Neuron- or glial-specific ablation of secreted renin does not affect renal renin, baseline arterial pressure, or metabolism

Di Xu,1 Giuliana R. Borges,2 Deborah R. Davis,3 Khrisofor Agassandian,4 Maria Luisa S. Sequeira Lopez,5 R. Ariel Gomez,5 Martin D. Cassell,4 Justin L. Grobe,3 and Curt D. Sigmund2,3

1Interdisciplinary Genetics Program, Departments of 2Internal Medicine, 3Pharmacology, and 4Anatomy and Cell Biology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa; and 5Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Virginia

Submitted 12 October 2010; accepted in final form 28 December 2010

Neuron- or glial-specific ablation of secreted renin does not affect renal renin, baseline arterial pressure, or metabolism. Physiol Genomics 43: 286–294, 2011. First published December 28, 2010; doi:10.1152/physiolgenomics.00208.2010.—The renin-angiotensin system (RAS), known for its roles in cardiovascular, metabolic, and developmental regulation, is present in both the circulation and in many individual tissues throughout the body. Substantial evidence supports the existence of a brain RAS, though quantification and localization of brain renin have been hampered by its low expression levels. We and others have previously determined that there are two isoforms of renin expressed in the brain. The classical isoform encoding secreted renin (sREN) and a novel isoform encoding intracellular renin (icREN), the product of an alternative promoter and first exon (exon 1b). The differential role that these two isoforms play in cardiovascular and metabolic regulation remains unclear. Here we examined the physiological consequences of neuron- and glia-specific knockouts of sREN by crossing mice in which the sREN promoter and isoform-specific first exon (exon-1a) is flanked by LoxP sequences (sRENflox mice) with mice expressing Cre-recombinase controlled by either the neuron-specific Nestin promoter or the glia-specific GFAP promoter. Resulting offspring exhibited selective knockout of sREN in either neurons or glia, while preserving expression of icREN. Consistent with a hypothesized role of icREN in the brain RAS, neuron- and glia-specific knockout of sREN had no effect on blood pressure or heart rate; food, water, or sodium intake; renal function; or metabolic rate. These data demonstrate that sREN is dispensable within the brain for normal physiological regulation of cardiovascular, hydromineral, and metabolic regulation, and thereby indirectly support the importance of icREN in brain RAS function.

blood pressure; energy; angiotensin; transgenic; knockout

There is a wealth of evidence supporting the function of an intrinsic renin-angiotensin system (RAS) in the brain. Every component of the RAS is expressed in the brain, although the localization of renin remains challenging because its level of expression is very low (12). Despite a vast literature supporting the function of the brain RAS, the mechanisms and location of de novo angiotensin generation and action remain unclear.

Renin activity was initially identified in rat and dog brains, and the presence of brain renin independent of its renal source was verified after bilateral nephrectomy (6–8). Renin-expressing cells have been identified via immunohistochemistry in rat and mouse brain, and primary cultures of rat neurons and glia express renin (14, 15). Moreover, the presence of neurons expressing renin and coexpressing both renin and angiotensinogen were identified using reporter genes driven by the renin and angiotensinogen promoters in transgenic mice (20, 21). That intracerebroventricular injection of antisense oligonucleotides targeting renin mRNA in spontaneously hypertensive rats decreases blood pressure provides direct evidence supporting an important role of brain renin in blood pressure regulation (19). This is further supported by studies showing that brain-specific overexpression of the human RAS genes leads to elevated blood pressure in transgenic mice (24).

Previous studies from our laboratory and others have revealed an alternative form of renin mRNA in the mouse, rat and human brain (23, 29). Translation of the alternative form of renin mRNA is predicted to produce a non-secreted form of renin. This form of renin, termed intracellular renin (icREN) cleaves angiotensinogen to angiotensin I in vitro and when overexpressed increases blood pressure in vivo (22, 23). We recently demonstrated that both the classic secreted renin (sREN) and icREN are expressed in the brain and that they employ discrete regulatory elements for transcription (29, 33). Supporting this conclusion was evidence that expression of icREN in the brain was preserved when the sREN promoter and first exon were selectively knocked out in inbred C57BL/6 mice (33). Interestingly, there appears to be a developmental shift in the expression of these isoforms in the brain. sREN mRNA is the predominant form during fetal development, whereas icREN mRNA becomes the predominant form in the adult brain (33). Despite a growing body of evidence for the expression of these distinct renin isoforms in the brain, their relative roles either during development or in adults remains very unclear. To begin to assess the relative importance of sREN and icREN in the brain, we generated a conditional allele of sREN that would be deleted selectively in response to cell-specific Cre-recombinase, while preserving expression of icREN. Herein we crossed mice harboring a floxed allele of endogenous sREN to mice expressing either neuronal- (Nestin-Cre) and glial-specific (GFAP-Cre) Cre recombinase to generate neuronal- or glial-selective knockouts of sREN. We then measured blood pressure, hydromineral balance, and baseline metabolism in these mice. The measurements in neuronal-specific and glial-specific knockout mice were compared with mice carrying a null allele for sREN. sREN-null mice exhibit no expression of sREN in any tissue including the brain and kidney (33).
METHODS

Generation of neuron- and glial-specific knockouts. Brain-specific knockouts of sREN were generated by crossing sREN\textsuperscript{floxed} with Nestin-Cre and GFAP-Cre lines to establish neuronal and glial-specific knockout mice, respectively. Nestin-Cre and GFAP-Cre transgenic mice were obtained from The Jackson Laboratory (stock numbers 003771 and 004600, respectively) and have been documented for their capability to target neuronal and glial cells, respectively (32, 34). Detailed information about the sREN\textsuperscript{floxed} line was previously reported (33). We first crossed Cre\textsuperscript{+} (either Nestin-Cre or GFAP-Cre) with homozygous sREN\textsuperscript{floxed/floxed} to give rise to sREN\textsuperscript{floxed/+} Cre\textsuperscript{+}. Subsequently, sREN\textsuperscript{floxed/+} Cre\textsuperscript{+} mice were bred to sREN\textsuperscript{floxed/floxed} to generate sREN\textsuperscript{floxed/floxed} Cre\textsuperscript{+} and sREN\textsuperscript{floxed/floxed} Cre\textsuperscript{−}. All mice used in this study are on a C57BL/6J background. Unless otherwise mentioned, all the mice are provided with standard chow and water ad libitum. All animal experimental procedures were approved by the University of Iowa Animal Care and Use Committee and were performed under the guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals.

PCR and gene expression. The primers specifically detecting the flox and null alleles of sREN in genomic DNA are 5′-CCCATGCCCTGC-CACCATCTGCG-3′ and 5′-CCCCACAGAGGACAGACTTGAGGT-3′. The primers specifically detecting only the flox allele of sREN are: 5′-CCCCATGCCCTGCACCATCTGCG-3′ and 5′-CCCTCTTTCATCCTGACCCAC-3′. The primers to detect sREN, total renin = 5′-CCCCACAGAGGACAGACTTGAGGT-3′ CACCACTCTGC-3′ = 5′-CCTCCTTTCA-CCTCCTCTTCTC-3′. The primers to detect sREN, total renin and GAPDH mRNA harvested from embryonic (embryonic day 18.5) and adult (2–3 mo) brains and adult kidney were described previously (33).

Tissue immunohistology. Brains from the Ren-Cre × ROSA and Nestin-Cre × ROSA mice were perfused-fixed with 4% paraformaldehyde and stained for β-galactosidase (β-Gal) and/or immunofluorescence staining against NeuN and GFAP. For β-Gal staining, 50 μm sections were incubated overnight at 37°C in potassium ferricyanide/ferrocyanide solution (with bromo-4-chloro-3-indolyl-β-D-galactoside in diethylformamide at 1 mg/ml). After three washes in PBS, sections were incubated for 2 h at room temperature in biotinylated mouse anti-NeuN antibody (Chemicon/Millipore; 1:500, in PBS plus 0.1% Triton-X). Following another three washes in PBS, the sections were incubated in avidin-horseradish peroxidase (Vector Laboratories) for 30 min, washed three times in PBS, and placed in a solution containing 0.03% 3,3′-diaminobenzidine in acetate buffer and 0.001% hydrogen peroxide. After appearance of reaction product (2–5 min), the sections were washed in tap water (pH 7.6) and mounted on slides. For double-immunofluorescence staining, sections were incubated in 4% normal horse serum for 30 min, followed by incubation with a mouse anti-β-gal antibody (Abcam; 1:1000, in PBS plus 0.1% Triton-X) overnight at 4°C. Following three washes in PBS, sections were incubated with a biotinylated goat anti-mouse antibody (1:200) for 2 h, washed again in PBS for another three times, and then incubated for 60 min in avidin Texas red (Vector, 1:60), which allowed labeling of β-Gal with red fluorescence. After three washes in PBS, the NeuN group was incubated in biotinylated mouse anti-NeuN (1:500) for 2 h, washed three times in PBS, and incubated with avidin-Fluorescein (Vector, 1:50) for 30 min. The GFAP group was incubated in rabbit anti-GFAP (Abcam, 1:1000) for 2 h, washed three times in PBS, and incubated with biotinylated goat anti-rabbit antibody (Vector Laboratories, 1:100) for 60 min, washed again, and then incubated with avidin AMCA (Vector, 1:50) for 30 min.

Kidneys from neuronal- or glial-specific sREN knockouts were removed and fixed in Bouin’s buffer overnight. Subsequently, immunostaining was carried out on 5 μm paraffin-imbbeded kidney sections for renin using a well-characterized rabbit anti mouse, polyclonal, 1:500 antibody directed against renin originally derived from Dr. T. Inagami using a Vectastain ABC kit (Vector Laboratories) as previously described (10, 28).

Blood pressure and heart rate. Blood pressure was recorded via radiotelemetry as described previously (33). Briefly, brain-specific knockouts and littermate controls were subjected to implantation of radiotelemetry catheters (TA11PA-C10, Data Sciences International) into the left common carotid artery under ketamine and xylazine anesthesia and allowed 10 days of recovery before recording. Data were recorded every 5 min for 30 s for 10 consecutive days.

Metabolic measurements. Oxygen consumption at thermoneutrality was utilized as a surrogate marker for basal metabolic rate. O2 intake and CO2 output were measured with instruments from Ametek/AEI (models S-3A/II and CD-31) as previously described (11).

Metabolic cage studies. Experimental and control littermates were single-housed in specialized cages (Nalgene) equipped with individual burettes for saline (0.15 M NaCl) and tap water for 4 days and 3 nights. Buret positions were alternated daily to account for side bias, and animals were allowed the first night to acclimate to the new cages. Standard powdered food (NIH-31 modified 6% diet from Teklab) was supplied in each cage.

Statistic analysis. All the analyses and comparisons are between sex- and age-matched animals. Data satisfying normality and equal variance tests were analyzed by ANOVA or Student’s t-test, otherwise by Friedman’s ANOVA or Mann-Whitney U-test, and plotted as means ± SE. P values < 0.05 were considered significant.

RESULTS

To verify the activity of the sREN promoter in neurons in the brain, we crossed mice carrying a knock-in allele of Cre-recombinase inserted into the Ren-\textsuperscript{1d} locus with ROSA26 mice (28). The Ren-cre mice are heterozygous for the Ren-\textsuperscript{1d} insertion and are maintained on a C57BL/6 background. Therefore, the preservation of renin from the intact Ren-\textsuperscript{1d} and Ren-2 alleles in the Ren-Cre (and Ren-cre × ROSA) mice prevents lethality. Cre-recombinase in these mice is under the control of the sREN promoter, whereas the icREN promoter is ablated. Since the activation of β-Gal expression in ROSA mice in response to Cre-recombinase occurs at the DNA level and is permanent, the presence of β-Gal staining in the resultant Ren-Cre × ROSA offspring is indicative of either concurrent or previous expression of the sREN promoter. β-Gal activity revealed that the sREN promoter is primarily expressed in the pons, medulla, and cerebellum with almost all of the sREN-expressing cells colabeling with the neuronal marker NeuN (Fig. 1A). In the pons and medulla, sREN-expressing cells were concentrated in the medial vestibular nucleus, the parvicular, and intermediate reticular formation, and the dorsal and ventral cochlear nuclei (Fig. 1, B and C). Much smaller numbers of β-Gal-stained cells were observed in the lateral reticular nucleus, the nucleus raphe pallidus, medial nucleus of the solitary tract, nucleus of the lateral lemniscus, and nucleus prepositus. In the cerebellum, β-Gal staining was only found in Purkinje cells (Fig. 1D). In the mesencephalon and diencephalon, sREN-expressing cells were consistently identified in the mesencephalic tegmental nucleus and were occasionally seen in the perifornical region of the hypothalamus. In the telencephalon, β-Gal staining was only found in the hippocampus where it was restricted to subfields CA3 and CA4 (Fig. 1E).

We previously reported the generation of a floxed allele of sREN in the Ren-\textsuperscript{1d} locus of inbred C57BL/6 mice, a strain that carries only a single renin locus (33). To assess the importance of sREN in neurons we backcrossed sREN\textsuperscript{floxed} mice to Nestin-Cre\textsuperscript{+} mice for two successive generations to obtain Nestin-Cre\textsuperscript{+} × sREN\textsuperscript{floxed/floxed} (neuronal sREN knockout) mice. Nestin-Cre\textsuperscript{−} × sREN\textsuperscript{floxed/floxed} mice served as littermate controls. The specificity
of the Nestin promoter for neurons in the brain was confirmed by breeding Nestin-Cre\(^+\) mice with ROSA26. Colabeling of \(\beta\)-Gal with NeuN was identified in the cerebral cortex and throughout the brain including those regions where the sREN promoter was active (Fig. 2). We also performed a breeding between sREN\(^{floxed}\) mice with GFAP-Cre\(^+\) to target the sREN gene in glial cells (glial sREN knockout) as a control.

We next assessed the specificity of Nestin-Cre and GFAP-Cre activity on sREN\(^{floxed}\) by assaying for the presence of a floxed or null allele in genomic DNA isolated from brain and kidney (Fig. 3A). The generation of the null allele occurs through the deletion of the intervening DNA between loxP sites in sREN. In glial-specific knockout mice, a null allele was detected in the brain but not kidney. The null allele was not
detected in the control mice from this cross. On the contrary, although we did not detect a null allele in the control mice from the neuronal-specific cross, a null allele was detected in both brain and kidney in neuronal-specific knockout mice. We next extended this analysis to other tissues (Fig. 3B). A null allele was detected in brain and brown adipose tissue in the glial-specific knockout mice, whereas a null allele was detected in all tissues of the neuronal knockout mice. Retention of the floxed allele was detected in all tissues except for brain, suggesting greater efficiency of Cre-mediated recombination in the brain over other tissues (see lanes marked * in Fig. 3B). It is important to note that the ratio of null-to-floxed alleles in this assay is not quantitative. This is due to the size differences in the PCR products, which makes amplification of the null allele more efficient. For this reason we developed a separate assay which only measures the floxed allele (Fig. 3C). This assay demonstrates retention of the floxed allele in all tissues. This is consistent with partial recombination in various tissues. We conclude that the GFAP-Cre is more restricted than the Nestin-Cre model, although in the brain, the Nestin-Cre is neuron specific.

Given that Nestin-Cre activity was evident in the kidney, and previous reports suggest the Nestin promoter may be expressed in renin-expressing juxtaglomerular cells (13), we assayed for the level of renin expression in the kidney of both the glial and neuronal knockout models. Standard (Fig. 4A) and quantitative (Fig. 4B) RT-PCR demonstrate retention of renin mRNA in the kidney, in both the glial and neuronal strains. Similarly, there was no change in juxtaglomerular expression of renin protein in the kidney of either model (Fig. 5). Further evidence that there was no significant effect on renal renin comes from genetic scoring. As we previously reported, sREN null mice exhibit 90% postnatal preweaning lethality (33). On the contrary, there was no decrease in survival in either the glial or neuronal knockout models. Consequently, despite a previous report to the contrary (13), we were not able to obtain any evidence that Cre-recombinase under the control of the Nestin promoter is active in renal JG cells.

In the brain, the level of renin mRNA is particularly low. In practice, normalized with GAPDH, there is as much as a 1,000-fold ($\Delta C_T \sim 10$) difference between total renin mRNA in the kidney and the brain. This low level of renin expression significantly complicates determinations of the efficiency of sREN-knockdown in the brain of the neuronal and glial knockout mice. We noted a trend toward a reduction in brain sREN mRNA in neuronal knockout versus control littermate mice both in utero (0.44 ± 0.3 vs. 1.0 ± 0.7, $n = 4$) and in adults (0.48 ± 0.57 vs. 1.0 ± 0.7, $n = 7$), although this was variable among mice.
We measured arterial blood pressure by radiotelemetry for 10 consecutive days in neuronal-specific and glial-specific knockout mice compared with their individual littermate controls derived from the same cross. The blood pressure and heart rates of neuronal-specific (Fig. 6A) or glial-specific (Fig. 6B) knockout mice were indistinguishable from their respective control littermates. Normal circadian rhythms were maintained in both models. This stands in contrast to our previous report that global sREN knockout mice (lacking sREN in all tissues including the kidney) exhibit a 20 mmHg ($P < 0.001$) decrease in arterial pressure compared with wild-type controls (33).

Food intake was unaffected by global, neuronal-specific, or glial-specific knockout of sREN (Fig. 7A). Total fluid intake, when offered both water and 0.15 M NaCl solution, was greatly increased in global sREN knockout mice but was unaffected in either the neuronal-specific or glial-specific knockout lines (Fig. 7B). Urine output paralleled the fluid intake, with robust effects in global knockout but not in neuronal- nor glial-specific sREN knockout (Fig. 7C). Total sodium intake (from both drink and food sources) was elevated in global sREN knockout mice but was unaffected in either cell-specific knockout mice (Fig. 7D). Mice from all strains exhibited the expected slight aversion to 0.15 M NaCl (vs. water, Fig. 7E) as we have been previously reported for C57BL/6 mice, and no differences were observed with global, neuronal-specific, or glial-specific sREN knockout mice (11).

Body masses exhibited a slight trend toward reduction in adult sREN knockouts of all three strains (Fig. 8A), though no significant effects were observed. Oxygen consumption at thermoneutrality (as a surrogate marker for basal metabolic rate) was significantly reduced in neuronal-specific sREN knockout mice (Fig. 8B), but this difference was lost when data were normalized for body mass (Fig. 8C). Similarly, when calculating total heat production (which takes into account shifts in respiratory quotient and thereby energy source), neuronal-specific sREN knockout mice showed a significantly reduced metabolic rate (Fig. 8D); however, normalization for body mass again ablated this difference (Fig. 8E).

**DISCUSSION**

Many lines of evidence support the existence of a local, autocrine/paracrine version of the RAS within the brain, and it has been postulated that angiotensin may be utilized as a neurotransmitter in selected brain regions that control cardiovascular, hydromineral, and energy homeostasis (5). Confounding the identification of angiotensin as a classical neurotransmitter is the low level of renin expression within the brain, because a mechanism for de novo neuronal synthesis of angiotensin is necessary to fulfill nearly any formal definition of a neurotransmitter (12).

Previously we and others identified a novel isoform of renin (icREN) that results from the use of an alternate promoter sequence, transcriptional start site, first exon, and alternative initiation codon (23, 29). As this variant of renin lacks an export sequence, the resultant renin protein should remain intracellular, whereas the classic secreted form of renin (sREN) is targeted to the cell’s export apparatus (12). Interestingly, icREN is the predominant form of renin mRNA in the adult brain, whereas sREN expression appears to be the predominant form during fetal development (33). Thus we hypothesize that icREN may constitute the mechanism for de novo neuronal synthesis of angiotensins for use as neurotransmitters. As a first step to examine the physiological significance of icREN versus sREN in vivo, we developed an sREN null mouse that ablated expression of sREN in all tissues but preserved icREN expression in the brain. Complete knockout of sREN (while maintaining icREN) resulted in poor survival due to severe renal
malformations. The surviving adults exhibited renal insufficiency and hypotension (33). This is consistent with previous reports of global knockout of all forms of renin in mice and illustrates the critical importance of secreted kidney-derived renin (31). Unfortunately, the severe phenotype and decreased survivability of sREN-deficiency hampered our examination of the role of sREN and icREN in the brain.

To circumvent these limitations, we next adapted a strategy to specifically ablate sREN only in the brain using promoters targeting Cre-recombinase expression specifically to neurons and glial cells. This would leave expression of renal sREN intact and likely prevent renal dysfunction and decreased survivability. Indeed, we showed that there was no change in renal sREN expression or localization to JG cells and there was no decrease in survival of the mice. Molecular analysis revealed that the GFAP promoter was far more specific than the Nestin promoter in directing a brain-specific ablation of sREN. The only other tissue with evidence of cre-recombinase activity in the GFAP-Cre mice was the brown adipose tissue, a site where we have previously reported no evidence for renin expression (11). On the contrary, there was a surprising level of Cre-recombinase activity in many tissues in Nestin-Cre mice, although the activity appeared to be highest in brain and, in the brain, was neuron-specific. Because the Nestin promoter was previously reported (13) to be active in renal JG cells and because we observed the presence of a null allele in genomic DNA from kidney we confirmed that there was no change in the level or cellular localization of sREN expression in the kidney of the Nestin-Cre mice. Based on our results, we are unable to confirm the activity of the Nestin promoter in renal JG cells.

Unlike sREN null mice, which exhibit hypotension and increased fluid and sodium turnover, there were no changes in blood pressure or in any of the hydromineral endpoints measured in neuronal-specific and glial-specific sREN knockout mice. Similarly, there were no changes in body mass or metabolic rate in these mice. At first, perhaps this is not surprising because icREN and not sREN is the major isoform expressed in the adult brain. However, the Nestin promoter is active as early as 9.5 days of gestation (35), and sREN is the predominant form of renin mRNA in the brain during fetal development (33). Therefore, it is likely that expression of Nestin-Cre was effective at ablating expression of sREN during the period when it is expressed during development. Initial studies suggest that expression of sREN was decreased by ~55% in the brain at 18.5 days of gestation. Consequently, this suggests the possibility that expression of sREN in the developing brain may not play a significant role in later cardiovascular regulation. One weakness of the current study revolves around the difficulty in completely validating the cell-specific-
ity and level of sREN ablation. This is due to very low levels of endogenous sREN expression in the brain. Factors affecting the level of sREN ablation would be the effectiveness of the promoters driving cre-recombinase, the level of cre-recombinase expression in neurons and glia, the relative distribution of sREN in neurons and nonneuronal cells, and the stochastic nature of the recombination reaction on a per cell basis. We also cannot rule out the possibility that some compensatory mechanism was induced in response to life long loss of sREN in neurons.

Activity of the sREN promoter (as indicated by the presence of β-Gal staining in the Ren-Cre × ROSA mice) was restricted to neurons in a relatively small number of brain regions. All of these brain regions, with the exception of a cluster of VIII cranial nerve-related structures (dorsal cochlear nucleus, nucleus of lateral lemniscus and medial vestibular nucleus), have been shown previously to contain neurons exhibiting activity of the renin promoter (21). The identification of these renin expressing cells was based on the use of a renin promoter-eGFP reporter transgene that was reported to be accurately expressed in the JG cells of the kidney and to respond appropriately to physiological and developmental cues (17). Because of the low level of renin expression, the eGFP reporter was used as a surrogate marker for renin-expressing cells. On the other hand, regions of the brain that have previously been reported to exhibit renin promoter activity on the basis of the same Ren-eGFP reporter gene such as the amygdala, cerebral cortex, and subfornical organ were not identified in the Ren-Cre × ROSA mice employed herein (21). Consequently, one possible interpretation of our data is that none of the brain nuclei containing neurons expressing sREN appear to play a direct role in blood pressure regulation and the association

Fig. 7. Intake and output behaviors in neuronal-specific and glial-specific knockout mice. A: daily food intake. B: daily total fluid intake during 2-bottle choice between water and 0.15 M NaCl. C: daily urine output. D: total daily sodium intake from food and 0.15 M NaCl drink solution. E: preference for 0.15 M NaCl vs. water. sREN-null control n = 3, sREN-null knockout n = 3; Nestin-Cre⁻ n = 7, Nestin-Cre⁺ n = 8; GFAP-Cre⁻ n = 6, GFAP-Cre⁺ n = 6.

Fig. 8. Energy output in neuronal-specific and glial-specific knockout mice. A: body mass. B: oxygen consumption. C: oxygen consumption, normalized for body mass. D: total heat production. E: total heat production, normalized to body mass. *P < 0.05 vs. control. sREN-null control n = 9, sREN-null knockout n = 9; Nestin-Cre⁻ n = 7, Nestin-Cre⁺ n = 7; GFAP-Cre⁻ n = 11, GFAP-Cre⁺ n = 11.
between sREN and neurons involved with sodium intake and/or thermogenesis is tenuous. Neurons containing mineralocorticoid receptors and 11-β hydroxysteroid dehydrogenase type 2 have been identified in the medial nucleus tractus solitarii, but sREN-expressing cells were only sparsely distributed there (9). sREN expressing neurons were identified in the nucleus raphe pallidus, a structure involved in thermogenesis but were greatly outnumbered by non-sREN-containing neurons (25). This brain distribution of sREN expressing neurons is certainly consistent with the failure to find significant physiological effects of sREN deletion.

Perspectives

The failure to observe any effects on blood pressure, hydromineral balance, or metabolism in the neuron- or glial-specific sREN-deficient mice, coupled with previous studies suggesting an important role for brain renin, indirectly supports our working hypothesis: that icRen may be of primary importance in brain RAS function. Validating this hypothesis will require the development and analysis of mice specifically lacking icRen. The development of this mouse model is currently in progress.

This begs the question, what is the role if any of sREN in the brain? One conservative conclusion from this study would be that the identification of sREN-expressing cells in the Ren-Cre × ROSA mice reflects involvement and expression of sREN during development. For example, AT1 and AT2 receptors are found in early brain development in the medullary reticular formation, cochlear, and vestibular nuclei (1, 26). Perhaps the secreted form of renin may be involved in forming the endogenous ligands to these receptors, including angiotensin II, which may be required for appropriate development. As pointed out above, one of the weaknesses of the present study was the difficulty in directly comparing sREN-expressing cells during development detected in the Ren-Cre × ROSA mice with those targeted for deletion by cre-recombinase in the Nestin-Cre × sRENflox/flox mice. Although we did not assay for this directly, we did not observe any obvious brain or behavioral abnormalities nor was there any lethality associated with the neuronal-specific deletion. Consequently it remains unclear if sREN is required for the generation of angiotensin during brain development that would serve to activate AT1 and AT2 receptors.

Certainly, many studies clearly demonstrate that overexpression of renin, presumably sREN, can have profound effects on arterial pressure, hydromineral balance, and most recently metabolism (11, 24, 30). Whether intracellular production of angiotensin can occur in these models remains unclear. These overexpression studies demonstrate that locally synthesized angiotensin in the brain can have robust pleiotropic effects. Few studies however, have addressed a role for endogenous brain renin, and our studies represent the only attempts to differentiate secreted from intracellular renin. Interestingly, we previously identified sREN promoter activity in the CA3 and CA4 subfields of the hippocampus (20, 21). Hippocampal subfield CA3 is the major input to subfield CA1, the main hippocampal area involved in long term potentiation (LTP). Angiotensin II injected into CA1 disrupts LTP (4) and disrupts passive avoidance behavior (3) by an AT2 receptor-dependent mechanism. This suggests the provocative hypothesis that sREN in CA3 could be involved in the generation of angiotensin II in CA1 to regulate synaptic plasticity associated with learning and memory. Overexpression of angiotensin II in the brain of transgenic mice was also reported to impair cognitive function (16). Interestingly, studies reporting an increase in the expression of RAS components in the brain of Alzheimer’s disease, and the beneficial effects of RAS inhibitors has led to a hypothesis that the RAS may affect the pathogenesis of this disease (reviewed in Ref. 18). Similarly, indirect evidence suggests the RAS may affect depression, stress, and alcohol intake (reviewed in Ref. 27). In many respects, sREN could have similar effects on brain function as brain-derived neurotrophic factor, which has been implicated in synaptic stabilization and neuron growth and differentiation as well as Alzheimer’s disease and depression (reviewed in Ref. 2). Whether renin activity and, in particular, differential activity of sREN or icRen plays a role in these processes will require many more studies, perhaps using models similar to the one described herein.

GRANTS

This work was supported NIH Grants to C. D. Sigmund (HL-48058, HL-61446, HL-084207), R. A. Gomez (HL-066242 and HL-096735), a fellowship from the American Heart Association to D. Xu (0910035G). J. L. Grobe was supported through K99/R00 Pathway to Independence Award HL-098276, and M. L. S. Sequeira Lopez was supported by NIH K08 Award DK-075481. We also gratefully acknowledge the generous research support of the Roy J. Carver Trust.

DISCLOSURES

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

REFERENCES