Renalase in hypertension and kidney disease

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Abstract

Renalase, a recently discovered flavoprotein, which is strongly expressed in the kidney and heart, effectively metabolizes catecholamines. It was discovered during the search to identify proteins secreted by the kidney that could help explain the high incidence of cardiovascular disease in patients with chronic kidney disease. Recent advances have led to more detailed knowledge of its biology, structure, enzymatic activity, mechanisms of action, associations with human disease states and potential therapeutic value. In this study, we review these advances with a focus on hypertension and kidney disease.

Characterization and Structure of Human Renalase

Renalase was first identified as we screened existing public databases using an algorithm that targeted proteins that were highly expressed in the kidney and were likely to be secreted. Among selected genes, one clone (we named it renalase) showed high renal expression, especially in the proximal tubule [1]. Renalase resides on chromosome 10 at q23.33 has nine exons spanning 311,000 base pairs and encodes a 342 amino acids protein with a calculated molecular mass of ~38 kDa. Aside from the kidney, it is also highly expressed in the heart, skeletal muscle and small intestine [1]. We have described three splice variants (renalase 2–4) [2, 3]. Human (h) hRenalase1 is very well conserved, with orthologs present in chimpanzee (95% amino acid identity) and cyanobacteria (23% identity). Its key structural features include a putative signal peptide at the N terminus (amino acids 1–17), a flavine adenine dinucleotide (FAD)-binding domain (amino acids 3–42) and an amine oxidase domain at amino acids 75–335. Although hRenalase3 and 4 have not been studied, their structure has shortened amine oxidase domains, making it unlikely that they have amine oxidase function.

The crystal structure of hRenalase1 is known (Figure 1) [4] and indicates that it is a member of the flavoprotein superfamily, which includes oxidase and monoxidase enzymes. A comparison of catalytic residues with other oxidases, such as monoamine oxidase (MAO)-A and MAO-B, indicates that some critical residues needed for amino-oxidation by the latter enzymes are absent in renalase, thus suggesting that hRenalase1 metabolizes catecholamines differently from the classical monoamine oxidases [5, 6]. A knowledge of its structure was an important breakthrough as it affords the opportunity for better characterization of the molecular mechanisms of its function and regulation, as well the development of synthetic analogues with potential therapeutic relevance.

Function of Human Renalase

hRenalase1 uses nicotine adenine dinucleotide (P)H as a cofactor to metabolize catecholamines (epinephrine >> 1-dihydroxyphenylalanine (1-DOPA) > dopamine = norepinephrine) [7–9], yielding a 6-fold faster catalytic activity than in its absence. The proposed reaction scheme is one where NADH reduces the FAD moiety of renalase, which reacts with oxygen to generate superoxide anion (O2•−), which in turn oxidizes epinephrine [7]. Superoxide dismutase, an O2•− scavenger, completely abolishes the epinephrine oxidation.

The administration of hRenalase1 decreases plasma epinephrine, 1-DOPA and dopamine by 82, 63 and 31%, respectively. It does not increase urinary levels of the
deaminated (DOPA carboxylase), methylated (3-MT) and deaminated plus methylated (homovanillic acid) metabolites, suggesting that renalase’s action profile is significantly different from that of catechol ortho-methyl transferase and MAOs [7, 10]. As suggested by the critical importance of superoxide for the action of renalase on catecholamines, aminochromes are the reaction products of renalase. In vitro studies demonstrate that renalase catalyzes the formation of adrenochrome, L-dopachrome, dopachrome and noradrenochrome from epinephrine, L-DOPA, dopamine and norepinephrine, respectively [7]. Additionally, dobutamine, isoproterenol and α-methyldopa are also potential substrates for renalase [7]. All the renalase substrates identified are currently aromatic amines. However, chemical screens have intrinsic limitations, and it is possible that other physiologically relevant substrates exist.

Renalase is secreted in blood and its levels are regulated by three key factors: renal function, renal perfusion and catecholamine levels. When measuring renalase levels by western blot using a polyclonal antibody raised against the whole protein, they hold a direct relationship with the glomerular filtration rate and kidney mass [1, 11], resulting in marked renalase deficiency in patients with end-stage renal disease (ESRD). Likewise, subtotal nephrectomy (5/6 Nx) in rats results in decreased renalase, thus suggesting that renal function plays a key role in regulating renalase secretion. In a model of unilateral renal artery stenosis, renalase expression and secretion are decreased in the ischemic kidney compared with the nonischemic side, thus implicating changes in renal perfusion as a determinant of renalase secretion [12]. In the isolated perfused rat kidney model, catecholamine infusions stimulate renalase secretion into the renal vein as well as its activation [12], thus linking renalase secretion to prevalent catecholamine levels. Taken together, these data suggest that the kidney regulates renalase secretion in plasma, both in basal and stimulated states.

It is important to report that studies using a commercially available ELISA kit (Uscn Life, China) have reported opposite relationships with renal function, i.e., markedly increased levels in patients with ESRD [13, 14]. In a study of 34 prevalent hemodialysis patients, serum renalase concentration was >4-fold higher than in healthy volunteers (17.51 ± 6.73 versus 3.99 ± 1.73 μg/mL, P < 0.001) [14]. Similar observations were made in a larger cohort of 104 hemodialysis patients in whom serum renalase levels were lower in hemodialysis patients than in 27 healthy controls (27.53 ± 7.18 versus 3.86 ± 0.73 μg/mL, P < 0.001) [13]. The same investigators studied 130 heart transplant recipients with moderate renal dysfunction (mean baseline serum creatinine 1.7 ± 1.1, estimated glomerular filtration rate (eGFR) by the CKD-EPI formula 55 ± 28 ml/min). They showed that blood renalase levels were higher in these patients than in healthy controls (8.41 ± 5.47 versus 3.86 ± 0.73 μg/mL, P < 0.001) and had a strong negative correlation with eGFR (r = −0.59, P < 0.001) [15]. In adjusted analyses, renal function (as serum creatinine) was the strongest predictor of renalase blood levels (β = 0.79, P < 0.0001). When renalase levels were reported according to the CKD stage, they demonstrated a stepwise increase with higher stages of kidney disease: CKD Stage 1, 4.57 ± 0.83; Stage 2, 5.97 ± 1.79; Stage 3, 7.64 ± 2.67; Stage 4, 12.17 ± 4.84 and Stage 5, 27.69 ± 8.84 μg/mL [15]. Similar results were observed among 89 prevalent kidney transplant recipients [16].

These different results in blood levels based on western blots and ELISA need further exploration and raise concerns about the interpretation of renalase levels in patients with kidney disease. The most relevant issue is that the two antibodies used in the commercial sandwich ELISA kit (Uscn Life) have not been fully validated. The identity of the antibodies and the epitopes they recognize, and information on how they behave in native western blots is not available. For example, they may recognize epitopes undetected by the
F I G U R E 2: Detection of renalase in human serum by western blot. (left panel) Serum proteins separated on a gradient gel (4–16%) under native (nondenaturing, nonreduced) conditions and probed with a monoclonal antibody (ProteinTech, #60128-1-lg, 1:300 dilution), weak bands detected at ∼300, and 75 kDa; (right panel) probed using a renalase polyclonal (R&D Systems, #AF5350, 1:200 dilution), detects dimeric form of renalase at ∼75 kDa.

Lessons from the renalase knockout mouse models

The global renalase knockout (KO) mouse model has normal renal function and an ∼3-fold increase in serum and catecholamine levels, with attendant lower body weight, tachycardia and moderate hypertension [17]. In addition, the animals have hypophosphatemia and display marked sensitivity to myocardial and renal ischemia. The hypertension is mediated by sympathetic activity, as suggested by the observation that the infusion of TOCRIS A-61603 hydrobromide (a highly specific alpha adrenergic receptor 1A/C agonist [$\alpha$AR1A/CJ]) provokes more severe blood pressure elevations in renalase KO mice than in wild-type (WT) animals. Furthermore, renalase causes a sustained (24 h), large (2.6-fold) decrease in epinephrine levels, and single amino acid mutations that alter its function result in lesser BP-lowering effect [7], thus further linking renalase’s hypotensive effects to its catecholamine metabolizing function. Renalase KO mice have poor tolerance to myocardial ischemia and develop more significant (3-fold greater) ischemic necrosis than WT littermates, a process that is abrogated by the coadministration of recombinant renalase suggesting an active role of renalase in process, likely due to the metabolism of catecholamines accumulated in the myocardial interstitium [17], though other processes yet unidentified are also possible. Likewise, renalase KO mice have increased susceptibility to renal ischemia/reperfusion injury resulting in lower renal function (serum creatinine 2.8 versus 2.3 mg/dL, P < 0.05) and greater tubulointerstitial inflammation, tubular necrosis and apoptosis, a process that was also ameliorated by the administration of recombinant renalase [18]. Changes in dietary phosphate (Pi) modulate the expression and activity of renalase in the WT kidney, along with MAO-A and MAO-B, suggesting that these enzymes participate in renal Pi metabolism by regulating the renal and urinary dopamine [19]. Renalase KO mice maintained on a normal diet develop moderately severe hypophosphatemia with increased urinary phosphate excretion, a process that is presumed to be related to increased urinary dopamine, a known phosphaturic mediator [20].

Renalase regulation of intrarenal dopamine and epinephrine systems

The proximal tubule generates dopamine, which contributes to the regulation of sodium excretion [21]. A low-salt diet decreases aromatic l-amino acid decarboxylase (AADC) activity and inhibits renal dopamine synthesis and excretion, while the opposite occurs under high-salt conditions. Once secreted, dopamine cannot re-enter the tubular cells and therefore, cannot be regulated by MAO and catechol-O-methyltransferase (COMT), which are intracellular enzymes. Prior to the discovery of renalase, the regulation of renal dopamine levels was thought to occur prior to secretion. Given the evidence for dopamine metabolism by renalase, there is support for its relevance in regulating the dopamine-dependent natriuresis and phosphaturia observed in 5/6 nephrectomized and sham rats [22, 23]. Epinephrine acts on tubular adrenergic receptors, facilitates the neuronal release of norepinephrine and enhances vasoconstriction induced by the firing of renal sympathetic nerves even in doses that are not hypertensogenic [24]. In humans, urinary epinephrine excretion is unchanged following bilateral adrenalectomy [25], and adrenal demedullation in rats decreases plasma epinephrine levels by >90%, but has no effect on renal epinephrine [26]. Therefore, there is direct regulation of epinephrine in the kidney, and renalase is well suited to be a major mediator of intrarenal levels (Figure 3).

ASSOCIATION OF RENALASE WITH HUMAN DISEASES

Several recent observational studies have explored the relationship between polymorphisms in the renalase gene and the risk of development of hypertension and/or several of its complications. While this evidence is purely associative and can only be viewed as hypothesis generating, the general
direction is one that links renalase with increased risk of complications as discussed in the following sections.

Renalase SNPs and essential hypertension

Two single-nucleotide polymorphisms (SNPs; rs2576178 GG genotype and rs2296545 CC) within the renalase gene were associated with essential hypertension (defined as BP ≥160/100 mmHg) among 2586 Chinese individuals [27]. After adjustments for age, gender, body mass index, glucose, lipids, renal function, smoking and alcohol use, these two SNPs were associated with increased odds of hypertension (odds ratio, OR = 1.58 for rs2576178 GG and 1.61 for rs2296545 CC, both P=0.0002) [28]. In another series of 892 subjects with Type 2 diabetes, the C allele of rs2296545 SNP was associated with hypertension after adjustments for age, gender, body mass index and lipid profile (OR = 2.58, P = 0.009) [29]. In one study of patients with coronary disease, however, no renalase SNPs were associated with blood pressure levels [9]. Overall, it appears that polymorphisms in the renalase gene confer an increase in risk of hypertension.

Renalase SNPs and coronary disease, left ventricular morphology and function

We have tested the association of renalase SNPs with cardiac disease [9]. When compared with other genotypes, we found that SNP rs2296545 CC (Glu37Asp, present in 184 of 590 subjects) is associated with increased left ventricular mass index (95.4 versus 90.0 g/m², P = 0.01), lower left ventricular ejection fraction (60.7 versus 63%, P = 0.01) and lower exercise capacity (6.2 versus 7.2 metabolic equivalent, P = 0.0003) after adjustment for age, gender, body mass index, systolic blood pressure, diastolic blood pressure and estimated glomerular filtration rate [9]. In addition, adjusted binary analyses demonstrated increased left ventricular hypertrophy [OR = 1.43, 95% confidence interval (95% CI) 0.99–2.06; P = 0.06], systolic dysfunction (OR = 1.72, 95% CI 1.01–2.94; P = 0.05), diastolic dysfunction (OR = 1.75, 95% CI 1.05–2.93; P = 0.03), poor exercise capacity (OR = 1.61, 95% CI 1.05–2.47; P = 0.03) and inducible myocardial ischemia (OR = 1.49, 95% CI 0.99–2.24; P = 0.06) among subjects with this genotype. SNP rs2296545 CC results in a conserved amino acid change at amino acid 37 (glutamic to aspartic acid) within the FAD binding domain (E37D). Interestingly, E37 has higher affinity for NADH and is more than 2-fold more active than D37. Therefore, there is biological plausibility to the observation that an SNP resulting in functional enzymatic changes leading to decreased catecholamine degradation is associated with left ventricular hypertrophy and lower function. It is of relevance that this SNP was not associated with blood pressure in this cohort. Several reasons are possible, most likely the high prevalence of hypertension (≈65%) and the aggressive management of hypertension (average BP 131/73 mmHg).

Renalase SNPs and stroke

In a study of 892 Type 2 diabetic patients and 400 controls genotyped with three SNPs in the renalase gene [29], the most interesting finding was a novel association of rs10887800 SNP with stroke, with 66% of patients being GG homozygotes. This association was confirmed in 130 stroke patients without diabetes (OR for risk allele was 1.79, 95% CI 1.33–2.41). Therefore, it appears that the rs10887800 polymorphism is associated with increased stroke risk in patients with and without diabetes.

Renalase SNP and Type 1 diabetes

Linkage and association studies have consistently revealed associations between Type 1 diabetes (T1D) and multiple loci within the HLA region, suggesting an immune basis for the
quality. A recent genome-wide association study and meta-analysis found that approximately 42 loci affect the risk of diabetes [30]. This study included 7514 cases of T1D and 9045 reference controls, and two replication cohorts, one from Denmark and another from Great Britain, confirmed linkage with most of the 24 previously identified loci, the strongest being with HLA, INS, PTPN22, CTLA4 and IL2RA. Moreover, it identified novel loci, so 27 novel regions were further tested in an independent set of 4267 cases and 4463 controls, and 2319 affected sib pair families. Of these, 18 regions were replicated (P < 0.01; overall P < 5 × 10−8), and the strongest evidence of association among these novel regions was achieved at rs10509540 (combined P = 1.3 × 10−28), located on chromosome 10q23.31 immediately upstream of the renalse (RNLS) gene, the only one located in the region. In another recent study, 21 SNPs that reached a genome-wide significance level in one or multiple GWA studies were examined in the southeast US Caucasian population [31]. Putative association was confirmed for 18 genes, with renalse, showing the seventh strongest association (PTPN22 > INS > SH2B3 > CTLA4 > ERBB3 > IFIH1 > Renalse). Renalse is expressed in the pancreas in insulin-secreting cells, but the mechanisms that underlie its possible role in the development of T1D have not been investigated. In summary, there is a possible relationship between the renalse gene and T1D. Further work is necessary to better identify this risk and evaluate mechanistic possibilities.

**Potential Therapeutic Utility of Renalse**

Since renalse metabolizes catecholamines, it is conceivable that it may be valuable in conditions accompanied by increased sympathetic activity. This hypothesis has been tested in several animal models of hypertension, CKD, myocardial ischemia and acute kidney injury.

Rats subjected to subtotal nephrectomy (5/6 Nx) develop hypertension and CKD. In these animals, a single dose of recombinant renalse (1.3 mg/kg) administered subcutaneously decreased both systolic (~22 mmHg) and diastolic BP (~20 mmHg) (Figure 4), an effect equivalent to 5 mg/kg of enalapril [7]. In the spontaneously hypertensive stroke-prone rats, both renalse and enalapril (1 mg/kg) decreased systolic and diastolic BP by approximately 7 mmHg by 12 h after treatment.

The longer-term effect of renalse on BP was analyzed using the 5/6 Nx model. Compared with buffer-treated control 5/6 Nx rats, those who received recombinant renalse subcutaneously daily for 4 weeks had significantly lower plasma norepinephrine, mean arterial pressure (MAP), left ventricular hypertrophy and cardiac fibrosis [32]. Plasma creatinine and blood urea nitrogen trended lower in the renalse-treated group, but the difference did not reach statistical significance. The effect of renalse treatment on renal histology was not examined in that study. Taken together, these data suggest that hRenalse1 may represent a useful therapeutic option in the treatment of hypertension associated with kidney disease.

Catecholamine levels increase markedly in the ischemic myocardium, and a >500-fold increase in norepinephrine levels has been documented in myocardial interstitial fluid of the ischemic pig heart [33]. Renalse deficiency in the KO mouse is associated with a marked increase in ischemic myocardial necrosis, but administration of recombinant renalse completely rescues this cardiac phenotype [7, 17].

The urinary excretion rate of renalse at baseline in rats was compared with that following mild and moderate bilateral renal ischemia (Desir, unpublished observations [3]). Urinary renalse levels were measured in urine samples collected hourly over several hours by the western blot using an antirenalse monoclonal antibody. The top panel of Figure 5 shows that renalse excretion is relatively constant over several hours under baseline conditions. After 5 min of bilateral renal ischemia, urine collected over the next hour showed a measurable decrease in renalse excretion, which immediately returns to presischemic levels over the next hour. The lower panel shows that more severe ischemic insults (global ischemia for 45 min) caused a dramatic and long-lasting decrease in urinary renalse excretion. These data suggest that urinary renalse may be an early biomarker for ischemic acute kidney injury.

Acute ischemic renal injury is associated with a marked and prolonged (>24 h) increase in renal sympathetic activity as reflected by a 1.5- and 7-fold increase in renal nerve sympathetic activity and renal vein catecholamines, respectively [34]. Since hRenalse1 administered subcutaneously reduces plasma epinephrine by ~80% at 24 h, we tested its effect on acute renal ischemic injury in WT and renalse KO mice.
The animals underwent uninephrectomy, and the remaining kidney was subjected to 20 min of global ischemia. Dialysis buffer (control) or hRenalase1 (1.5 mg/kg) was subcutaneously administered immediately prior to the ischemic injury, and the animals were sacrificed 24 h later. Mice subjected to renal ischemia reperfusion injury had significantly reduced kidney and plasma renalase levels compared with the sham-operated mice. Consistent with this, mouse plasma norepinephrine levels increased significantly after renal ischemia reperfusion injury. Furthermore, renalase-deficient mice subjected to renal ischemia and reperfusion had exacerbated renal tubular inflammation, necrosis and apoptosis with higher plasma catecholamine levels compared with the renalase WT mice. The administration of recombinant human renalase reduced plasma catecholamine levels and ameliorated ischemic acute kidney injury in renalase WT mice by reducing renal tubular necrosis, inflammation and apoptosis. Taken together, our data show that renalase serves to protect against ischemic acute kidney injury by reducing renal tubular necrosis, apoptosis and inflammation. Recombinant renalase therapy may provide a novel therapeutic approach for the prevention and treatment of acute kidney injury. In addition, as plasma renalase decreases after ischemic acute kidney injury, it may serve as a novel and sensitive biomarker for the detection of acute kidney injury. Taken together, these observations indicate that renalase deficiency increases the susceptibility to both cardiac and renal ischemic injuries, and that increasing plasma renalase by administering the recombinant protein is protective.

The impact of renal denervation on blood pressure and renalase expression and secretion was evaluated in spontaneously hypertensive (SH) rats [35]. Compared with control rats, SH rats had increased blood pressure and tyrosine hydroxylase (TH) protein expression, and decreased kidney and plasma renalase. One week after renal denervation, MAP and TH protein expression fell to control levels, and plasma (Figure 6a) and kidney (Figure 6b) renalase levels increased markedly. The hypotensive effect of renal denervation was short lived in this model as blood pressure increased, and renalase expression decreased to presurgical levels.

In conclusion, developments in the understanding of the structure and function of renalase, and early clinical studies exploring its relationship with different human diseases have raised it as a substance with potential pathophysiological relevance and diagnostic/therapeutic utility. Further work is necessary to better define these observations. These include, for example, better standardization of methods for its measurement in serum and urine, evaluation of its activity in serum, assessment of its protective role in renal and myocardial injuries once damage has already occurred and

**FIGURE 5:** Rapid inhibition of renalase urinary secretion by renal ischemia—levels in urine measured by western blot at indicated time. Representative data.

**FIGURE 6:** Renal denervation increases renalase expression in SH rats. See text for details. Adapted from ref. [35].
comparison of its therapeutic performance in long-term comparisons to standard therapies in models of hypertension, CKD or ischemic heart failure. If the results of such experiments corroborate the early findings, renalase may be well positioned for larger diagnostic/prognostic studies and exploratory treatment trials in humans.

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