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Upregulation of neprilysin activity is a promising strategy for therapy and prevention of AD and type 2 diabetes

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صدق الله العلي العظيم

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Dedication

To the fountain of patience, optimism and hope.

To each of the following in the presence of god and his messenger my mother dear.

To those who have demonstrated to me what is the most beautiful of my Brothers life.

To the big heart my Father dear.

To the people who paved our way of science and knowledge all our teachers distinguished.

To the taste of the most beautiful moments with my friends.

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CHAPTER ONE

INTRODUCTION

1-Introduction

1-1-General Properties of Neprilysin

Neutral endopeptidase, or neprilysin (NEP), was first described as a neutral proteinase in rat kidney brush border membranes and then purified from rabbit kidney and characterised as a zinc metallopeptidase[1]. Although NEP is abundant in the kidney (about 4% of all membrane proteins), its content in other organs, including the brain, is much lower. NEP was later rediscovered as a brain enzyme responsible for inactivation of the enkephalin family of neuropeptides
and given the name enkephalinase[2]. However, it was subsequently shown that NEP is not enkephalin specific but that it can cleave a wide range of biologically relevant peptide substrates, for example, substance P, and as such it was given the common name, endopeptidase-24.11[3]. NEP is also known as the common acute lymphoblastic leukaemia antigen (CALLA or CD10) since it turned out to be identical with this leukocyte cell surface antigen[4] . NEP was also reported to be identical with a recently described activity termed skin fibroblast elastase which plays a role in skin aging and UVA-induced skin damage[5].
NEP is an oligopeptidase which cleaves peptides containing up to 40–50 amino acids and the most efficiently hydrolyzed substrate is substance P[6]. The principal substrates of NEP *in vivo* appear to be enkephalins, atrial natriuretic peptide, tachykinins, bradykinin, endothelins, adrenomedullin, members of the vasoactive intestinal peptide family, glucagon, thymopentin, and, most significantly in pathophysiological terms, the Alzheimer's disease (AD) amyloid β-peptide (Aβ) .

NEP is a type II integral membrane zinc metalloprotein and does not have a proenzyme form. It is an ectoenzyme with the bulk of its structure,

including the active site, facing the extracellular space[7]. The cDNA cloning of NEP revealed that rat and human enzymes consist of 742 amino acids[8]. The high similarity between human and rodent NEP proteins makes the rat a useful animal model for studying NEP functions and regulation.

The first human NEP inhibitor isolated from saliva was opiorphin which had some pain-suppressive potency[9]. The most potent and widely used NEP inhibitors include phosphoramidon and thiorphan, and the 3D structure of the extracellular domain of NEP in a complex with hosphoramidon has been resolved allowing better understanding of the catalytic properties of the enzyme[10].

One particular feature of the NEP catalytic site is its restricted size which prevents access of large peptides and proteins but allows peptides containing up to 50 amino acid residues. This is consistent with Aβ as a preferred substrate of NEP. Another characteristic feature of NEP is its sensitivity to inhibition by phosphoramidon and thiorphan at nanomolar concentrations. Although a closely related NEP homologue endothelin-converting enzyme (ECE-1) is also inhibited by phosphoramidon, it is only sensitive to micromolar concentrations of the inhibitor and is not affected by thiorphan.

In the brain, NEP appears to have mostly neuronal localization[11] although it was recently reported to be expressed by activated astrocytes[12] and microglia[13] . In peripheral tissues NEP was also found to be transiently expressed on the surface of certain haematopoietic cells and increased NEP levels were found on mature lymphocytes in certain disease states[14] . It has also been implicated in the progression of a number of cancers, including prostate[15], renal[16] , and lung[17] cancer. Another important role of NEP is related to inactivation of the natriuretic

peptides *in vivo* and as such NEP inhibitors have been explored as potential cardiovascular and renal therapeutics.

The human *NEP* gene is located on chromosome 3 and exists in a single copy which spans more than 80 kb. It is composed of 24 exons and is highly conserved among mammalian species[18]. Expression of the *NEP* gene is controlled through two distinct promoters[19] whose role differs between cell types, although both promoters show similar characteristics and activity. Three distinct NEP mRNAs have been identified in human and rat which differ only in their 5_-noncoding regions . A gene knockout of *NEP* in mice has been reported in which the animals appeared developmentally normal but the NEP null mice were highly sensitive to endotoxic shock[20] . This observation may reflect a general role of NEP in the metabolism of proinflammatory peptides. NEP knockout mice also showed enhanced aggressive behaviour in the resident-intruder paradigm and altered locomotor activity as assessed in the photobeam system[21] . They also had an increased alcohol and food consumption[22] .

1-2-Type 2 diabetes and Alzheimer's disease :

There is a wide activity of NEP but in this review I shall focus about two pathological patterns type 2 diabetes and Alzheimer's disease (AD) because

first : They share a number of clinical and biochemical features [23]. Both are characterized by functional tissue loss associated with accumulation and aggregation of a small peptide—islet amyloid polypeptide (IAPP, also known as amylin) in pancreatic islets in type 2 diabetes and amyloid β peptide (Aβ) in AD brain. Human IAPP (hIAPP) aggregation leads to pancreatic beta cell loss, while Aβ aggregation leads to neuronal cell loss[24,25] . Similarly to the synthesis of Aβ from the amyloid precursor

protein, IAPP is derived from an 89- residue prohormone precursor (prepro-IAPP) to form the mature 37-amino acid peptide hormone[26]. IAPP is co-secreted with insulin by pancreatic beta cells [27-29]. hIAPP, like human Aβ but not rodent IAPP (rIAPP) or rodent Aβ, forms oligomers and then fibrils leading to the amyloid deposits seen in association with type 2 diabetes and AD, respectively. In the case of IAPP it is thought that three proline residues within the region of amino acids 20–29 of rIAPP hinder its aggregation. hIAPP lacks these proline residues and aggregates under pathological circumstances[30].

Second, more recently neprilysin (NEP), another amyloid-degrading enzyme (ADE), was demonstrated to be involved in the regulation of IAPP aggregation[31],[32] through what was proposed to be a non-catalytic mechanism. NEP has been shown to play a major role in the clearance of Aβ in brain[33], and thus NEP playing a role in preventing IAPP aggregation would represent another parallel between type 2 diabetes and AD.

NEP has been reported to be present in both mouse and human pancreatic islets [32]. In experiments conducted in transgenic mice, inhibition of NEP increased amyloid formation [31]. Conversely, upregulation of NEP decreased amyloid formation and beta cell apoptosis in cultured hIAPP transgenic mouse islets [31]. However, since NEP degradation of hIAPP was not observed, it was suggested that NEP acted by inhibiting hIAPP fibrillisation through a non-catalytic protein– protein interaction [31].

Third, insulin accelerates Aβ intracerebral circulation and elimination, and increases of the brain insulin resistance have been reported in many AD patients. Showing insulin resistance during midlife increases the risk of suffering AD in subsequent years[34] , so do not be surprised that AD may consider **type 3 diabetes** . For this reason, the effectiveness of rosiglitazone has been researched in treatment of AD; it reduces such

resistance, is anti-inflammatory and stimulates mitochondrial biogenesis[35] .

NEP delivery to peripheral tissues has also proved effective in reducing the amyloid polypeptide deposits which contribute to type 2 diabetes. Thus, adenoviral delivery of NEP to the pancreas reduced these amyloid deposits and cell death in the islets[31] . Surprisingly, however, in this case, NEP did not degrade the amyloid peptide but appeared to act by inhibiting the amyloid fibril formation through protein–protein interactions involving the enzyme active site, rather than by hydrolyzing the peptide.

Finally, the **fourth** reason focusing about idea in my mind (if there is drug(s), dietary supplement(s) or strategy for therapy and prevention of AD and type 2 diabetes together ? and what are the mechanisms that lead to that magical effects ?) I select ginkgo Biloba extract(GBE), is a well-defined mixture of active compounds (24% ginkgo flavonoid glycosides, 6% terpene lactone) [36]

extracted from Ginkgo biloba leaves has been studied extensively, with regard to its effects on both behavior and physiological parameters [37]. It has been shown to exert a broad range of biochemical and pharmacological actions. These include antioxidant and free radical-scavenging effects, as well as hemodynamic and neurotransmitter effects[37]. While the mechanisms underlying the neuroprotective actions of GBE are unclear, there is some evidence showing that GBE can regulate the levels of neurotransmitters, such as serotonin [38], influence neurotransmitter receptors [39], regulate structural changes in hippocampal circuitry[40], affect neuronal excitability [41] and trigger neurogenesis in the hippocampus. GBE also possesses **antihyperglycemic**, , and antihyperlipedimic activities in streptozocin induced chronic diabetic rats[42].

So, the choosing of GBE in this review for their known effect in treatment of type 2 diabetes and AD and the proposal mechanism for GBE I put here based upon reducing and/or preventing the progressive β-cell failure associated with type 2 diabetes and Aβ in AD may involve modulating neprilysin activity suggesting that upregulation of neprilysin activity is a promising strategy for therapy and prevention of AD and type 2 diabetes.

CHAPTER TWO

NEPRILYSIN ACTIVITY

2-Upregulation of neprilysin activity is a promising strategy for therapy and prevention of AD and type 2 diabetes:

Apart from genetic manipulations of NEP expression, a number of studies have aimed at pharmacological upregulation of the enzyme which have demonstrated that NEP activity can be increased by, among other compounds, a component of green-tea extract, Epigallocatechin-3-gallate (EGCG)[43] and other plant extracts and polyphenols[44],so concomitant with these finding GBE may have the same effect (up regulation of NEP). Some researchers tested the hypothesis that elevated levels of neuropeptide substrates of NEP could themselves up-regulate NEP as a feedback control mechanism. They hence screened a wide range of NEP substrates demonstrating that, of those tested; only somatostatin was capable of up-regulating NEP expression in primary neuronal cells in a mechanism possibly mediated through somatostatin receptor sub-types 2 or 4[45]. Another potential strategy for pharmacological up-regulation of NEP was reported in a study analysing the effects of a selective peroxisome proliferator activated receptor-δ (PPAR-δ) agonist, GW742, which activated the NEP promoter driving luciferase expression in transfected HEK293 cells[46] .

A completely new turn in development of strategies for modulation of NEP expression and activity , demonstrated that the C-terminal APP (amyloid precursor protein) intracellular domain (AICD) was able to up-regulate NEP transcription and to reduce accumulation of Aβ [47],[48].

Following these initial observations further attempts were made to up-regulate NEP expression using this AICD-dependent mechanism and it was reported that the tyrosine kinase inhibitor, Gleevec® (imatinib, STI-571), could elevate AICD levels and, in turn, increase NEP mRNA and protein levels and decrease Aβ secretion[49],[50].(fig 3)

While investigating the epigenetic mechanisms of NEP regulation by AICD it was observed that the NEP promoter is competitively regulated by AICD and the histone deacetylase histone deacetylase (HDAC)1 and that HDAC inhibitors such as trichostatin A and valproic acid (VA) could upregulate NEP at the mRNA, protein and activity levels[51],[52] .

CHAPTER THREE

DISCUSSION

3-Discussion :

Pharmacological approaches to enzyme upregulation (allosteric modulators, activators, epigenetic regulators, etc.) might provide more economic approaches than gene delivery strategies but targeted delivery may be important given the alternative substrates for some of these enzymes, hence GBE considered more cheaper , available and less side effect.

In any case, it is important to bear in mind that only a relatively small but prolonged increase in the activity of any ADEs could result in a positive shift of amyloid balance towards its clearance. A single focus for treatment of AD is unlikely to emerge so a combination therapy including targeting other links in the chain of pathological events leading to AD is likely to be optimal. But all depends on the primacy of the amyloid cascade hypothesis (Fig 1 and 2).

The level of NEP activity decreases with age, and patients with AD have low levels of this enzyme in the hippocampus and cortex. Thus, upregulation of NEP has been explored as a therapeutic approach to treating AD. Insulin degrading enzyme (IDE) can also degrade Aβ, and it has been suggested that insulin resistance results in impaired clearance of Aβ. That being said, decreased NEP and IDE levels may be a result and not a cause of Aβ pathology. A study of postmortem tissue from the frontal cortex of patients of different ages and at different pathological stages of AD was analyzed for IDE and NEP levels and activities. The results suggested that downregulation of NEP and IDE activity is not a primary cause of AD but rather can increase Aβ accumulation. Other enzymes such as endothelin-converting enzymes and angiotensin-converting enzymes are under investigation for their ability to reduce the risk of AD or delay AD progression .

there is an increasing need of new natural antihyperglycemic products with less side effects, safe, and high antihyperglycemic potential and the GBE is preferred to be used and we advised to be used before the occurrence of type 2 diabetes and AD

Twenty years of research may not have produced the long-awaited antiamyloid ‘magic bullet’ but the search continues to prove that GBE have this mechanism of action.(fig. 4)

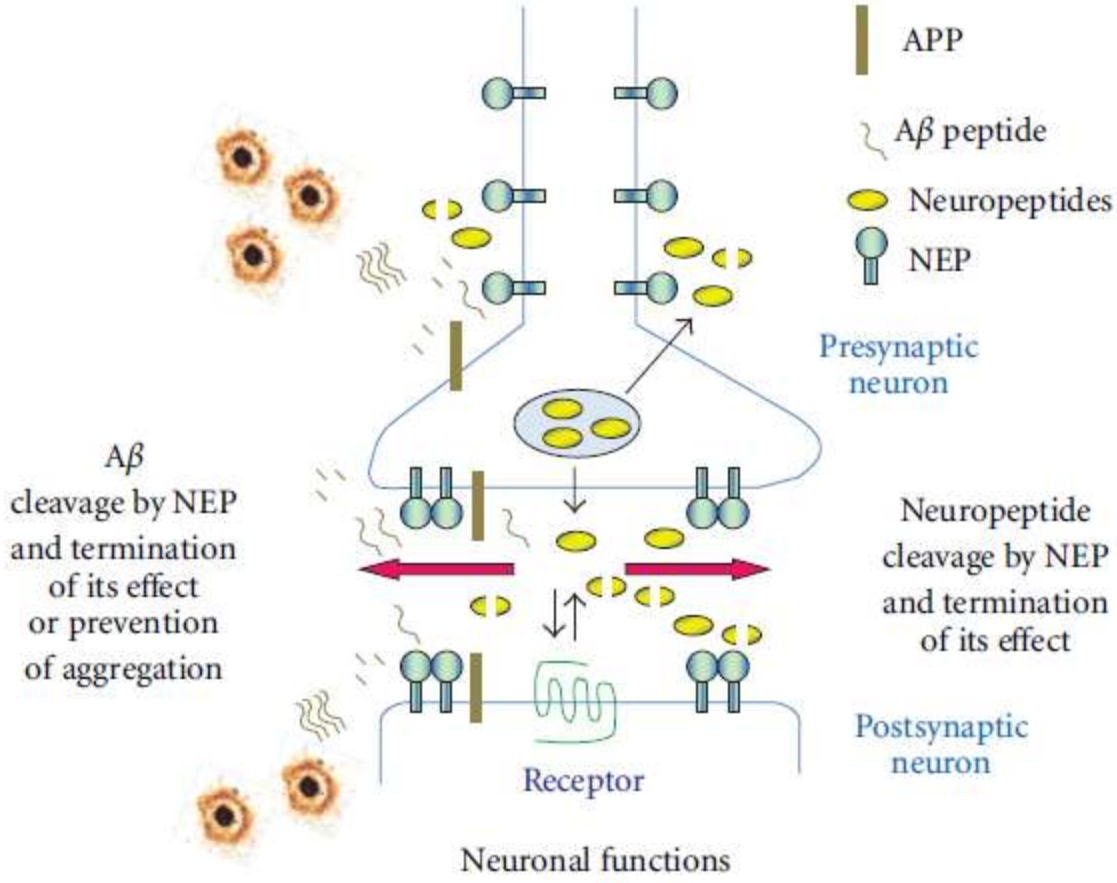

Figure 1: Schematic presentation of NEP localization and functional activity in the brain. NEP being localised pre- and postsynaptically in neuronal cells cleaves its neuropeptide substrates (including Aβ) terminating their properties and as such regulating cellular response to their action and neuronal functions. In the case of Aβ, NEP also prevents accumulation and aggregation of toxic amyloid oligomers. All symbols are explained in the figure.

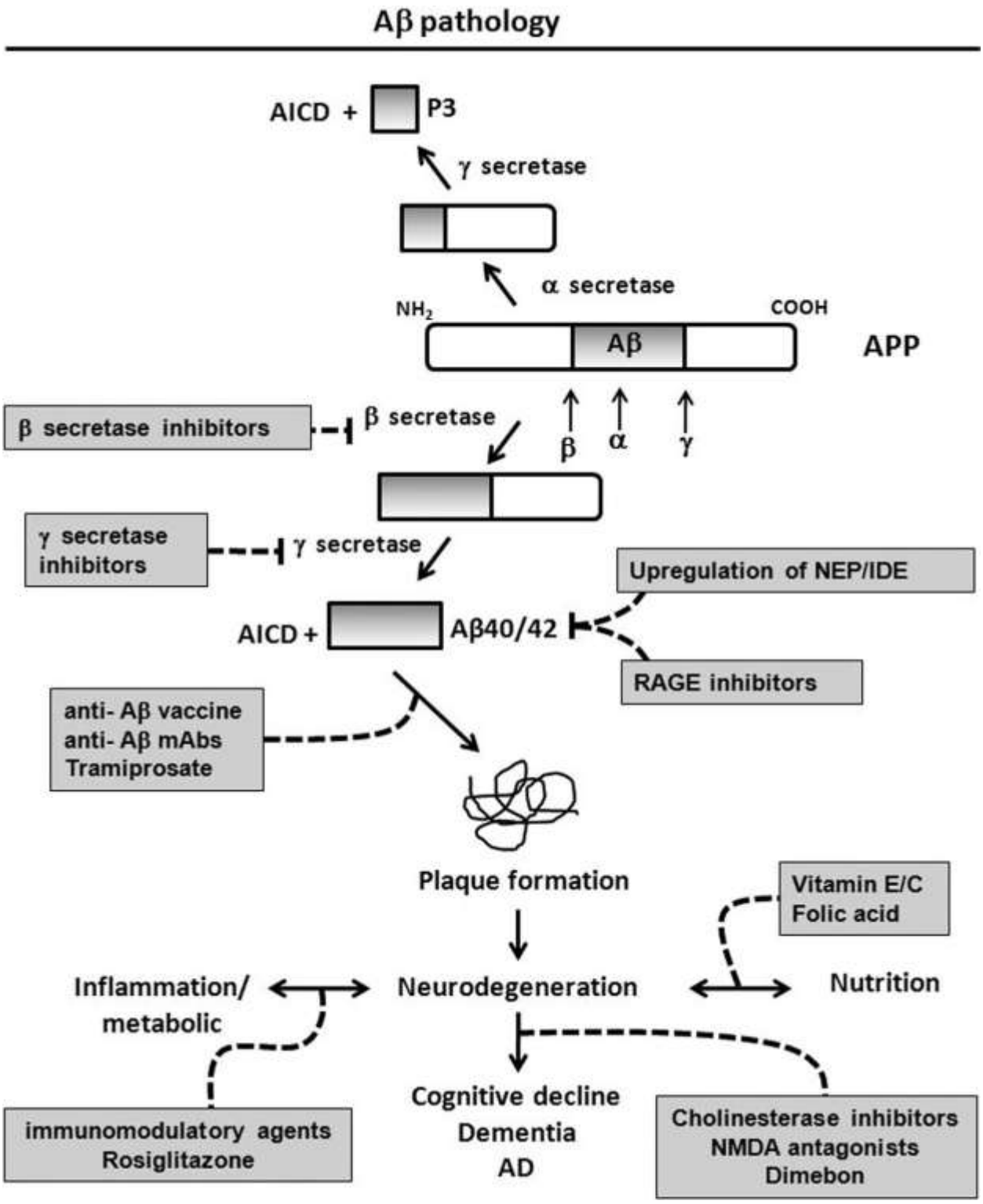

Figure 2. Schematic of the amyloid precursor protein processing pathway that leads to neurodegeneration and Alzheimer's disease (AD). Gray boxes indicate emerging therapeutic strategies for targeting β-amyloid peptide (Aβ)-mediated pathology. AICD indicates APP intracellular domain; APP, amyloid precursor protein; IDE, insulin degrading enzyme; mAbs,

monoclonal antibodies; NEP, neprilysin; NMDA, N-methyl-D-aspartic acid; RAGE, receptors for advanced glycation end products.

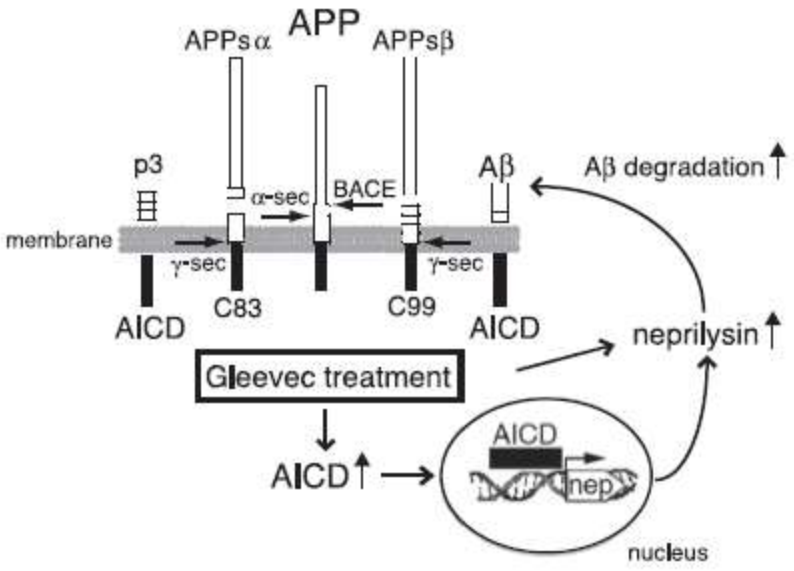

Figure 3 .working Model of Gleevec Mechanism. Gleevec treatment increases AICD levels via a slowed turnover of AICD. Neprilysin expression is increased by Gleevec, mediated by transcriptional activation, which probably involves AICD signaling. Increased neprilysin expression may lower A levels by enhanced degradation. α-sec, α-secretase; γ-sec,γ-secretase; nep, neprilysin gene.

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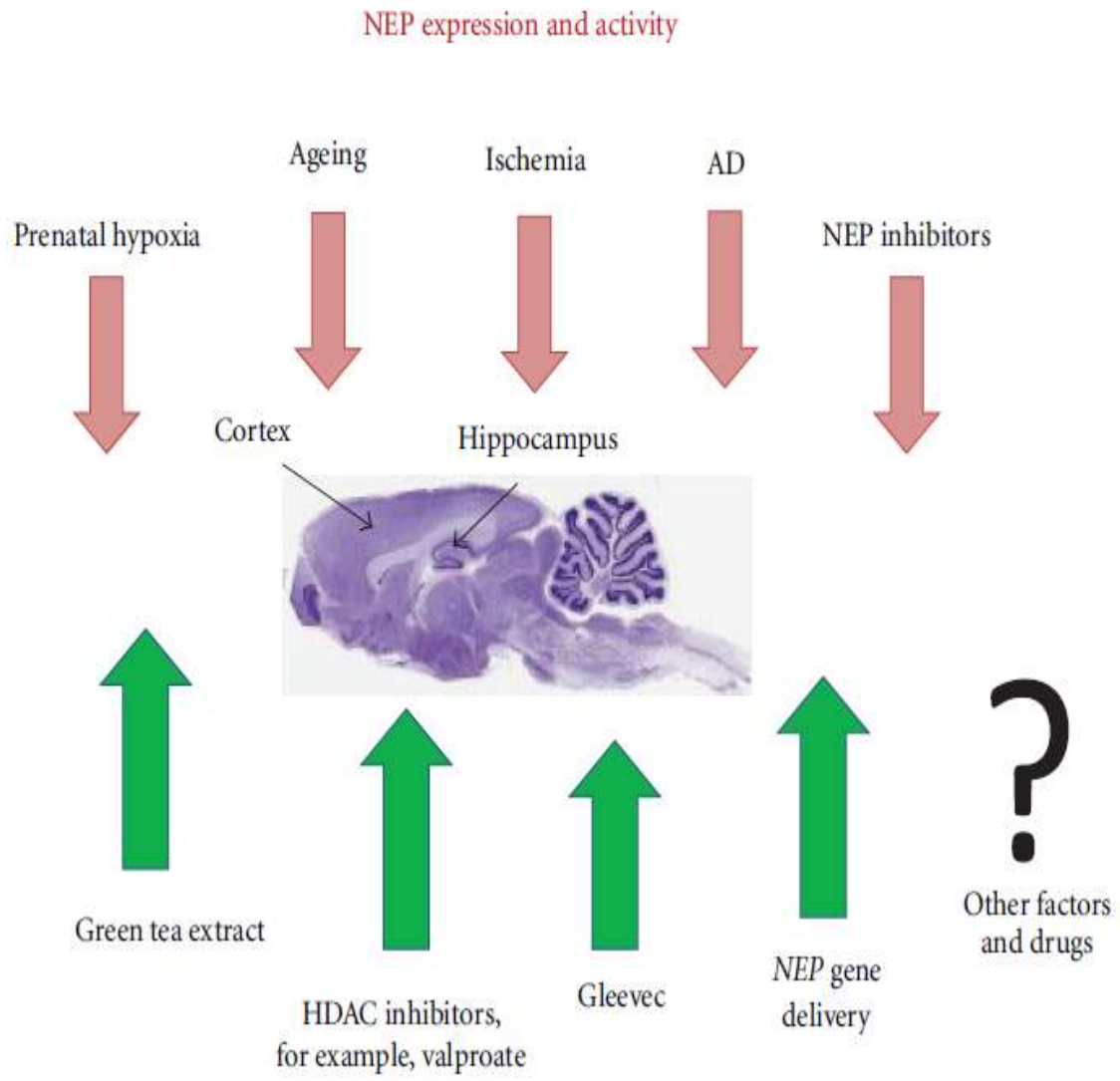

Figure 4: Effects of various experimental conditions on NEP activity in vivo. As explained in the text, NEP expression and activity in brain cortex and hippocampus (the structures which are characterised by accumulation of amyloid deposits) decreases with age and is also decreased after prenatal hypoxia, ischemia, or in the case of AD. In animal models, NEP activity can be modulated by its inhibitors affecting such brain functions as learning and memory. Mechanisms which can control and upregulate NEP expression and increase its activity include targeted NEP gene delivery, regulation of its promoter via inhibition of HDACs or pharmacologically by green tea extract (or EGCG) or Gleevec.

CHAPETER FOUR

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