Control of renin secretion from kidneys with renin cell hyperplasia

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Kurt B, Karger C, Wagner C, Kurtz A. Control of renin secretion from kidneys with renin cell hyperplasia. Am J Physiol Renal Physiol 306: F327–F332, 2014. First published November 27, 2013; doi:10.1152/ajprenal.00536.2013.—In states of loss-of-function mutations of the renin-angiotensin-aldosterone system, kidneys develop a strong hyperplasia of renin-producing cells. Those additional renin cells are located outside the classic juxtaglomerular areas, mainly in the walls of preglomerular vessels and most prominently in multilayers surrounding afferent arterioles. Since the functional behavior of those ectopic renin cells is yet unknown, we aimed to characterize the control of renin secretion from kidneys with renin cell hyperplasia. As a model, we used kidneys from mice lacking aldosterone synthase (AS−/− mice), which displayed 10-fold elevations of renin mRNA and plasma renin concentrations. On the absolute level, renin secretion from isolated AS−/− kidneys was more than 10-fold increased over wild-type kidneys. On the relative level, the stimulation of renin secretion by the β-adrenergic activator isoproterenol or by lowering of the concentration of extracellular Ca2+ was very similar between the two genotypes. In addition, the inhibitory effects of ANG II and of perfusion pressure were similar between the two genotypes. Deletion of connexin40 blunted the pressure dependency of renin secretion and the stimulatory effect of low extracellular Ca2+ on renin secretion in the same manner in kidneys of AS−/− mice as in wild-type mice. Our findings suggest a high degree of functional similarity between renin cells originating during development and located at different positions in the adult kidney. They also suggest a high similarity in the expression of membrane proteins relevant for the control of renin secretion, such as β1-adrenergic receptors, ANG II type 1 receptors, and connexin40.

aldosterone synthase knockout; isolated perfused kidney; renin cell hyperplasia; renin secretion

RENIN-EXPRESSION CELLS during kidney development appear in a characteristic temporal and spatial pattern. First, they appear as mural cells of the developing preglomerular vascular tree. With ongoing maturation of vessel segments, renin-expressing cells are visually replaced by smooth muscle-like cells not expressing renin (24). Along with the formation of glomeruli renin cells appear at the junction site between afferent arterioles and glomeruli (so-called juxtaglomerular cells), which maintain production and secretion in the adult kidney (8). These cells are few in number and contain a great number of renin storage vesicles, leading to the cuboid appearance of the cells (8). Renin secretion from these cells occurs via compound exocytosis (27). It is physiologically mainly regulated via β1-adrenergic and ANG II type 1 (AT1) receptors and by the perfusion pressure in preglomerular arterioles (5, 14), thus managing a controlled activity of the renin-angiotensin-aldosterone system (RAAS) in the sense of a negative feedback. Renin secretion from cells displays a peculiarity in the way that decreasing the availability of Ca2+ enhances rather than impedes secretion (1, 19). In addition, a yet unexplained function of the gap junction protein connexin (Cx)40 is essential for the proper control of renin secretion from juxtaglomerular cells (13, 32).

In states of a genetically defective RAAS, many more renin cells than normal develop, and this renin cell hyperplasia is maintained in the adult kidney (6, 7, 9, 17, 22, 28, 29). In these kidneys, renin cells are not only located in the juxtaglomerular area, as typical, but also in the media layer of preglomerular arterioles and most prominently in cellular multilayers around afferent arterioles (7, 9, 17, 22, 28, 29). This is in contrast to normal adult mice with pharmaceutical inhibition of the RAAS, which leads to reversible expression of renin in smooth muscle cells of preglomerular vessels but not to perivascular multilayers, suggesting a developmental component of renin cell hyperplasia.

Those additional cells in states of renin cell hyperplasia also develop renin storage granules similar to those seen in typical juxtaglomerular cells (22). There is indirect evidence showing that at least newly recruited renin cells in the adult kidney may control renin secretion as juxtaglomerular cells do (23). However, if renin secretion from hyperplastic renin cells is regulated in a similar fashion as in juxtaglomerular cells is yet unknown.

To address the issue of control of renin secretion from hyperplastic renin cells, we studied renin secretion from kidneys isolated from mice lacking aldosterone synthase (AS). These kidneys contain a strongly increased number of renin-producing cells, as determined by immunohistochemistry (10, 17, 20, 21) and also as reflected by highly elevated levels of renin mRNA (20, 21). Accordingly, in addition, the secretion of renin into the circulation is enhanced in these kidneys, as indicated by the marked increased plasma renin concentration in aldosterone-deficient mice (20, 21). We chose AS-deficient (AS−/−) mice as a model since these mice have an intact machinery to generate ANG II, which is lacking in renin-, angiotensinogen-, or angiotensin-converting enzyme-deficient mice, and have intact AT1 receptors, which are lacking in AT1-deficient mice. Moreover, the structural deterioration of the kidney is much less in AS−/− kidneys than in any other of the afore-mentioned RAAS-deficient mice.

A comparison of renin secretion from kidneys isolated from wild-type (WT) and AS−/− mice revealed a 10-fold difference in absolute renin secretion but no clear difference in the regulation of secretion by the parameters mentioned above. These findings indicate that hyperplastic renin cells behave very similarly to classic juxtaglomerular cells.

MATERIALS AND METHODS

Mice. Experiments were performed with inbred 129/SvEv genetic background (17, 21) WT AS and AS−/− female mice at an age of 8 wk. This mouse model was generously provided by Prof. O. Smithies
Kidneys of AS−/− mice had 10-fold higher levels of renin mRNA (Fig. 1, top), which was reflected by a marked increase in the number of renin-expressing cells (Fig. 2). Renin cells were not only located in the classical juxtaglomerular position but were also seen in the vessel walls of afferent arterioles and in cuff-like structures surrounding afferent arterioles. Cells contained numerous electron-dense vesicles, assumed to be renin storage vesicles (Fig. 2C). Plasma renin concentrations, as an indicator of in vivo renin secretion, were under basal conditions about 10-fold higher in AS−/− mice compared with WT mice (Fig. 1, bottom).

RESULTS

Kidneys of AS−/− mice were perfused ex situ with mod-
ified Krebs-Renseleit solution supplemented with 6 g/100 ml BSA and human red blood cells (10% hematocrit) at a constant pressure of 90 mmHg. Using an electronic feedback control, perfusion pressure could be changed stepwise in a pressure range between 40 and 140 mmHg to assess the pressure control of renin secretion. Renin secretion was further modulated by infusing stock solutions of isoproterenol, ANG II, or EGTA into the arterial limb of the perfusion system. For the determination of renin secretion rates, three samples of the venous effluent were taken every 2 min. Renin activity in the venous effluent was measured by incubating the perfusate with plasma from bilaterally nephrectomized male rats as a renin substrate for 1.5 h at 37°C. The produced ANG I (in ng·ml−1·h−1) was determined by radioimmunoassay (RENCSTK, DiaSorin). The renin secretion rate was determined as perfusate flow (in ml·g organ weight−1·min−1) multiplied by renin activity (in ng ANG I·ml−1·h−1) of the perfusate.

Statistics. Values are denoted as means ± SEM. Differences within the same kidneys were analyzed by Student’s paired t-test. Differences between experimental groups were analyzed by ANOVA and Bonferroni’s adjustment for multiple comparisons. P values of <0.05 were considered statistically significant.
In line, basal renin secretion rates from isolated kidneys perfused under standard conditions at a constant pressure of 90 mmHg were about sixfold higher in AS /-/- mice compared with WT mice (321 ± 35 ng ANG I·h⁻¹·min⁻¹·g⁻¹ in AS /-/- mice and 54 ± 5 ng ANG I·h⁻¹·min⁻¹·g⁻¹ in WT, means ± SE of 10 mice each, P < 0.05). Renin secretion was stimulated by the β-adrenoreceptor ligand isoproterenol in a concentration-dependent fashion in kidneys from both WT and AS /-/- mice (Fig. 3, top). At any concentration of isoproterenol, renin secretion rates in AS /-/- kidneys were about 10-fold higher than in WT kidneys (Fig. 3, top). The stimulatory effect of isoproterenol in kidneys of both genotypes was clearly further enhanced by a factor of two to three when the Ca²⁺ concentration in the perfusate was lowered into the submicromolar range by the addition of 3.1 mmol/l EGTA (Fig. 3, bottom).

The stimulatory effect of isoproterenol (10 nmol/l) was reversed by ANG II in a concentration-dependent fashion with IC₅₀ values of about 50 pmol/l in kidneys from both genotypes (Fig. 4). Perfusion flow rates (at a constant pressure of 90 mmHg) in the presence of isoproterenol (10 nmol/l) and absence of ANG II were about 20% higher in AS /-/- kidneys compared with WT kidneys (Fig. 4, bottom). In the presence of ANG II, perfuse flow rates concentration dependently decreased to similar values in kidneys from the two genotypes.

Renin secretion in isolated perfused WT kidneys is typically inversely related to the perfusion pressure of the kidneys (25), as also found in this study (Fig. 5, top). The pressure-secretion relationship was clearly shifted to higher pressure values in AS /-/- kidneys compared with WT kidneys. Pressure-dependant flow rates were up to 40% higher in AS /-/- kidneys compared with WT kidneys in this experimental setting (3 nmol/l isoproterenol; Fig. 5, bottom).

It has been previously shown that an intact function of Cx40 in renin-secreting cells is important for the control of renin secretion by perfusion pressure (31). In line, we confirmed that the characteristic inverse relationship between perfusion pressure and renin secretion was absent in Cx40 /-/- kidneys (Fig. 6, top). We therefore wondered if hyperplastic renin cells express Cx40 like juxtaglomerular cells do and, if so, if Cx40 is of relevance for the control of renin secretion also in these cells. It turned out that ectopic hyperplastic renin cells also express Cx40 (Fig. 7) and that this Cx subtype is also of relevance for the control of renin secretion also in these cells. It was shown that in kidneys lacking both AS and Cx40, the relationship between perfusion pressure and renin secretion as determined in AS /-/- kidneys (Fig. 5, top) was blunted in the absence of Cx40 (Fig. 6, top). In addition, lack of Cx40 prevented the enhancement of renin secretion by the Ca²⁺ chelator EGTA.

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DISCUSSION

Our measurements of about 10-fold increases of renin mRNA levels and a greatly increased number of renin-expressing cells in the kidneys of aldosterone-deficient mice is in accordance with previous reports (10, 17, 20). An ectopic localization of additional renin-expressing cells outside the juxtaglomerular apparatus in the media layer of preglomerular vessels or in multilayers surrounding afferent arterioles has also been previously described in mice lacking angiotensin-converting enzyme (9) or AT1A receptors (22). In accordance with those studies, we found that the ultrastructure of those ectopic renin-expressing cells was similar to those of typical juxtaglomerular cells, which are characterized by numerous electron-dense renin storage vesicles (8). In juxtaglomerular cells, renin is released from these vesicles by compound exocytosis (27) triggered by the cAMP signaling pathway (5, 14), which is further enhanced by lowering of the extracellular concentration of Ca\(^{2+}\) (1, 14, 19). Renin secretion from AS\(^{-/-}\) and WT kidneys was dose dependently stimulated by isoproterenol, which activates the cAMP pathway in juxtaglomerular cells by binding to β₁-adrenergic receptors (8). Renin secretion rates elicited by isoproterenol from isolated kidneys of AS\(^{-/-}\) mice were about 10-fold higher than those from WT kidneys, in kidneys of mice with either intact or deleted AS (Fig. 6, bottom).

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Discussion

Fig. 3. Effects of increasing concentrations of isoproterenol on renin secretion from kidneys isolated from AS\(^{-/-}\) and WT mice (top). Data are means ± SE of 5 kidneys/genotype. *P < 0.05 by ANOVA. At the end of the dose-response experiments with isoproterenol (10 nmol/l), EGTA at a final concentration of 3.1 mmol/l was added to the perfusate. Bottom: renin secretion rates before the start of dose-response experiments (co), during isoproterenol (10 nmol/l) alone, and in the presence of both isoproterenol and EGTA. Data are means ± SE of 5 kidneys/genotype. *P < 0.05 by paired t-test.

Fig. 4. Effects of graded concentrations of ANG II on renin secretion (top) and on perfusate flow (bottom) at a constant pressure of 90 mmHg in the continuous presence of isoproterenol (10 nmol/l) in kidneys isolated from AS\(^{-/-}\) and WT mice. Data are means ± SE of 5 kidneys/genotype. *P < 0.05 by paired t-test.

Fig. 5. Effect of perfusion pressure on renin secretion (top) and on perfusate flow (bottom) in kidneys from AS\(^{-/-}\) and WT mice. In the presence of isoproterenol (3 nmol/l), isolated kidneys were initially perfused at a constant pressure of 90 mmHg. Pressure was then reduced to 40 mmHg and then subsequently increased to 140 mmHg in 25-mmHg steps. Each pressure level lasted for 5 min. Data are means ± SE of 5 mice/genotype. *P < 0.05 by paired t-test.
which corresponds well with the 10-fold higher renin mRNA levels and 10-fold higher plasma renin concentrations in AS\(^{-/-}\) mice. Although we could not measure renin secretion from individual renin cells, as it was previously done by Rasch and coworkers (23), we infer from the similarity of the in vivo and in vitro data that the higher values of renin secretion rates from kidneys of AS\(^{-/-}\) mice resulted from increased levels of renin gene expression paralleled by higher numbers of renin-expressing cells. Previously, we (18) have provided evidence showing that changes of renin mRNA levels in the kidney are, in fact, closely paralleled by the number of renin-expressing cells.

If so, then our results would suggest that renin secretion from ectopic renin cells is also stimulated by the cAMP signaling pathway and enhanced by low extracellular Ca\(^{2+}\) like in the juxtaglomerular cell. Moreover, the response to isoproterenol further suggests that ectopic renin cells are also equipped with \(\beta_1\)-adrenergic receptors. Similar conclusions hold for the well-known inhibitory action of ANG II on renin secretion. ANG II strongly inhibited renin secretion from WT and AS\(^{-/-}\) kidneys with very similar IC\(_{50}\) values, suggesting that the inhibitory pathway of renin secretion acts very similarly in juxtaglomerular and ectopic renin cells. In addition, ectopic renin cells appear to be equipped with AT\(_1\) receptors.

Together with \(\beta_1\)-adrenergic receptors and AT\(_1\) receptors, Cx40 is also expressed by ectopic renin cells. Cx40 is a strong characteristic for renin-expressing cells and distinguishes them from vascular smooth muscle cells in afferent arterioles (15).

Renin secretion from AS\(^{-/-}\) kidneys is also controlled by the baroreceptor mechanism, which establishes a reciprocal relationship between renal perfusion pressure and renin secretion (4, 11). This observation suggests that the yet unknown mechanisms that couple renin secretion to perfusion pressure are also active in ectopic renin cells, at least in principle. This conclusion is supported by the finding that inactivation of Cx40 blunted the influence of pressure on renin secretion also in AS\(^{-/-}\) mice, as has been previously reported for other mouse genotypes (32).

Compared with WT kidneys, however, the pressure-secretion relationship was shifted to higher pressure values. The working mode of this so-called baroreceptor mechanism, along with how increasing perfusion pressure inhibits renin secretion, is not well understood. It may involve vascular stretch (30) linked to Ca\(^{2+}\) influx (16) and inhibition of renin secretion. It could eventually also involve a contribution of the macula densa in a way that increasing pressure could increase glomer-
ular filtration rate and thus activate the macula densa mechanism causing inhibition of renin secretion (2, 26). Even without knowing the detailed working mechanism of the baroreceptor, we can speculate about possible explanations about the rightward shift of the pressure-secretion curve. One would be that preglomerular arteries in AS -/- kidneys have a higher resistance than that in WT kidneys, leading to lower pressure values inafferent arterioles. This explanation, however, is not supported by functional data in AS -/- mice (20) and by the increased perfusate flow rates measured in isolated AS -/- kidneys. Another speculation, therefore, could be that the multilayers of renin cells dampen the spreading of the initial signals arising from the intraluminal pressure and/or from the macula densa.

In summary, our findings suggest that renin cells that develop by renin cell hyperplasia are functionally very similar to juxtamelaglomerular cells, which are considered as the classic renin producers in the normal organism.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.K., C.W., and A.K. conception and design of research; B.K. and C.K. performed experiments; B.K., C.K., C.W., and A.K. approved final version of manuscript; C.W. and A.K. edited and revised manuscript; A.K. analyzed data; A.K. interpreted results of experiments.

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