei in proximal tubules but no BrdU incorporation into nuclei of renin-immunoreactive cells, neither in wt nor in Cx40−/− kidneys (data not shown).

Because the extent of periglomerular renin expression was difficult to estimate from single histologic sections, we performed a 3-D reconstruction of 80 consecutive 5-μm sections that stained for renin and for α-SMA. With the use of this technique, no obvious differences between preglomerular vessel trees of wt and Cx40−/− kidneys were noted (Figure 4, A and B). The number of glomeruli within this defined kidney volume was similar between wt and Cx40−/− kidneys. In low-salt/ACEI-treated wt mice, renin expression was exclusively associated with the walls of the distal parts of the afferent arterioles, where the renin-producing cells formed cuff-like

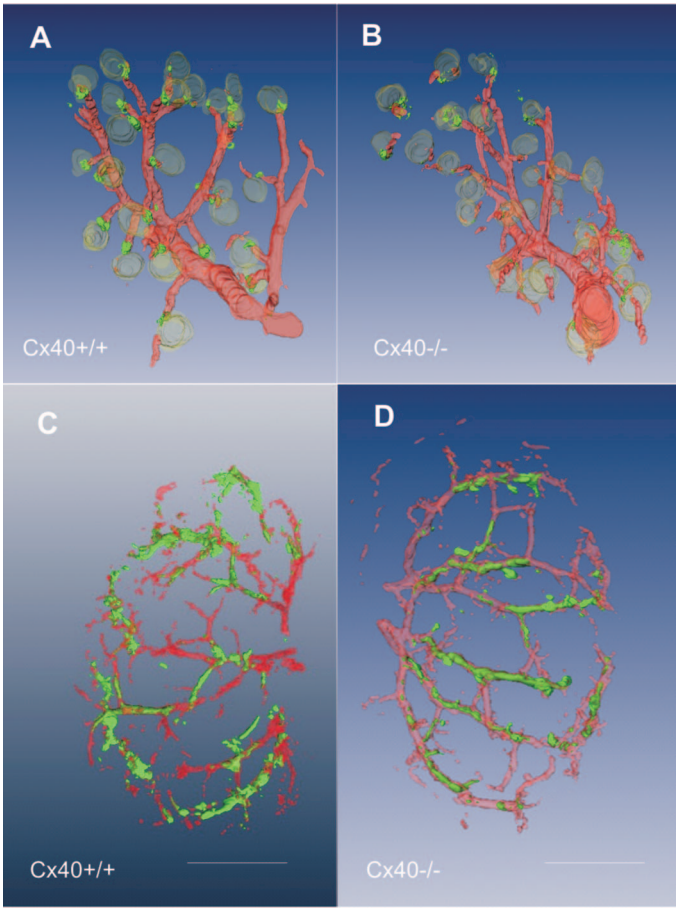

Figure 4. (A and B) Three-dimensional (3-D) reconstruction of serial kidney sections stained for renin/α-SMA of adult wt (A) and Cx40−/− (B) kidneys. Mice were pretreated with low salt and ACEI (green, renin; red, α-SMA). (C and D) 3-D reconstruction of serial kidney sections stained for renin and for α-SMA of wt (C) and of Cx40−/− (D) mice at embryonic day 18 (green, renin; red, α-SMA). Bars = 500 μm.

structures at the vascular pole of virtually all glomeruli. In Cx40−/− kidneys, no cuff-like structures were observed. Instead, 30% of glomeruli showed a prominent periglomerular spreading (Figure 4B). In the remaining glomeruli, the number of renin-expressing cells was markedly lower, but again these cells were not integrated into the walls of the afferent arterioles.

In view of the aberrant position of renin-producing cells in the kidneys of adult Cx40−/− mice, it was of interest to study the localization of renin-producing cells during the development of the kidneys. Therefore, we performed a 3-D analysis of the vascular tree and of renin-producing cells of kidneys at day 18 after conception. As shown in Figure 4, C and D, the different arteries and arterioles could clearly be identified in embryonic kidneys of wt and Cx40−/− mice. There were no obvious differences in the vascular morphology in the two genotypes.

Similarly, the distribution of renin-producing cells in these fetal kidneys was very similar between the genotypes. The renin-producing cells were associated with the walls of the larger arteries. Most notable, renin expression in Cx40−/− kidneys was restricted to the vessel wall and did not appear in the interstitium (Figure 4, C and D). The first clearly recognizable divergence in the localization of renin-producing cells between the two genotypes appeared with the development of renin expression in the glomerular regions. Figure 5, B through D, shows that in the kidneys of 1-d-old Cx40−/− pups, renin-expressing cells are still integrated into the walls of afferent arterioles, although they start to spread out at the vascular poles. In wt kidneys of the same age, renin expression remained restricted to the vessel wall of the preglomerular arteries (Figure 5A).

Considering these apparent changes of the architecture of the JGA raises the question about functional changes. It was of particular interest for us to see whether the normal signaling of the macula densa cells to renin-producing cells is altered in the absence of Cx40. To study the macula densa control of renin secretion, we used a classical maneuver, the acute administration of loop diuretics, which blocks salt transport in the thick ascending loop of Henle including the macula densa segment. In Cx40+/+ mice, application of furosemide led to a 15-fold increase of PRC (Figure 6A). Similarly, also in isolated perfused kidneys of Cx40+/+ mice, bumetanide caused a strong enhancement of renin secretion (Figure 6B). In Cx40−/− mice, basal PRC were sixfold elevated when compared with Cx40+/+ mice (Figure 6A). Administration of furosemide increased PRC values only two-fold in Cx40−/− mice (Figure 7A). Renin secretion from isolated perfused kidneys of Cx40−/− mice responded well to isoproterenol, whereas the response to bumetanide was absent (Figure 6B).

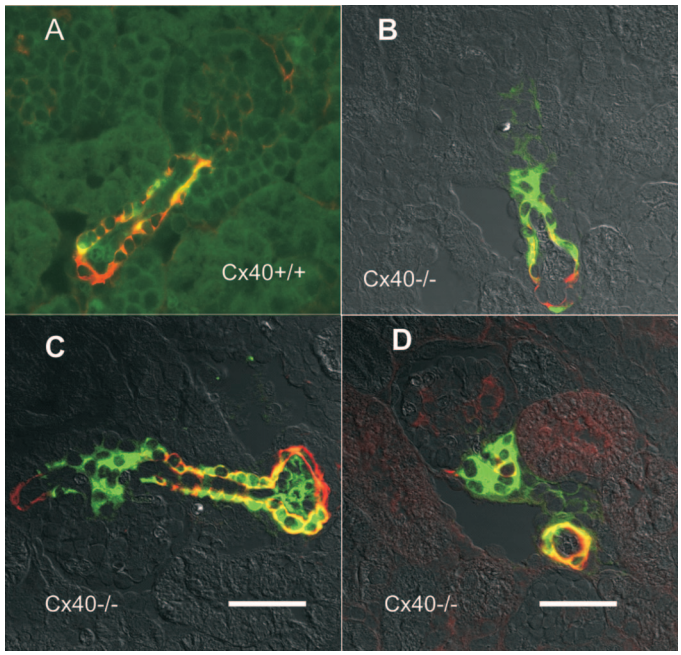

Figure 5. Immunohistochemistry for renin (green) and α-SMA (red) on kidney sections of wt (A) and Cx40−/− (B through D) mice 1 d after birth. Yellow indicates overlap of renin and α-SMA. Bars = 20 μm.

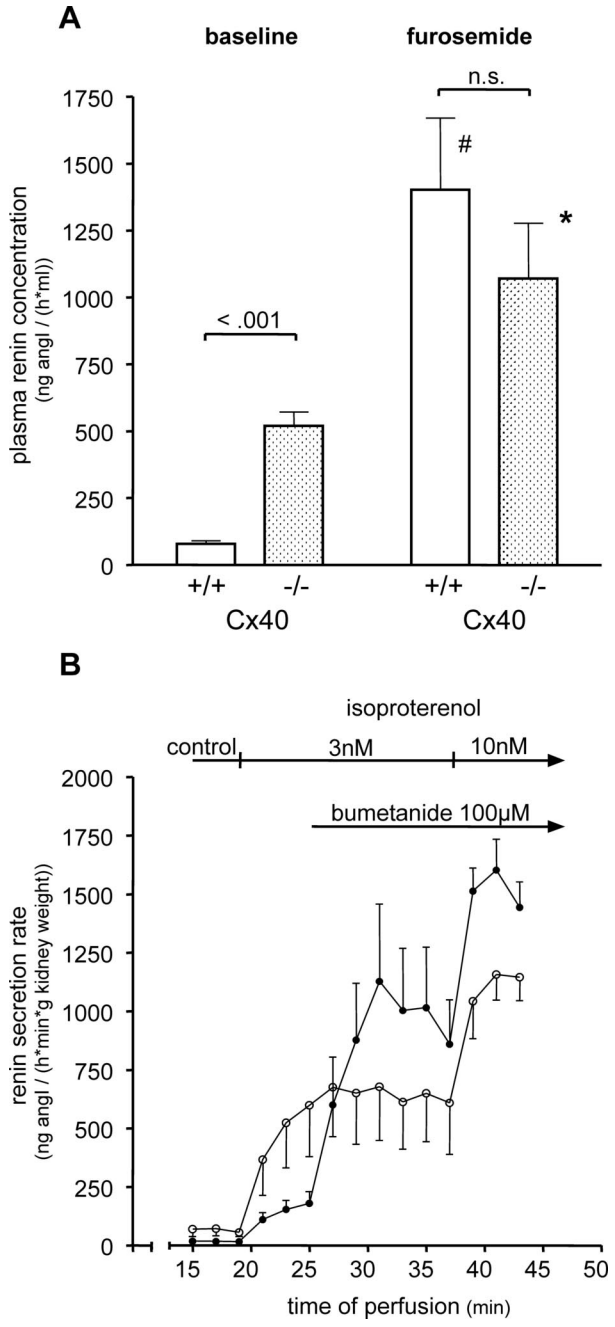

Figure 6. Effect of loop diuretics on renin secretion *in vivo* (A) and from isolated perfused kidneys (B) in Cx40+/+ and Cx40−/− mice. (A) Data are means ± SEM of 14 and 16 Cx40+/+ and Cx40−/− mice, respectively. Furosemide (40 mg/kg) was injected 10 d after baseline blood collection of each mouse. Plasma renin concentration values were determined 1 h after injection of furosemide. #$P < 0.001$ *versus* baseline Cx40+/+; *$P < 0.05$ *versus* baseline Cx40−/−. (B) For isolated perfused kidneys, five kidneys of each genotype were used. Data are means ± SEM. ○, Cx40−/−; ●, Cx40+/+. The increases of renin secretion that were elicited by isoproterenol (iso; 3 nM) were significant ($P < 0.05$) for both genotypes. The change of renin secretion that was elicited by bumetanide (bum) was significant for Cx40+/+ only ($P < 0.05$ *versus* iso 3 nM alone). The change of renin secretion that was elicited by iso (10 nM) was significant ($P < 0.05$ *versus* iso 3 nM + bum 100 μM) for both genotypes.

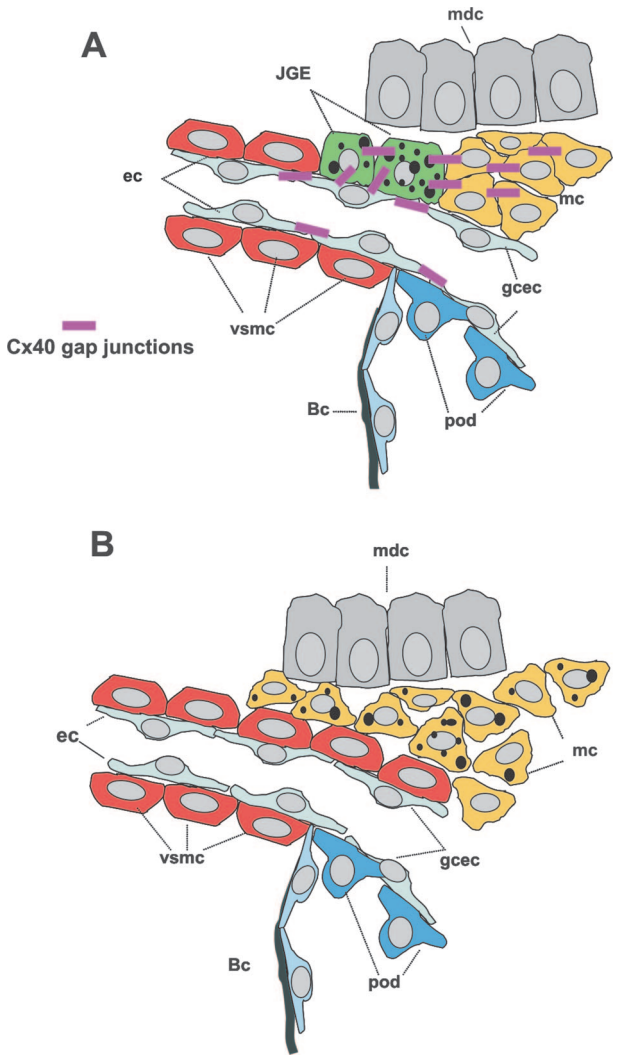

Figure 7. Sketch summarizing the morphology of the JGA in the presence (A) and absence (B) of Cx40. mdc, macula densa cells; JGE, juxtaglomerular epithelioid cells; ec, endothelial cells; mc, mesangial cells; gcec, glomerular capillary endothelial cells; pod, podocytes; vsmc, vascular smooth muscle cells; Bc, Bowman capsule.

Discussion

A main finding in the present study is that the absence of Cx40 is associated with a major disturbance in the location and identity of renin-expressing cells. Cell-to-cell communication through Cx40 seems to be required for the maintenance of the architecture of the JGA and the direction of renin expression to cells in the walls of afferent arterioles in a typical juxtaglomerular location (Figure 6, A and B). In the absence of Cx40, renin-expressing cells are found outside the terminal parts of the afferent arterioles and extending into the juxta- and periglomerular interstitium. Furthermore, stimulation of renin synthesis and secretion does not induce the normal recruitment of renin-expressing cells in the walls of upstream preglomerular arteries.

Given that Cx40 is the main gap junctional protein in the JGA (25–28), especially in the region of renin production (Figure

6A), our observations are in accordance with numerous findings of a central role of gap junctions in the differentiation of a variety of cells in the cardiovascular, nervous, or reproductive system (13–19). There is general agreement that gap junctional communication favors structural and functional differentiation, whereas loss of gap junctions leads to dedifferentiation (13–19). There is also agreement that gap junctional communication is important for the correct formation of endo- and exocrine glands and for coordinating the responses of individual gland cells (39).

Our data suggest that the mislocation of renin-producing cells in Cx40−/− kidneys may begin with typical expression of renin in the juxtaglomerular region. Endothelial cells of the afferent arterioles, renin-producing cells, and mesangial cells form a network of cells that are connected *via* Cx40 gap junctions (23–26) (Figure 6C) and that are derived from the juxta/periglomerular mesenchyme as a group of pericytes. Notably, all of these cell types, including the extraglomerular and intraglomerular mesangial cells (40,41), have the capability to synthesize renin, although the expression of renin in the adult mesangium is a very rare event (1,42–44).

Because renin expression in the EGM of Cx40−/− kidneys is abundant, Cx40 gap junctions do not seem to be a general prerequisite for the expression of renin in the pericytes, a conclusion that is confirmed by normal fetal development. Because endothelial cells in the afferent arteriole normally form Cx40 gap junctions with renin-producing cells but not with VSMC, one may speculate that the lack of Cx40 gap junctions leads to a transformation of originally renin-producing pericytes into VSMC. As a consequence, remaining renin-producing cells are displaced to the periphery of the afferent arterioles, initiating the process of dispersion of renin expression to cells outside the afferent arteriolar vessel wall. Our findings suggest that this proliferation period ends once the formation of the glomeruli is complete.

One may hypothesize that contact between endothelial cells and renin-producing pericytes *via* Cx40 gap junctions could provide an essential anchor for the juxtaglomerular position of renin-producing cells. Once a stable contact is established, endothelium-coupled pericytes may send a signal through Cx40 gap junctions to neighboring pericytes to suppress their proliferation and renin secretion. The inherent capability to produce renin, however, would be preserved. In fact, it is known that long-lasting stimulation of the renin-angiotensin system can lead to the recruitment of renin production not only in the larger vessels but also in the EGM (42–47).

The displacement of renin-producing cells raises the question of whether the physiologic regulation of the renin system is affected by the absence of Cx40. It is known that Cx40−/− mice are hypertensive (48). Although first evidence suggests that the hypertension in Cx40−/− mice is not primarily renin dependent (48), our observation of elevated PRC values (Figure 7A) could suggest that an exaggerated renin secretion could contribute to the hypertension. The enhanced basal renin secretion in Cx40−/− mice could result from an interruption of the inhibitory macula densa signaling to renin-producing cells. Such an explanation would be supported by our findings that the stimulatory effect of loop diuretics on renin secretion is absent in isolated kidneys of Cx40−/− mice (Figure 7B) and is markedly attenuated *in vivo* (Figure 7A). A possible explanation for why the renin response to loop diuretics *in vivo* is not blunted as *in vitro* is that additional factors may contribute to the effects of loop diuretics *in vivo*, such as inhibition of NKCC1 (49,50) or activation of the sympathetic nervous system, which may be less relevant *in vitro*. In fact, our *in vitro* findings suggest that Cx40−/− kidneys respond more sensitively to β-adrenoreceptor activation than do Cx40+/+ kidneys (Figure 7B). Altogether, our data show that the macula densa signaling to renin-producing cells is markedly deteriorated in the absence of Cx40−/−, suggesting an important role of this connexin in macula densa signaling and supporting the conclusions of a recent elegant imaging study (32).

For further elucidation of the possible underlying mechanisms of the crucial effect of Cx40 for the position of renin-producing cells and for the macula densa control function, two strategies will be used. First, in mice with an endothelium-specific deletion of Cx40, we would expect an interruption of the communication between endothelial cells and renin-producing cells without affecting the communication between renin-producing cells and mesangial cells. Second, replacement of Cx40 with other related connexins should provide information about the connexin subtype that is causal in determining the position of renin-producing cells.

Acknowledgments

This work was financially supported by the Sonderforschungsbereich 699, by the Deutche Forschungsgemeinschaft grant Wi 2071/1-1, and by the "Prof. Dr. Karl und Gerhard Schiller-Stiftung, Frankfurt/Main."

We thank Jurgen Schnermann for critical reading of the manuscript and for helpful comments. The expert technical assistance provided by Anna Bángui, Katharina Machura, and Hiltraud Hosser is gratefully acknowledged.

Disclosures

None.

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