Inducible deletion of connexin 40 in adult mice causes hypertension and disrupts pressure control of renin secretion

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Genetic loss-of-function defects of connexin 40 in renal juxtaglomerular cells are associated with renin-dependent hypertension. The dysregulation of renin secretion results from an intrarenal displacement of renin cells and an interruption of the negative feedback control of renin secretion by blood pressure. It is unknown whether this phenotype is secondary to developmental defects of juxtaglomerular renin cells due to connexin 40 malfunction, or whether acute functional defects of connexin 40 in the normal adult kidney can also lead to a similar dysregulation of renin secretion and hypertension. To address this question, we generated mice with an inducible deletion of connexin 40 in the adult kidney by crossing connexin 40–floxed mice with mice harboring a ubiquitously expressed tamoxifen-inducible Cre recombinase. Tamoxifen treatment in these mice strongly reduced connexin 40 mRNA and protein expression in the kidneys. These mice displayed persistent hypertension with renin expression shifted from the media layer of afferent arterioles to juxtaglomerular periglomerular cells. Control of renin secretion by the perfusion pressure was abolished *in vitro*, whereas *in vivo* plasma renin concentrations were increased. Thus, interruption of the connexin 40 gene in the adult kidney produced very similar changes in the renin system as had embryonic deletion. Hence, impairments of connexin 40 function in the normal adult kidney can cause renin-dependent hypertension.

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The protease renin is the key regulator of the activity of the renin–angiotensin–aldosterone system involved in body fluid and blood pressure homeostasis. Its synthesis and secretion is controlled by a number of systemic and intrarenal parameters, which mainly act in the sense of negative feedback loops. One important negative feedback loop in this context is the influence of the systemic blood pressure and the intrarenal perfusion pressure on renin secretion. As activation of the renin–angiotensin–aldosterone system elevates blood pressure, high blood pressure suppresses renin secretion and vice versa, resulting in a characteristic inverse relationship between blood pressure and plasma renin concentrations.[1] There is common agreement that a defective feedback regulation of renin secretion by the blood pressure can be a major reason for hypertension.[2–4] The mechanisms along which the blood or renal perfusion pressure control renin secretion from the juxtaglomerular cells of the kidney were rather speculative, until the discovery that genetic defects of the gap-junction protein connexin 40 (Cx40) interrupts the feedback control of renin secretion by blood pressure[2,3,5] and as a consequence they are associated with high blood pressure.[2,4–6] In addition, those defects are also associated with a translocation of renin expression from juxtaglomerular cells of afferent arterioles to the periglomerular interstitium[7,8] and with an abolition of the so-called 'calcium paradox' of renin secretion.[2,3] The 'calcium paradox' circumscribes the phenomenon that lowering of the extracellular concentration of calcium stimulates renin secretion from juxtaglomerular cells,[9–11] which is unexpected, as calcium is in general considered a positive trigger for secretory events.[12] The abundant expression of Cx40 in preglomerular endothelial cells and renin-producing cells raises the question in which cell type the Cx40 expression is required to enable negative feedback of blood pressure on renin secretion and positioning of renin-producing cells in the juxtaglomerular area. Selective deletion of Cx40 in renin-producing cells exactly mimics the phenotype of global Cx40 deletion, whereas endothelial deletion of Cx40 has no effect on renin secretion or blood pressure.[3]

It is unclear so far whether these profound changes of the function of renin-expressing cells induced by genetically

determined malfunctions of Cx40 is a consequence of defective differentiation or maturation of renin-producing cells, or whether acutely occurring defects of Cx40 function in the normal adult kidney can produce similar changes in the function of renin-producing cells in the kidney. Proper function of Cx40 is dependent on the synthesis of a correct protein on one hand and of correct insertion into the plasma membrane. Although little is known yet about the intracellular control of Cx40 synthesis and trafficking,[13,14] changes of Cx40 expression have already been described for clinically relevant situations such as hypertension, sepsis/inflammation, diabetes, or hypercholesteremia.[15–19]

Therefore, it appeared of interest for us to find out whether changes of Cx40 function in the normal adult kidney can also induce fundamental changes of the function of renin-producing cells of the kidney and thus provide a basis for renin-dependent hypertension. To address this aim, we have generated and characterized mice with an inducible deletion of Cx40 in the adult animals.

RESULTS

To find the best treatment protocol for tamoxifen-induced Cre recombinase activation and secondary Cx40 deletion, adult $Cx40^{fl/fl}Cre^{ER}$ mice and $Cx40^{fl/fl}$ controls were either injected with tamoxifen (40 mg/kg/day) every other day for 10 days or were fed a tamoxifen-containing chow (40 mg/kg/day) over different time periods. Most efficient and reproducible deletion of Cx40 in the kidneys was achieved with feeding the tamoxifen-containing chow for 2 weeks. Animals were then analyzed 2 and 14 weeks after stop of tamoxifen feeding. Already 2 weeks after completing the tamoxifen treatment, renal Cx40 mRNA abundance and Cx40 protein levels had dropped to 20 and to 10% of the respective values of $Cx40^{fl/fl}$ control mice (Figure 1a). The reduction of Cx40 expression by tamoxifen treatment of $Cx40^{fl/fl}Cre^{ER}$ mice was also clearly visible by immunohistochemical analysis of the kidneys. In kidneys of $Cx40^{fl/fl}$ control mice, Cx40 immunoreactivity was clearly detectable in intraglomerular (presumably mesangial) cells, in juxtaglomerular renin-producing cells, and in the endothelial cell layer of afferent arterioles (Figure 1b). In kidneys of $Cx40^{fl/fl}Cre^{ER}$ mice, Cx40 immunoreactivity was occasionally seen as a residual faint intraglomerular staining, but renin-producing cells were frequently free of Cx40 immunoreactivity (Figure 1c). Determination of the renal mRNA abundance of the other vascular connexin isoforms Cx37, Cx43, and Cx45 revealed no obvious differences between the two genotypes (Table 1).

Already 2 weeks after the completion of tamoxifen treatment, $Cx40^{fl/fl}Cre^{ER}$ mice had developed robust hypertension, which sustained throughout the time of observation, whereas tamoxifen-treated $Cx40^{fl/fl}$ control mice remained normotensive (Figure 2c; Table 2). Treatment with enalapril (10 mg/kg/day), an angiotensin I–converting enzyme inhibitor, for a time span of 12 days reduced the blood pressure of $Cx40^{fl/fl}$ mice significantly and decreased blood pressure in $Cx40^{fl/fl}Cre^{ER}$ mice to the levels observed in control littermates (Table 2). Plasma renin concentrations were approximately doubled in $Cx40^{fl/fl}Cre^{ER}$ mice relative to $Cx40^{fl/fl}$ control mice throughout the experimental period (Figure 2b), whereas renin mRNA abundance in the kidneys was not different between the two genotypes (Figure 2a).

Hypertension in combination with elevated plasma renin concentrations suggests abnormalities in the negative feedback control of renin secretion by the blood pressure. We therefore analyzed the pressure control of renin secretion from isolated kidneys of tamoxifen-treated $Cx40^{fl/fl}Cre^{ER}$ mice and from tamoxifen-treated $Cx40^{fl/fl}$ control mice. In kidneys from $Cx40^{fl/fl}$ control mice, renin secretion was inversely related to the perfusion pressure as normal, whereas in kidneys from $Cx40^{fl/fl}Cre^{ER}$ mice renin secretion showed no dependency on the perfusion pressure (Figure 3a). As the normal pressure control of renin secretion is a calcium-sensitive process,[10] we next considered the calcium sensitivity of renin secretion in isolated kidneys of tamoxifen-treated $Cx40^{fl/fl}$ control mice and of tamoxifen-treated $Cx40^{fl/fl}Cre^{ER}$ mice. In kidneys of $Cx40^{fl/fl}$ control mice, renin secretion is stimulated by the β-adrenoreceptor activator isoproterenol, and this stimulation of renin secretion is strongly enhanced by lowering the extracellular concentration of calcium owing to the addition of the calcium chelator ethylene glycol-bis (aminoethyl ether) tetraacetic acid (EGTA) to the perfusate. In kidneys of $Cx40^{fl/fl}Cre^{ER}$ mice, isoproterenol induced a similar stimulation of renin secretion as in kidneys of $Cx40^{fl/fl}$ control mice, but further addition of EGTA to the perfusate produced no additional effect on renin secretion (Figure 3b).

The abnormalities of renin secretion in tamoxifen-treated $Cx40^{fl/fl}Cre^{ER}$ mice prompted us to study the morphology of renin-secreting cells in more detail. In kidneys of tamoxifen-treated $Cx40^{fl/fl}$ control mice, renin immunoreactive cells were restricted to the media layer of afferent arterioles at the juxtaglomerular junction sites of afferent arterioles and glomerular capillaries as normal (Figure 1b). In kidneys of tamoxifen-treated $Cx40^{fl/fl}Cre^{ER}$ mice, a more variable localization pattern of renin immunoreactive cells emerged, which could be described as a gradual shift of renin expression from the media layer of afferent arterioles into the extraglomerular mesangial (lacis) cell field accompanied with a local increase in the number of ectopic renin immunoreactive cells (Figures 1c and 4). The development of such larger ectopic renin cell fields increased with the age of the animals. In the older tamoxifen-pretreated $Cx40^{fl/fl}Cre^{ER}$ mice (24 weeks), about 15% of glomeruli showed extended ectopic renin cell fields, as illustrated in Figure 4b and c, whereas 30% of glomeruli displayed no renin immunoreactive cells.

DISCUSSION

A number of previous studies have shown that genetically fixed loss of functions of Cx40 in renin-secreting cells of the kidneys cause major functional and structural alterations of the cells.[2–4,7,20] Most prominent is an interruption of the