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Ontogeny of renin gene expression in the chicken, *Gallus gallus*

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Abstract

Renin or a renin-like enzyme evolved in ancestral vertebrates and is conserved along the vertebrate phylogeny. The ontogenic development of renin, however, is not well understood in nonmammalian vertebrates. We aimed to determine the expression patterns and relative abundance of renin mRNA in pre- and postnatal chickens (*Gallus gallus*, White Leghorn breed). Embryonic day 13 (E13) embryos show renal tubules, undifferentiated mesenchymal structures, and a small number of developing glomeruli. Maturing glomeruli are seen in post-hatch day 4 (D4) and day 30 (D30) kidneys, indicating that nephrogenic activity still exists in kidneys of 4-week-old chickens. In E13 embryos, renin mRNA measured by quantitative polymerase chain reaction in the adrenal glands is equivalent to the expression in the kidneys, whereas in post-hatch D4 and D30 maturing chicks, renal renin expressions increased 2-fold and 11-fold, respectively. In contrast, relative renin expression in the adrenals became lower than in the kidneys. Furthermore, renin expression is clearly visible by in situ hybridization in the juxtaglomerular (JG) area in D4 and D30 chicks, but not in E13 embryos. The results suggest that in chickens, renin evolved in both renal and extrarenal organs at an early stage of ontogeny and, with maturation, became localized to the JG area. Clear JG structures are not morphologically detectable in E13 embryos, but are visible in 30-day-old chicks, supporting this concept.

Keywords

Renin ontogeny; renin gene expression; renin-angiotensin system; nephrogenesis; juxtaglomerular apparatus; chicken embryo

1. Introduction

Renin is a hormone and substrate-dependent enzyme secreted mostly by the juxtaglomerular (JG) cells of the afferent arteriole of the kidney located at the vascular pole of the glomerulus. Renin acts on renin substrates (angiotensinogen, Agt) and forms angiotensin (Ang) I which is subsequently converted to Ang II by Ang converting enzyme (ACE). The renin-angiotensin system (RAS) is crucial for the regulation of blood pressure and fluid

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Conflict of Interest

The authors declare that no conflict of interest exists.

electrolyte homeostasis. In addition, the RAS has a role in erythropoiesis, hematopoiesis, and vascular development of the kidney (Savary et al., 2005; Sequeira-Lopez et al., 2015; Gomez and Sequeira-Lopez, 2016). The renin gene is initially expressed in renal arteries and arterioles but is concentrated in the JG area with maturation (Gomez et al., 1989). Renin or a renin-like enzyme evolved in ancestral vertebrates (Nishimura et al., 1973; Takei et al., 1993; Fournier et al., 2012) and is conserved throughout the vertebrate phylogeny (for review, see Nishimura, 2017). The renin gene and/or protein has been characterized in several species of teleost fishes (Liang et al., 2004) and mammals (Prokop et al., 2015). Also, complete genomic information, including the sequence of the renin gene, is available for the chicken, *Gallus gallus* (ENSGAL00000000545) (Wallis et al., 2004). In rodents and zebrafish, renin and other components of the RAS are expressed early in embryonic development of the kidney as well as in extrarenal tissues (Rider et al., 2015; Gomez and Sequeira-Lopez, 2018).

While the morphology of various developmental stages of chick kidneys is fairly well understood (Davey and Tickle, 2007), the expression pattern and possible roles of renin and renin-producing cells throughout development remain to be elucidated. We therefore aimed to determine the expression pattern and relative abundance of renin mRNA in renal and extrarenal tissues from pre- and postnatal chickens, *Gallus gallus*. We hypothesize that in chickens, renal and extrarenal renin evolve at early embryonic periods.

2. Materials and Methods

2.1. Animals

Fertilized eggs of *Gallus gallus*, White Leghorn breed, Hy-line W36, were purchased from a commercial hatchery (Hy-line North America; Warren, IN, USA) and were incubated at 37.5°C and 60% humidity with periodic rotation. Hatched birds were kept at 37°C for the first three days and then gradually moved to 25°C temperature-controlled brooders and group pens. Commercial chow and tap water were given ad libitum. The present study includes both males and females. Embryonic day 13 (E13) embryos (body weight, BW: 5.55 ± 0.4 g, $n = 5$), newborn chicks (4 days after birth, D4; BW: 36.8 ± 2.7 g, $n = 4$), and maturing chicks (30 days after birth, D30; BW: 277.3 ± 14.0 g, $n = 3$) were used. We selected E13 embryos because the metanephric kidney appears then (Davey and Tickle, 2007). Also, the kidney and adrenal gland can be separately collected at E13. Post-hatch day 4 (D4) newborns were chosen because adaptation to the new environment requires a number of functional and hormonal changes. In chickens, nephrogenesis continues after birth; by four weeks of age, although some maturing nephrons still remain near superficial (cortical) regions, most organogenesis has been completed in chickens.

Partial or whole left-side kidneys were used for morphological studies (fixed in Bouin's fixative or in 4% paraformaldehyde [PFA]), and the rest were snap-frozen for biochemical measurements. In D4 and D30 chicks all measurements (morphology, in situ hybridization [ISH], quantitative polymerase chain reaction [qPCR], reverse transcription polymerase chain reaction [RT-PCR]) were determined in the same individual birds. E13 embryo kidneys are small (40–96 mg), so different embryos were used for different measurements. However, the kidney, liver and adrenal from the same embryo were always used.

All animals were handled in accordance with the National Institutes of Health guidelines for the care and use of experimental animals, and the protocol pursued in this study (UVA 1835) was approved by the Institutional Animal Care and Use Committee of the University of Virginia.

2.2. Primers

Primers were designed using the National Center for Biotechnology Information (NCBI) PrimerBlast Tool. The following primers were used for both RT-PCR and qPCR: renin forward 5'-TACTACAGCCGGAAGTCTCC-3' and renin reverse 5'-TGATCTGCCAGTACCCACTC-3'; GAPDH forward 5'-GAAAGTCATCCCTGAGCTGAA-3' and GAPDH reverse 5'-GTCAGGTCAACAACAGAGACA-3'. The primers used to generate riboprobes for ISH [section 2.6] were as follows: renin forward 5'-TAATACGACTCACTATAGGGAGAGAGAGTTCCGGCTGTA-3' and renin reverse 5'-AATTAACCCTCACTAAAGGGGCCAATGGTACAGGCTTTGC-3'.

2.3. RNA riboprobe generation

The riboprobe primers detailed above [section 2.2] were amplified using wild type embryonic chick cDNA with polymerase chain reaction (PCR) conditions of 95°C, 60°C, and 72°C for 40 cycles. RNA was then synthesized from the PCR product via in vitro transcription and purified using Micro Bio Spin Columns (Bio-Rad; Hercules, CA, USA) according to the manufacturer's instructions. The purified product was run on a 1% agarose gel and confirmed by Sanger Sequencing.

2.4. RNA extraction and quantitative PCR (qPCR) analysis

Kidney, liver, and adrenal were removed and placed in *RNAlater* (Ambion; Austin, TX, USA) overnight at 4°C and then stored at -20°C. Total RNA was extracted and qPCR was performed as we previously described (Belyea et al., 2015). cDNA was prepared from 3 µg RNA for kidneys and livers and 1.5 µg RNA for adrenals. PCR conditions were run at 95°C, 55.3°C, and 72°C for 40 cycles. Technical and biological triplicates were used, and all samples were standardized relative to values from D30 kidneys. Renin mRNA expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression; changes in expression were determined using the Ct method. Expression was shown relative to a D30 kidney (average, 1.105, n = 3).

2.5. RNA reverse transcription PCR (RT-PCR)

To confirm qPCR results, RT-PCR was performed. PCR conditions were run at 95°C, 55°C, and 72°C for 40 cycles. Aliquots (10 µl) of the PCR product were electrophoresed on a 1% agarose gel. GAPDH was included as the comparison housekeeping gene. Sequencing confirmed that the bands amplified corresponded to authentic renin mRNA.

2.6. In situ hybridization (ISH)

Kidneys, livers, and adrenals were removed and immediately fixed in 4% PFA at 4°C for 24 hours, and embedded in paraffin. ISH was performed on 7-µm-thick sections as we

previously described (Medrano et al., 2014). Hybridization was conducted at 50°C for 18 hours using 500 ng/mL digoxigenin (DIG)-labeled riboprobes as detailed above (section 2.2.) in hybridization solution. Sites of hybridization were detected using alkaline phosphatase (AP)-conjugated DIG antibody (Roche Diagnostics Corp.; Indianapolis, IN, USA) at a 1:4000 dilution followed by BM Purple AP substrate color development (Roche; Basel, Switzerland). Sections were post-fixed in 4% PFA/0.2% glutaraldehyde and counterstained with hematoxylin and eosin (H&E) for better visualization. Negative controls were performed by omitting the probe in the hybridization step and with the use of a sense riboprobe that was not complementary to the sequence of interest.

2.7. Histology

Kidneys, livers, and adrenals were removed from chick embryos at 13 days of gestation, E13 (n = 3), and postnatal days D4 (n = 3) and D30 (n = 3), then fixed overnight in Bouin's fixative at room temperature and embedded in paraffin. Sections (5 μ m thick) were deparaffinized and stained with H&E to examine overall morphology. Alpha-smooth muscle actin (α -SMA) immunohistochemistry was performed using an anti α -SMA antibody raised against mouse clone IA4 (Sigma; St. Louis, MO, USA). This α -SMA antibody specifically binds to α -SMA1 from human, mouse, rat, bovine, chicken, frog, goat, guinea pig, rabbit, dog, sheep, and snake.

2.8. Statistical analysis

Results were expressed as means $\pm$ SD. Significance was determined by Student's *t*-test (two-tailed). The difference was considered significant at a *P* value of < 0.05 .

3. Results

3.1. Histology of JG apparatus (JGA; Fig. 1)

In E13 kidneys (Fig. 1A), unidentified immature structures, some developing glomeruli, possibly mesonephric kidney, and renal tubules are seen. Dense podocytes are lined on the surface of the glomerular tuft. The structures of afferent and efferent arterioles are recognized by α -SMA staining but are not well distinguished in H&E staining. In newborn chick (D4) kidney (Fig. 1B), renal tubules and developing glomeruli, such as S-shaped bodies (not shown), are seen. A short arteriole abruptly derived from arteries (arrow) drains into the glomerulus. A distal tubule is seen adjacent to the glomerulus, but the vascular pole demonstrates no characteristics of a fully developed JGA. In D30 kidneys (Fig. 1C), more mature tubules and glomeruli are seen. In superficial regions, however, developing glomeruli are seen, indicating that nephrogenesis is still ongoing. In both H&E and α -SMA staining (not shown) longer afferent and efferent arterioles connect to the vascular pole where the renal tubule (presumably distal; Fig. 1C, arrow) is attached, forming the JGA. Characteristics of macula densa cells, however, were not noted in D30 kidneys.

3.2. Renin gene expressions detected by ISH (Fig. 2)

E13 embryos (n = 3) were examined for renin ISH signals in the kidney, liver, and adrenal. Renin gene expression was not seen in the kidney (Fig. 2A), liver, or adrenals (not shown) from E13 embryos. In D4 newborn chicks (n = 3), in two of three chicks, renin gene

expression signals were identified by ISH adjacent to glomeruli (Fig. 2B arrows, 2C, 2D), but not in the liver or adrenals (not shown) examined in the same D4 chicks. ISH signals in the JGA appeared to connect to the signals in the arteriole (Fig. 2C, 2D). On D30, renin gene expression detected by ISH was clearly seen adjacent to glomeruli ($n = 3$) (Fig. 2E, 2F, arrows), but not in the liver or adrenals (not shown). In the kidney, expression was most clearly seen slightly away from the surface of the kidney where glomeruli are usually aligned. While the majority of signals were seen on or near the vascular pole of the glomerulus, a small number of signals were also seen in renal tubules.

3.3. Relative renin gene expression levels detected by qPCR (Fig. 3)

Renin mRNA detected by qPCR demonstrates that at E13, renin gene expression in the adrenal glands was comparable to that in the kidney, whereas expression in the liver was minimal (Fig. 3A). By D4, the renin gene expression in the kidney had doubled, whereas expression in the adrenals relative to the kidney had decreased (Fig. 3B, 3D). By D30, renin gene expression in the kidney was 11 times higher than that in the embryonic kidney, whereas expressions in the D30 liver and adrenal were lower than in the D30 kidney (Fig. 3C, 3D).

3.4. Renin gene expression detected by RT-PCR (Fig. 4)

In kidneys from E13 embryos ($n = 3$) and in kidneys from D4 ($n = 3$) and D30 chicks ($n = 3$), the RT-PCR products, which reflect renin mRNA, show bands in the kidney, liver, and adrenal. D4 chicks that show clearly positive RT-PCR products (#1 and #3) agree with those that show clear positive kidney ISH signals. In embryonic and D30 kidneys, although the levels varied somewhat, the presence of RT-PCR bands in the expected regions (124 bp) confirmed the results of qPCR. The bands were confirmed to be authentic renin by sequencing.

4. Discussion

4.1 Renin gene expressions (Table 1)

While renin gene expression and abundance in the kidney during development and after birth have been characterized in zebrafish (Rider et al., 2015) and in rodents (Gomez, 1998; Gomez and Sequeira-Lopez, 2018), as well as in humans (Schutz et al., 1996), the ontogeny of renin in birds is not well understood. New findings of the present study are that in E13 chicken embryos, renin mRNA is clearly detected in the kidney, adrenal gland, and liver by qPCR, although the levels were low. In E13 embryo kidneys, no renin signals were seen by ISH, suggesting that individual cells near the vascular pole of the glomerulus may not express renin in an amount sufficient to be detected by ISH. Renin may also be expressed in other renal tissues such as pericytes and renal tubules. Hence, the expression was detectable by qPCR and RT-PCR for which the homogenate of whole kidney was used. In newborn (4 days of age) chicks, short afferent arterioles, abruptly derived from arteries, enter the vascular pole of the glomerulus. Renin mRNA was detected in the developing JG areas by ISH and by RT-PCR in the same two out of three birds. This agrees with the finding that renin expression levels determined by qPCR were doubled from those at E13. At D30, more distinct afferent and efferent arterioles connect to the JG area, and renin mRNA was detected

clearly at the vascular pole by ISH in all three birds examined. Renin gene expression (qPCR) in the kidney was 11 times higher than that at E13. This agrees with the findings that in mature chickens, plasma (Nishimura et al., 1981) and kidney renin activities and Bowie-staining positive granules (Sokabe et al., 1969) are seen on the afferent arteries and arterioles of the kidney. Immunoreactive renin using anti-mouse renin was also noted in the glomerular mesangial regions along the glomerular capillaries of the chicken kidney (Kon et al., 1986). The specificity of the renin antibody, however, was not clear.

In mice, *Foxd1*⁺ progenitor cells and renin precursor cells appear at E11.5-E12 in stroma-mesenchymal tissues, whereas renin protein can be seen at E14.5 (Sequeira-Lopez and Gomez, 2011). The number of renin cells in the embryo are initially very few, depending on the age examined; hence renin levels detected by PCR may be low. However, in the last days of gestation in the rat (fetal age 20), the mRNA signals (assessed by Northern blots) are higher than in newborns, which are higher than in the adult (90 days) (Gomez et al., 1989). Renin-expressing cells and other components of the RAS were observed along the renal arteries and arterioles, and within the glomeruli (Harris and Gomez, 1997; Sequeira-Lopez and Gomez, 2004). As the renal arterial tree develops, renin mRNA-containing cells are localized progressively to more distal blood vessels and finally to the JGA (Sequeira-Lopez and Gomez, 2004, 2011). While some of the renin cells retain renin gene expression, others differentiate into smooth muscle cells, epithelial cells, and mesangial cells (Sequeira-Lopez et al., 2015; Gomez and Sequeira-Lopez, 2018).

The piscine renin gene was characterized in zebrafish and fugu (Liang et al., 2004). In zebrafish, their transparency, early organogenesis, and ex utero development allow investigation (Hwang and Chou, 2013) including that of the RAS (Hoffmann et al., 2018). In zebrafish embryos, the pronephron is functioning from 76 hours postfertilization (76 hpf). Renin gene expression is noted as early as 48 hpf within the location of the anterior mesenteric artery (Rider et al., 2015, 2017). In zebrafish, the final functioning nephron is the mesonephros.

Renin expression is high in rats toward the end of gestation and in newborns but decreases after maturation, whereas renin gene expression continues to increase in chickens and zebrafish after hatch and with maturation. Taken together, the available information indicates that in rodents, chickens, and zebrafish, renin gene expression evolves early in ontogeny (Table 1). Since the time scale of nephron development differs among zebrafish, chickens, and rodents, however, the comparison of renin expressions among these species at the comparable ontogenic stages of these species is difficult.

4.2 Extrarenal renin gene expressions (Table 1)

In chickens, we noted renin gene expression in the liver and adrenals during development. At E13, the renin mRNA level in the adrenal is nearly the same as that of the kidney. Renin mRNA was detected during blood island differentiation of the chicken yolk sac mesoderm (Savary et al., 2005). These findings are consistent with our hypothesis that renal and extrarenal renin are expressed early in embryonic development and that the expression becomes more specific to the JG area with maturation. This finding in chickens is the same as that in mice in which renin gene expression in the adrenal glands is observed as early as

E13.5 but is no longer detectable after birth (Gomez and Sequeira-Lopez, 2018; Sequeira-Lopez et al., unpublished observation). It thus appears that the ontogenetic expression of renin is not limited to the kidney; rather, renin widely evolved in extrarenal organs, including the adrenals and liver, in which the role of renin may be different from that observed in later life. Cells from the bone marrow and spleen contain pre-B lymphocytes that express renin; renin cells decrease in number as development proceeds (Gomez and Sequeira-Lopez, 2016).

Fetal adrenal function in birds has not been studied. We also noted considerable renin expression in embryonic chicken liver. Although the function of the hepatic renin is currently unclear in birds, it is presumably related to hematopoietic action of the RAS (Harvey, 2012).

Maturation of the kidneys is apparently slower in fowl than in rodents, and nephrogenesis continues at four weeks after hatching. It is thus not surprising that renin expression remains in the adrenals and liver in D4 newborn and D30 maturing chickens. The early appearance of renin expression has been reported in zebrafish aorta and anterior mesenteric arteries (Rider et al., 2015, 2017).

4.3 Regulation of renin expression and function of the renin-angiotensin system during development (Table 1)

In rodents, the RAS is essential for JG granulation, vascular and nephron development (Tufro-McReddle et al., 1995; Clark et al., 1997; Gomez, 1998; Yosypiv, 2008; Sequeira-Lopez and Gomez, 2004; Gomez and Sequeira-Lopez, 2018). Blockade of the RAS by ACE inhibition in adult rats increased kidney renin gene expression by increasing the renin mRNA level of the JGA as well as increasing the number of JGAs that express the renin gene (Gomez et al., 1990). Blockade of AT1, but not AT2, inhibited nephrovascular maturation (Tufro-McReddle et al., 1995) in newborn rats and metamorphosis and glomerular development in frog tadpoles (Gomez, 1988; Tufro-McReddle et al., 1995) possibly via tyrosine phosphorylation of the epidermal growth factor receptor (Yosypiv, 2008). Likewise, ACE inhibition decreased renal vascular resistance in sheep fetuses (Robillard et al., 1983), suggesting that the RAS may have physiological significance during maturation. Vascular smooth muscle cells can be recruited to re-express renin when homeostasis is threatened (Gomez and Sequeira-Lopez, 2016).

In chicken embryos, the angiogenic effect of Ang II was shown in the chorioallantoic membrane (Le Noble et al., 1991). Renin mRNA, as well as mRNAs encoding ACE, Agt, and Ang II receptor were present in the chicken yolk sac (Savary et al., 2005). Furthermore, during the first 56 hrs of development, ACE activity is inhibitable by a ACE inhibitor is detected in blastodisc (embryo plus yolk sac) extracts (Savary et al., 2005). In conscious young mature chickens, treatment with ACE inhibitor raised PRA, but had no effect on blood pressure (Kamimura et al., 1995). The role of the RAS in vascular and renal development, however, remains to be examined in chickens.

The molecular structure of chicken type 1 Ang receptor (cAT₁R) has been identified (Kempf et al., 1996). The fact that cAT₁R mRNA was markedly seen by ISH in the glomeruli (most

likely mesangium) on E9 (Nishimura et al., 2003) as well as in mouse embryos (E14) suggests that enin or the RAS has a role in early kidney development (Norwood et al., 1997). The cAT₁R expression decreased in E19 embryos and after birth.

Renin in developing zebrafish promotes angiogenesis in which endothelium and the Notch pathway are required (Rider et al., 2015). Following seven days treatment with ACE inhibitor, larval zebra fish were not able to survive in a low-Na environment, presumably due to the loss of Ang II (Rider et al., 2015). Also, in adult zebrafish, application of ACE inhibitor increased renin expression (Rider et al., 2017). After ACE inhibitor (quinaprilat) injection (i.p.) to adult zebrafish (24 hr, 48 hr), kidney renin activity (Ang I generation from porcine plasma and zebra fish kidney extract) increased by three-fold (Chen et al 2000). These findings suggest that the role of renin or the RAS in vascular morphogenesis may be conserved across the phylogenetic scale.

5. Summary and Perspectives

In summary, we demonstrated the presence of renin in the kidney, liver, and adrenals in chickens as early as E13. With maturation, renin gene expression is more specific to the kidney. This early ontogenic appearance of the RAS both in the kidneys and extrarenal organs is seen in rodents and zebrafish and now in chickens, suggesting that this may be a fundamental pattern of embryogenic renin expression in vertebrates.

In the vascular system of rodent fetal kidneys, renin-secretory cells (Gomez et al., 1989), renin transcripts (Jones et al., 1990, 2000), and renin/Agt gene expression (Gomez et al., 1989) are widely distributed in renal arteries and arterioles. As the renal arterial tree develops, renin mRNA-containing cells are localized progressively to more distal blood vessels and finally to the JGA (Gomez et al., 1989; Jones et al., 1990; Sequeira López and Gomez, 2011). Likewise renin-secretory cells are widely distributed along renal arteries and arterioles in bony fishes (Sokabe and Ogawa, 1974; Nishimura et al., 1973), including aglomerular teleosts (Nishimura et al., 1979). Granulated cells are more localized in the JG area with phylogeny of vertebrates. This trend indicates some resemblance of the ontogenic and phylogenic expression patterns of renin cells.

Likewise, stimulation of arteriolar branching by the RAS and inhibition of arterial growth and development by its blockade are similar in developing zebra fish and mice. The early and broad appearance of renin (or RAS) in the renal vascular system during early phylogenetic and ontogenic processes may suggest that renin has evolved as a local hormone regulating vascular growth and possibly functions such as the control of vascular tone. The fact that in aglomerular toadfish renin release is controlled by calcium concentration in renin-secretory cells rather than by renal sympathetic nerve or by systemic humoral substances (Nishimura and Madey, 1989; Nishimura 2017) supports this concept. In this context, the hematopoietic action of renin/RAS is worthy of investigation in nonmammalian vertebrates. In adult bony fish, hematopoietic tissues remain in the kidney (Ellett and Lieschke, 2010; Kobayashi et al., 2019) and other organs. The role of the RAS as a systemic hormone in regulating blood pressure and fluid-mineral balance appeared later in ontogeny

and phylogeny accompanied by morphological establishment of the macula densa and sympathetic nerve involvement in the JGA (Nishimura, 2017).

Although earlier attempts to elucidate whether ontogeny recapitulates phylogeny remain largely without success, and it is not certain whether some characteristics of the RAS in the mammalian fetus/embryo may be a repeat of the adult stage of ancestral vertebrates or whether they merely represent a common feature of the early ontogeny of all vertebrate animals, comparison of these two time-dependent processes may provide new insights into the interpretation of existing findings, and provide perspective for future investigations.

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List of abbreviations:

α-SMA	alpha-smooth muscle actin
Ang	angiotensin
Agt	angiotensinogen
AP	alkaline phosphatase
AT₁R	angiotensin type 1 receptor
cAT₁R	chicken angiotensin type 1 receptor
ACE	angiotensin converting enzyme
BW	body weight
D4	four days of age (post-hatch day 4)
D30	30 days of age (post-hatch day 30)
DIG	digoxigenin
E13	embryonic day 13
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
H&E	hematoxylin and eosin
hpf	hours post fertilization
ISH	in situ hybridization

JG	juxtaglomerular
JGA	juxtaglomerular apparatus
NCBI	National Center for Biotechnology Information
PCR	polymerase chain reaction
PFA	paraformaldehyde
qPCR	quantitative polymerase chain reaction
RAS	Renin-angiotensin system
RT-PCR	reverse transcription polymerase chain reaction

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Fig. 1.

Histology and immunohistochemistry of chicken kidneys at various developmental stages. Alpha-smooth muscle actin (α -SMA) staining of E13 kidney (A) and newborn (4 days of age, D4) kidney (B) are shown. In D4 kidney, short afferent arterioles that branched from a larger artery at a right angle drain into the vascular pole (arrow). D30 kidney (C), shown with hematoxylin and eosin staining, demonstrates a longer afferent arteriole that drains into the glomerulus at the JG area. Characteristic nucleated erythrocytes are aligned along the major axis of the vessel. A distal tubule is attached to the vascular pole (arrow). Scale bars, 50 μ m.

Fig. 2.

In situ hybridization for renin mRNA in the superficial area of kidneys at ages E13 (A), 4 days of age (B, C, D), and 30 days of age (E, F). For Figs. 2 A, B, E and F, after having confirmed the DIG signal, we counterstained with eosin and very light hematoxylin to better visualize the kidney morphology. For Fig. 2 C and D there was no counterstaining. Renin gene expression (blue signals) is observed at the superficial region (E). D is an expanded view of C (inset). F is an expanded view of the adjacent area of E. Black arrows indicate positive renin mRNA signals at JG areas (B, E and F). Scale bars, 100 μ m.

Fig. 3. Renin mRNA measured by qPCR in the kidney (solid column), liver (gray column), and adrenal (open column) of E13 embryo (A), D4 newborn (B), and D30 (C) chicks ($n = 3$ each). Expression was standardized to D30 kidney. Instead of using the average of 3 kidney values, we selected one kidney value as a standard, and relative values to the standard are calculated (1.105, average of $n = 3$). Comparison of relative kidney renin expressions is shown in D. Note that the scale of the ordinate varies. Data are expressed as means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

Fig. 4.

Representative gel electrophoresis of RT-PCR products from embryos (E13, top left), newborn chicks (middle left), and maturing chicks (D30, bottom left). Numbers 1, 2, and 3 are the results from different birds. While D4 and D30 are the same birds as those used for ISH and qPCR assays, E13 embryos represent different individuals due to their small sizes. Embryonic kidney, liver and adrenals are from the same individuals. All RNA preparations were free of DNA contamination (no band from RT-minus incubation). NC, negative control in which RT reaction mixture excludes cDNA.

Table 1**Renin Expression, Regulation, and Function during Development**

Species and Ages	Renin Gene Expression		Function and Regulation	Major References*
	Kidney	Extrarenal Organ		
Mouse, Rat				Gomez et al., 1988, 1989,1990; Gomez, 1998; Gomez and Sequeira-Lopez, 2016, 2018;Tuflo et al., 1995
Embryo	Rat, Mouse: Appears at E11.5-E12 at vascular pole, afferent, interlobular, arcuate arteries; Strong expression at E20 (rat) 20 times of adult	Mouse; adrenal, bone marrow and spleen	Mouse; Nephro-vascular differentiation and growth; Promotes hematopoiesis	
Newborn	Rat: whole length of afferent arterioles, 10 times that of adult		Rat, ACE inhibition inhibited nephrovascular maturation	
Mature	Rat Confined to JG area, Lower than in newborn	Rat: adrenal, brain	Rat, BP control and fluid-electrolyte homeostasis, catechoiamine release, ACE inhibition increased renin mRNA	
Chicken, <i>Gallus gallus</i>				Le Noble et al., 1991; Savary et al., 2005; Nishimura et al., 2017
Embryo	**E13 Kidney	** Adrenal (equivalent to kidney), liver; Yolk sac	Angiogenic effect, hematopoiesis In vivo ACE blockade decreased hematocrit	
Newborn	**Doubled from E15 embryo	**Adrenal; lower than kidney		
Mature	**4 wks; 11 times higher than in E15 embryo	**Adrenal; lower than kidney;	BP control; catecholamine release; endothelial nitric oxide release, ACE inhibition increased PRA	
Zebrafish, <i>Danio rerio</i>				Liang et al., 2004; Kumai et al., 2014; Rider et al., 2015; Hoffmann et al., 2018
Embryo	48 pfh; detect renin on anterior mesenteric artery (glomerular artery)	24–48 hpf, pectoral artery, ventral aorta	Vascular development	
Larva	Renin mRNA at anterior mesenteric artery			
Mature			Water and electrolyte homeostasis, tissue regeneration Renin expression increased by ACE inhibition	