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RENALASE: NOVEL HORMONE WITH POTENTIAL IN CARDIOVASCULAR DYSFUNCTION & RELATED ORGAN DAMAGE

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Abstract-Metabolic and humoral mechanisms have been known to be involved in the development and progression of cardiovascular-related sympathetic overdrive. Sympathetic overactivity in the genesis of major complications of cardiovascular system and associated organ damages has been reported as one of the major participant. Evidences has been provided that sympathetic neural mechanisms may participate in the development of cardiovascular -related target organ damage, such as left ventricular hypertrophy, diastolic dysfunction, impaired arterial compliance vascular atherosclerosis and renal failure.

Renalase, a circulating FAD-dependent amine oxidase, has been reported as a secretion in blood form the kidney as well as from heart, skeletal muscle and small intestine and known to alter systemic blood pressure, cardiac & renal function in health and diseases. It has been identified in two isoforms of the human protein and is produced by alternative splicing. In vitro studies have demonstrated that Renalase triggers the release as well as degrades catecholamines such as dopamine, norepinephrine and epinephrine and thus has been claimed to have casual link between sympathetic overdrive and increase risk of cardiovascular dysfunction & related organ damage.

This paper discussed the main features of physiological functions of renalase, including its sources of secretion, expression and circulating level and their relevance with adrenergic function in cardiovascular dysfunctions & related organ damage. The paper also reviewed the potential role of renalase in the diagnostic and /or therapeutic approach to cardiovascular disorders & renal diseases, aimed at regulation of sympathetic deactivation.

Keywords: Sympatho-neural system Renalase, Amine oxidase, Cardiac function, Myocardial Ischemia,

INTRODUCTION

The catecholamines are the neurotransmitters of the sympatho-neural system, released at sympathetic nerve endings and trigger its physiological actions by way of adrenergic receptors on target tissues[1]. In the last three decades, with over 35000 publications on various aspects of cardiovascular disorders, sympatho-neural system [SNS] has moved to centre stage in cardiovascular medicine and still awaiting answers to some serious gaps in understanding of the activity of sympatho-neural system and increased cardiovascular risk and related involvement of other organ damage[2]. Ability of the catecholamine to affect the lipid metabolism and cholesterol synthesis and its heterogenic nature of sympatho-neural system [SNS] activation via both direct and indirect mechanisms has been reported to be main determinants of [1] alterations of the respiratory pattern leading to chronic intermittent hypoxia, hence chemoreceptor stimulation[3]; [2] elevation of plasma osmolality, which activates hypothalamic vasopressinergic areas[4]; [3] vascular inflammation of the brainstem[5]; [4] angiotensin II-mediated sympathoexcitation[6,7] and [5] modifications of the pressure–natriuresis relationship mediated by renal sympathetic activation[8]. These findings support the hypothesis that the sympathetic overdrive resulting in to cardiovascular dysfunctions & related organ damage has a multifactorial origin in human.

In recent years, number of studies has been carried out to examine role of sympathetic factors in the development as well as in the progression of the renal organ damage observed in hypertensive subjects[9]. Sympathetic neurohormonal overactivity and consequent accumulation of circulating catecholamines associated with heart failure and sustained sympathetic activation leading to deterioration of cardiac function through a variety of mechanisms such as stimulation of cardiomyocyte apoptosis[10], direct toxicity to cardiomyocytes[11], and induction of ventricular dysrhythmias[12] have been studies and attempted to

explain the catecholamine accumulation due to desensitization of arterial baroreflexes[13] , cardiopulmonary reflexes[14], or elevated central and circulating levels of angiotensin II[15-16]. However, depletion of norepinephrine in the nerve terminal with increasing sympathetic activation in such conditions remains a question to be answered and points towards the unknown underlying mechanism responsible for depletion of catecholamine in conditions associated with increased sympathetic overdrive[17, 18].

The kidneys have dense afferent sensory and efferent sympathetic innervations and presumed to be origin as well as target of sympathetic activation[19]. Postganglionic sympathetic fibers innervate all essential renal structures, including the renal vasculature, the tubules, and the juxtaglomerular apparatus. Renal sympathetic activation leads to volume retention through sodium reabsorption and stimulation of rennin release from the juxtaglomerular apparatus. Conversely, angiotensin II too stimulates sympathetic nerve activity by facilitation of adrenergic neurotransmission at the sympathetic nerve terminal. Renal ischemia, observed in experimental animal with acute renal artery stenosis, as probable important primary event leading to increased SNS activity[20]. Recent studies suggested that, among others, renal ischemia, nitric oxide [NO], and oxidative stress are also involved in sympathetic activation associated with hypertension and chronic renal failure [21].It has been demonstrated that increase in arterial blood pressure is prevented by abrogation of afferent sensory signals[20-22] in rat model of CRF with 5/6th nephrectomy, suggesting that afferent signals from diseased kidneys to central integrative structures in the brain cause increased sympathetic nerve discharge and contribute to hypertension and deterioration of renal function after renal impairment. Animal studies demonstrated that renal sympathetic activation contributes significantly abnormal natriuresis and diuresis seen in hypertension related disorders[23, 24, 25]. Marked adrenergic overdrive has been documented in end-stage renal failure in human studies[26]. These finding suggest that sympathetic abnormalities and renal dysfunctions in hypertension closely and directly relate to [1] plasma level of asymmetrical dimethylarginine presumably leading to endothelial dysfunction and [2] left ventricular mass[27], but failed explain the relevance of these two variables to conclude that the degree of sympathetic overdrive related to cardiovascular organ dysfunctions & mortality[28, 29].

Renalase, recently known circulating amine oxidase in plasma with catecholamines mobilizing property has drawn attention of many research groups working on renalase and its link with regulation of sympatho-neural system. Infusion of catecholamine has been shown to release the renalase and suggest its role in cardiovascular health[30]. Kidney has been reported to be the major source of renalase secretion, but its expression in the heart, skeletal muscle, and liver, has also been demonstrated. Reduced renalase levels observed among patients with end-stage renal failure or in subnephrectomized rats[31, 32] did not demonstrated compensatory rise in renalase level. Down regulation of Renalase is associated with elevated blood pressure[33, 34, 35, 36] supports the hypothesis that renalase might be involved in the regulation of blood pressure. Patients with essential hypertension in a northern Han Chinese population have been found to be associated with two single nucleotide polymorphisms [rs2576178 and rs2296545] having possible functional impact of one of them in genesis of hypertension[37]. Recombinant renalase has been shown to dose dependently lower blood pressure, heart rate, and contractility and to protect the myocardium against ischemia–reperfusion injury[38].These findings indicate that increasing renalase concentration could reduce blood pressure, and that a population of patients with hypertension might be associating with renalase deficiency or renalase polymorphism. Further variations in renalase level associated with different conditions of cardiovascular dysfunction and related organ damage, characterized by altered circulating catecholamine, suggests that renalase could be one of the biomarkers could be used to trace the unrecognized pathway responsible for mediating effects of catecholamines released by sympathetic overdrive in cardiovascular health and diseases. This paper review the advances made in recent years targeting the physiology of renalase, a new flavin-adenine dinucleotide [FAD] containing hormone, and its relevance with cardiovascular dysfunction & related organ damage by modulation of prolonged SNS hyperactivity. It is also attempted to explore potential link between mechanisms underlying sympathetic activation in chronic cardiac and kidney disease, the range of adverse consequences associated with this activation, and potential therapeutic implications resulting from this relationship.

WHAT IS RENALASE?

Renalase is a FAD-dependent amine oxidase. Called also MAO-C, renalase has 13% and 12% identity at the amino acid level to MAO-A and MAO-B, respectively. It has a distinct substrate specificity and inhibitor profile to that of MAO-A and MAO-B, indicating that it represents a brand new class of unique FAD-containing monoamine oxidases[33] . The human renalase gene resides on chromosome 10, contains 9 exons and spans about 311 Kb. Renalase was reported to be an amine oxidase with significant activity towards the catecholamines noradrenaline [NA], adrenaline [ADR] and dopamine [DA], but with little or no activity towards other physiologically occurring amines, such as serotonin, tyramine, benzylamine, methylamine and spermidine[39].

RENALASE- CHEMICAL STRUCTURE;

Renalase resides on chromosome 10 at q23.33 and encodes a 342–amino acid protein with a calculated molecular mass of ≈38 kDa. It is a distant relative of monoamine oxidase [MAO]-A with <14% amino acid identity. The crystal structure of human MAO-B has been determined at a resolution of 3.0 Å and reveals a dimer

with the FAD cofactor covalently bound to a cysteine side chain [Cys-397]. The renalase protein has been very well conserved throughout evolution, with orthologs not only in chimpanzee [95% amino acid identity] but also in Cyanobacteria [23% identity][34]. The human renalase gene contains an amino-terminal signal sequence, followed by a flavin-adenosine-dinucleotide [FAD]- containing domain and an amino oxidase domain. The nucleic acid sequence of human renalase is 27.7% homologous to that of human MAO-A and 38.2% homologous to that of MAO-B [isoforms].

RENALASE- METABOLISM:

Amine oxidases are enzymes that metabolize biogenic amines and are classified according to the nature of the attached cofactor, such as FAD or topaquinone [TPQ]. MAO-A and MAO-B are FAD-containing, mitochondrial enzymes that metabolize intracellular catecholamines. MAO-A and MAO-B have overlapping substrate specificity, catabolize neurotransmitters such as epinephrine, norepinephrine, serotonin, and dopamine and are specifically inhibited by clorgyline and deprenyl, respectively while neither MAO-A nor MAO-B inhibitors [pargyline and clorgyline, respectively] could block renalase. Although the amine oxidase activity of renalase was dependent on its FAD content, it has been reported to be insensitive to the FAD-containing monoamine oxidase [EC 1.4.3.4] inhibitors pargyline and clorgyline. Thus this reported amine oxidase activity of renalase differs in specificity and inhibitor-sensitivity from the known FAD-containing amine oxidases.

RENALASE- SECRETION;

Renalase is secreted into the blood, where it is detectable by Western blotting of plasma proteins separated by SDS-PAGE[33]. It is secreted by the kidney into the blood in humans, demonstrates highest renalase gene expression, but also detectable in the heart, skeletal muscle and small intestine. Renalase is most abundant in the proximal tubules and it presents in the circulation of normal individuals, suggesting that renalase protein in the proximal tubules can be secreted via the basolateral membrane into the circulation where it catabolized its substrate[s], and thus, regulating catecholamine homeostasis at a systemic level. It is argued that Renalase also exerts its biological function at the lumen of renal tubules, since renalase is small protein which can be easily filtered to the lumen of nephron. In addition, renalase can be directly secreted via the apical membrane by the proximal tubules, where it metabolizes its substrate[s] that filtered through the glomeruli and generated de novo by the renal tubular cells such as dopamine. Detection of renalase in plasma and urine of healthy individuals suggests secretion by renal cells[34, 35]. Presence of detectable level of Renalase in venous plasma of healthy individuals but not in the plasma of patients with uremia, suggest secretion of Renalase by the intact kidney.

RENALASE-REGULATION

Renalase is secreted in an inactive form known as prorenalase and circulates in blood as protein hormone. Prorenalase has half life of 30-60 s and is rapidly activated by increased plasma catecholamines and converted to renalase, which in turn degrades catecholamines. Catecholamine administration promotes the secretion of preformed renalase within 5 min. It has been demonstrated that circulating renalase lacks significant amine oxidase activity under basal conditions [prorenalase] and a brief surge of epinephrine lasting <2 minutes causes renalase activity to increase from 48±18 to 2246±98 arbitrary units. Enzyme activation is detectable within 30 seconds and sustained for at least 60 minutes. Analysis of epinephrine-mediated hemodynamic changes in normotensive rats indicates that prorenalase becomes maximally activated when systolic pressure increases by >5 mm Hg. The catecholamine surge also leads to a 2.8-fold increase in plasma renalase concentration. Cultured cells exposed to dopamine increase steady-state renalase gene expression by >10-fold. The time course of prorenalase activation is abnormal in rats with chronic kidney disease[38]. Excess catecholamine facilitates the conversion of prorenalase into renalase, which can degrade catecholamine. These data reveal a mechanism responsible for the regulation of circulating catecholamine as well as activation of prorenalase & promote secretion and synthesis of Renalase[40].

RENALASE: MECHANISM OF ACTION

It has been suggested that renalase act as a catecholamine-degrading enzyme, via either O[2]-dependent or NADH-dependent mechanisms. The renalase crystal structure at 2.5 Å resolutions interacts with nicotinamide dinucleotides [NAD]. Renalase adopts the p-hydroxybenzoate hydroxylase fold topology-comprising a Rossmann-fold-based flavin adenine dinucleotide [FAD]-binding domain and a putative substrate-binding domain-containing a five-stranded anti-parallel β-sheet. A large cavity [228 Å], facing the flavin ring, presumably represents the active site. Compared to monoamine oxidase or polyamine oxidase, the renalase active site is fully solvent exposed and lacks an 'aromatic cage' for binding the substrate amino group. Renalase has an extremely low diaphorase activity, displaying lower k[cat] but higher k[cat]/K[m] for NADH compared to NADPH. Moreover, its FAD prosthetic group becomes slowly reduced when it is incubated with NADPH under anaerobiosis, and binds NAD [+] or NADP [+] with K [d] values of ca 2 mM. The absence of a recognizable NADP-binding site in the protein structure and its poor reactivity towards, NADH and NADPH suggest that these are not physiological ligands of renalase. However it is yet to be answered the question on the catalytic activity of renalase, which provides a firm framework for testing hypotheses on the molecular mechanism of action of renalase[41].

RENALASE- POTENTIAL ROLE IN SYMPATHO-NEURAL REGULATION AND CARDIOVASCULAR & RENAL ORGAN DAMAGE

Though the mechanism of role of renalase in regulation of blood pressure, sodium and phosphate excretion, and cardioprotectant action is barely understood to date, but its catecholamine metabolising action has been established by various studies[36-40]. It has been demonstrated that chronic kidney disease has been found to be associated with a number of systemic abnormalities, including activation of the sympathetic nervous system, increased catecholamines levels, cardiac hypertrophy, and hypertension. Further patients with chronic kidney disease [CKD], end-stage renal disease [ESRD] as well as animal models of chronic kidney disease and salt-dependent hypertension demonstrates markedly reduced level of plasma renalase. Low plasma and cardiac renalase, and abnormal renalase activation has been exhibited by the rats subjected to subtotal nephrectomy[41, 42].It is suggested that physiological function of renalase is attributed to its role in catecholamine metabolism. The claim of catecholamine-metabolising activity of renalase was based on the generation of H_2O_2 during incubation of the enzyme with catecholamines. It is critically dependent on FAD for oxidase activity, the renalase, in conjunction with MAO-A and MAO-B that catabolize intracellular amines, is an important enzyme to oxidase extracellular catecholamine, and thus contributing to the regulation of overall sympathetic tone by altering intra-lumen catecholamine level, thus regulating salt and water re-absorption. Clinical tests on rats showed that intravenous renalase decreased their blood pressures by 25% with dose dependent decrease in heart rate and cardiac contractility. The effects were dissipated within minutes. In another study, Injections of renalase in to the rats have shown significantly altered cardiovascular actions and reported to be ascribed to its catecholamine-metabolising activity. It was speculated that these findings may be due to massive α and β adrenoceptor blockade[43]. Increased frequency of hypertension and cardiovascular dysfunction in patients with end-stage renal diseases have shown higher plasma catecholamine concentrations but with absence of renalase in blood. It is hypothesized that poor or loss of secretion of Renalase by the kidney of ESRD might be responsible for increased plasma concentration of catecholamines and sympathetic manifestations[44].

Cardiac hypertrophy is frequently encountered in patients with renal failure and represents an independent risk factor for cardiovascular morbidity and mortality. The increased catecholamines such as norepinephrine [NE] may act on cardiac adrenergic receptors to cause cardiac hypertrophy which may lead to heart failure. These observation suggests that pathogenesis of cardiac hypertrophy is related to adrenergic over-stimulation[45,46]. Ghosh et al[44] studied norepinephrine transporter protein [NET] and renalase responsible for regulating the plasma catecholamine concentration and demonstrated that animals with 5/6 nephrectomy [Nx], had significantly higher plasma urea nitrogen, creatinine and blood pressure. The heart weight body weight ratio of the Nx cohort was higher than Sham and controls [$p<0.001$] groups indicating Nx animals had hypertrophied heart. It was concluded in this study that enhancement of cardiac GRK2 and NE acting on alpha-1 adrenergic receptors can contribute to cardiac hypertrophy seen in Nx animals.

Renalase is virtually non-detectable in patients with ESRD, whereas it is expressed in the blood of healthy individuals at concentration of about 5-10mg/L. Renalase deficiency observed in salt-sensitive rats as they develop hypertension, while Renalase inhibition by antisense RNA increases baseline blood pressure, and leads to an exaggerated blood pressure response to adrenergic stress. This relationship between Renalase levels and renal function make renalase an ideal candidate for a diagnostic marker for cardiovascular dysfunction and associated renal disease. Its identification may also lead to important implications in the development of therapeutics and diagnostics for end-stage renal diseases [ESRD] to treat/prevent hypertension, cardiovascular diseases such as asymptomatic left ventricular dysfunction, chronic congestive heart failure and atherosclerosis. Thus the identification of Renalase, its detection & expression in different cardiovascular & renal diseases condition makes it possible to identify a human patient afflicted with a disease, disorder or condition if it is associated with altered circulating level or expression of Renalase, comparing it with the level of expression of renalase in a normal human not afflicted with a disease, disorder or condition or increased sympathetic output.

SUMMARY

It is concluded from the above findings that renalase plays a key role in the regulation of sympathetic tone, blood pressure and cardiac function. The identification of renalase is not only an important step in development of a more detailed understanding of cardiovascular physiology, but also an important step in the quest for providing optimal treatment for patients with kidney disease and/or heart disease and their related complications. The identification and characterization of renalase provides a frame work for further study of renalase and its role in pathology of cardiovascular diseases such as chronic heart failure, myocardial infarction, cardiac arrhythmias as these diseases are invariably associated with increased sympathetic activity. Perhaps more importantly like MAO-A and B and renalase may provide a potentially useful target for modulating sympathetic activity in human. In addition to its potential therapeutic role, renalase may serve as a diagnostic marker for acute renal failure.

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