

## Role of Neprilysin in Various Diseases

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### Abstract

Neprilysin is a membrane bound zinc metallo-protease enzyme which catalyses a number of endogenous peptides. Hence exploring the physiological and biological role of these NEP family enzymes may provide novel approaches for treatment of major diseases. Neprilysin inhibitors have shown promising results for treatment of hypertension, heart failure, renal failure, hyperplasia, analgesia and improvement of  $\beta$ -cell function in obese type 2 diabetes mellitus. However decrease in neprilysin levels may lead to dementia, neurogenic inflammatory condition and also contributes to prostate cancer. Hence neprilysin being widely distributed in the body it is crucial to maintain its normal physiological levels since its up or down regulation may lead to various disorders.

**Keywords:** Neprilysin, natriuretic peptides, cancer, dementia

### 1. Introduction

Neprilysin is a cell membrane-bound zinc metallo-protease enzyme. It is also known as neutral endopeptidase (NEP), membrane metallopeptidase (MME), EC 3.4.24.11, arteriopeptidase, enkephalinase, cluster of differentiation 10 (CD10) cells, common acute lymphoblastic leukemia antigen (CALL or CD 10). It was first identified in rat kidney brush border membrane and later purified from rabbit kidney<sup>1</sup>. It was also discovered as brain enzyme which inactivates enkephalin family of neuropeptides and hence named as enkephalinase.<sup>2</sup> Later it was shown that NEP cleaved a wide range of biological peptides. NEP is most abundantly found in kidney. It is also found in human fibroblasts, human genital tract, brain, nerve ending and blood cells. In brain it is expressed by activated astrocytes<sup>3</sup> and microglia<sup>4</sup> NEP is identical to skin fibroblast elastase which plays important role in aging and UV induced skin damage.<sup>5</sup> It is expressed on surface of certain hematopoietic cell and on mature lymphocytes in certain diseased state.<sup>6</sup> It also plays an important role in number of cancers such as prostate<sup>7</sup> renal and lung cancer.

Neprilysin catalyzes the degradation of a number of endogenous vasodilator peptides such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), endothelin-1 (ET-1), angiotensin II, substance P, bradykinin, glucagon, gastrin, cholecystokinin, adrenomedullin, members of the vasoactive intestinal peptide family, amyloid  $\beta$  peptide (A $\beta$ ), enkephalin, neurotensin, oxytocin, bombesin like peptides and thymopentin. Therefore, the physiological actions of NEP will depend on the balance of its action on the breakdown of vasodilators and vasoconstrictors.<sup>8</sup>

#### 1.1 Genetics

The human NEP gene is located on chromosome 3 and it is composed of 24 exons.<sup>9</sup> The human genome has at least 7 NEP related enzymes of which endothelin converting enzyme – 1 (ECE-1) is well characterized. Genomic studies revealed that *Caenorhabditis elegans* and *Drosophila melanogaster* contain 22 and 24 NEP like genes and hence they are most frequently used as models for studying genomic expression and functional properties of NEP. In *Drosophila melanogaster* it has been reported that the Nep2 gene codes for a secreted endopeptidase which is involved in renal function and in spermatogenesis.<sup>10</sup>

#### 1.2 Biochemistry

NEP belongs to the family of exoenzymes such as aminopeptidase A and N, dipeptidyl peptidase, carboxypeptidase M and angiotensin converting enzyme (ACE).<sup>11</sup> Human NEP is an oligopeptidase expressed as a single chain of 742 amino acids and has a molecular weight 85000-110,000.<sup>12</sup> It is a 90 – 100 Kd transmembrane type II molecule with intra cytoplasmic N- terminus and extracellular C- terminus. The intracellular tail contains only 24 amino acids. The amino acid of extra cellular portions has 12 cysteine residues, many glycosylation sites and 3 zinc binding sites.<sup>11</sup>

#### 1.3 Endogenous inhibitors of NEP

Spinorphin obtained from bovine spinal cord was the first endogenous inhibitors of NEP. Next was sialorphin,

synthesized in sub-mandibular gland and prostate gland of rats.<sup>13</sup> The first human NEP inhibitor was opiorphin obtain from saliva.<sup>14</sup> NEP is inhibited by several exogenous agents such as candoxitrilat, phosphoramidon, thiorphan and its prodrug sinorphan.<sup>8</sup>

## 2. Physiological and pathological role of neprilysin

### 2.1. Central Nervous System

#### 2.1.1 Memory

Neprilysin is located pre and post synaptically in neuronal cells and cleaves its neuropeptide substances such as A $\beta$  thus terminating its action and neuronal function. Long-term reduction in neprilysin activity leads to Alzheimer's disease by promoting accumulation of A $\beta$  which leads to impaired synaptic plasticity and cognitive function in the brain. Studies showed that when rats were continuously infused with thiorphan, a specific neprilysin inhibitor, into the hippocampus region of brain developed cognitive impairments through accumulation of A $\beta$ . These results indicate that reduction of neprilysin activity contributes to the deposition of A $\beta$  and development of Alzheimer's disease. Thus, decrease in neprilysin levels is partly responsible for the memory-related symptoms of Alzheimer's disease and up-regulation of neprilysin activity may play a therapeutic role for treatment of Alzheimer's disease.<sup>15</sup>

#### 2.1.2 Stress

NEP genetically deprived, stress induced mice showed increased intake of alcohol without affecting the degradation of alcohol. Thus increasing NEP levels can be a potential target for treatment of alcohol abuse in the society.<sup>16</sup>

#### 2.1.3 Sleep

Studies in rats have showed that sustained sleep disturbance leads to increased NEP expression and activity in the intermediate lobe of the pituitary gland. The increase in neprilysin expression and activity was prolonged as long as the sleep disturbance continued and then returned to the basal level when rats were allowed to sleep freely. Thus the results showed that NEP processing and degradation in the pituitary gland plays a vital role in sleep deprivation.<sup>17</sup>

#### 2.1.4 Stroke

During stroke, brain levels of endothelin-I, a potent vasoconstrictor is raised. SLV338, an endothelin converting enzyme/neutral endopeptidase blocker, has shown anti inflammatory and neuroprotective effect, in salt-loaded, stroke-prone, spontaneously hypertensive rats model.<sup>18</sup> Thus combined endothelin converting enzyme/neutral endopeptidase inhibition could offer a new therapeutic approach for primary stroke prevention.

### 2.2. Cardiovascular System

#### 2.2.1 Hypertension and heart failure

Natriuretic peptides such as ANP, BNP, CNP and bradykinins have potent natriuretic and vasodilator properties, decrease sympathetic activity, inhibit aldosterone secretion and have anti-proliferative and anti-hypertrophic properties.<sup>19</sup> Neprilysin degrades these natriuretic peptides and studies in animal models of hypertension showed that ACE inhibitors effectively decreased blood pressure in renin dependent animal models but were ineffective in volume dependent animal hypertension models. However NEP inhibitors were effective in volume dependent models but were ineffective in renin dependent animal models of hypertension. Thus the antihypertensive effect of NEP inhibitor, candoxitrilat in humans was found to be variable. Hence dual ACE and NEP inhibition were found to be useful in the treatment of hypertension.<sup>20</sup>

Vasopeptidase inhibitors are drugs which can inhibit both NEP and ACE enzymes. Thus it decreases BP by preventing degradation of natriuretic peptides and decreasing angiotensin II levels. It also preserves renal blood flow and glomerular filtration rate and promotes diuresis without kaliuresis.<sup>21</sup> Four vasopeptidase inhibitor that are currently under clinical development are discussed below. Sompatriat showed sustained anti-hypertensive effect in humans<sup>21</sup> and improved cardiac function in chronic heart failure models of rats.<sup>22</sup> Fasidotril also known as alatriopril/aladotril is a prodrug and is converted into the body into fasidotrilate.<sup>20</sup> Both fasidotril and gemopatriat showed antihypertensive effect. Omapatriat showed significant blood pressure reduction but unfortunately caused high incidence of angioneurotic oedema since both enzymes were involved in bradykinin degradation.<sup>23</sup> Hence its use is still controversial. LCZ696 was the first chemical moiety which provided inhibition of both NEP and the angiotensin II type I receptor. This led to dual inhibition of RAAS and natriuretic peptides degradation without significantly affecting bradykinin catabolism.<sup>24</sup>

#### 2.2.2 Hyperplasia

CNP and adrenomedullin are natriuretic peptides that are degraded by NEP. It has been found that both CNP and adrenomedullin prevented atherogenesis by inhibiting vascular smooth muscle cells migration, proliferation and reducing intimal thickening and macrophage inflammation. Thus NEP inhibition will increase the level of adrenomedullin and CNP leading to improved endothelial functions. Thus a study showed that NEP inhibitor candoxitrilat coated stent increased the local concentration of CNP and adrenomedullin and helped to prevent hyperplasia in adventitial therapies leading to restenosis.<sup>25</sup>

### 2.3. Renal

In cirrhosis and renal failure patients sodium retention is present and studies showed that thiorphan, a NEP inhibitor induced natriuresis by acting directly on Na<sup>+</sup>K<sup>+</sup>ATPase without affecting systemic hemodynamics. Thus natriuresis without hemodynamic changes by NEP inhibitor is enviable in patients with cirrhosis and ascites.<sup>8</sup>

### 2.4. Metabolic effects

Studies show that increased NEP level is associated with cardio metabolic risk in the presence of insulin resistance.<sup>26</sup> Islet levels of NEP is increased on prolonged exposure to free fatty acids and this led to decreased insulin

secretion and cellular dysfunction. Thus neprilysin inhibition may be useful to improve  $\beta$ -cell function in obese type 2 diabetes mellitus patients.<sup>27</sup>

### 2.5. Inflammation

NEP inactivates tachykinins, substance P, bradykinins released during stimulation of sensory nerves and thus prevents neurogenic inflammation. Thus drugs that increase NEP levels may play a therapeutic role by suppressing these neurogenic responses. Substance P induces inflammation in acute pancreatitis by binding with neurokinin-1 receptor. NEP the cell-surface enzyme degrades substance P in the extracellular fluid. Studies show that acute inhibition of NEP increases substance P levels in caerulein-induced acute pancreatitis, causing severe inflammatory responses in the pancreas.<sup>28</sup>

Neprilysin exists in the basal cells of the airway epithelium, nerves, smooth muscles, glands, blood vessels and other cells. It modulates smooth muscle contraction, gland secretion, cough, vascular permeability and neutrophils adhesion. Decreased NEP activity occurs in epithelial removal, during respiratory viral infections, and during exposure to irritants such as cigarette smoke. The decrease in NEP activity will lead to exaggerated neurogenic inflammation and may play an important role in inflammatory diseases in airways.<sup>29</sup>

### 2.6. Pain

Endogenous opioid peptides such as Met-enkephalin, Leu-enkephalin and dynorphins are inactivated by aminopeptidase N and NEP. WBC and peripheral nerves are main sources of aminopeptidase N and NEP in inflamed tissue. The blockade of both of these enzymes prevents opioid peptide metabolism and promotes analgesic effect. Thus NEP inhibitor can be used for treating inflammatory pain.<sup>30</sup>

### 2.7. Immunity

CD10 is now known as NEP. CD10 is present transiently during early B and pre-B lymphoblastic stages and also expressed in developing organs such as breast and lungs. It is expressed in endometrial stroma cells, myo-epithelial layer of the breast, proximal tubule and glomeruli of kidney, in epithelial and stromal cell of prostate, canaliculi cells of liver, and epithelial cells of stomach and colon. CD10 and oxytocin cleaved peptides play an important role in milk secretion by inducing myoepithelial cell contraction. CD10 inactivates pro-inflammatory peptides involved in immune system. In rheumatoid arthritis and osteoarthritis notch signalling regulates expression of several genes including CD10 which in-turn hydrolyses IL  $\beta$ . Smokes and allergen also decrease CD10 levels leading to neurogenic inflammatory response. In melanoma CD10 along with other genes are involved in antigen processing and presentation.<sup>11</sup>

CD10 has diagnostic and prognostic value in acute lymphoblastic leukemia. CD10 also has therapeutic role in acute lymphoblastic leukemia and is used to assess minimal residual disease (MRD). Children with low MRD can receive less intense chemotherapy. It is also expressed by other solid tumors such as neuroblastoma and neuroblastoma in children and melanoma and several other carcinomas in adults.<sup>31</sup>

### 2.8. Cancer

NEP is expressed on benign prostate epithelial cells and its function is to reduce local concentrations of neuropeptides such as bombesin and endothelin available for receptor binding and signal transduction. Studies show that NEP can inhibit Focal Adhesion Kinase (FAK) phosphorylation on tyrosine and prostate cell migration. Decreased level of NEP in prostate cancer cells contributes to tumour progression by allowing neuropeptides to bind to their receptors and induce androgen-independent cell division and promote growth stimulatory pathways.<sup>7</sup>

## 3. Conclusion

NEP plays an important role in many physiological and biochemical process of the body and hence can provide potential treatment strategies for various diseases. Neprilysin inhibitors have shown promising results for treatment of hypertension, heart failure, renal failure and improvement of  $\beta$ -cell function in obese type 2 diabetes mellitus. However decrease in neprilysin levels may lead to dementia, neurogenic inflammatory condition and also contributes to prostate cancer. Hence it is necessary to maintain the level of NEP inhibitors at optimal levels to avoid possible adverse effects.

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